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ASPECTOS COMPORTAMENTAIS, BIOQUÍMICOS E MOLECULARES IMPLICADOS NO PAPEL DO MAGNÉSIO NOS DISTÚRBIOS MOTORES INDUZIDOS EXPERIMENTALMENTE EM RATOS

Santa Maria, RS, Brasil
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Defesa de Tese apresentado ao Curso de Pós-Graduação em Farmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Farmacologia.**

Orientadora: Prof[a] Dr[a]. Marilise Escobar Bürger

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Aprovado em 08 de fevereiro de 2019:

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Marilise Escobar Bürger, Dra. (UFSM)
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Santa Maria, RS

2019

Dedico esta tese aos meus queridos pais Nelsa e Wilmar.

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RESUMO

ASPECTOS COMPORTAMENTAIS, BIOQUÍMICOS E MOLECULARES IMPLICADOS NO PAPEL DO MAGNÉSIO NOS DISTÚRBIOS MOTORES INDUZIDOS EXPERIMENTALMENTE EM RATOS

Autor: Maikel Kronbauer
Orientadora: Marilise Escobar Bürger

O uso crônico de antipsicóticos típicos têm sido relacionados ao desenvolvimento de distúrbios do movimento, que se manifestam por movimentos involuntários repetitivos, tremores e discinesia tardia, manifestações que podem atingir níveis incapacitantes, prejudicando o tratamento farmacológico da patologia original. O objetivo inicial do presente estudo foi investigar a influência da suplementação de magnésio (Mg) (Mg; 40 mg / kg, via oral, uma vez ao dia) na prevenção (28 dias antes) e reversão (12 dias após), como também em suplementação concomitante, sobre a discinesia orofacial (DO) induzida por haloperidol (HAL) (12 mg / kg; im, uma vez por semana, durante 4 semanas) em ratos. Os resultados mostraram que a suplementação de Mg antes, após e concomitante à administração de HAL, respectivamente impediu, reverteu e preveniu o aumento da frequência dos movimentos de mascar no vazio (MMV) e cataplepsia. A suplementação de Mg: i) impediu a geração de espécies reativas (ER) em ambos córtex e *substantia nigra* (SN), reduzindo os níveis de proteínas carboniladas (PC) em todas as áreas cerebrais avaliadas; ii) reverteu a geração de ER induzida pelo HAL em ambos córtex e no estriado, diminuindo os níveis de PC em ambos SN e estriado; iii) preveniu a geração de ER no córtex, estriado e SN, como também o aumento dos níveis de PC na SN. A partir destes resultados, foi realizado sequencialmente um estudo comparativo entre a suplementação de Mg e a administração de nifedipina (NIF- 10mg/Kg/mL, via oral, uma vez ao dia), sobre os distúrbios do movimento induzidos pelo HAL em ratos, afim de determinar se ação do Mg é semelhante a NIF. Ambos Mg e NIF reduziram a DO induzida pelo HAL. Também, enquanto o HAL aumentou a atividade da Ca^{2+}-ATPase no estriado, a suplementação de Mg reverteu. No córtex, tanto o Mg quanto a NIF reduziram tal atividade. A imunorreatividade dos receptores dopaminérgicos D1 e D2 e do receptor glutamatérgico NMDA, foram modificadas de diferentes maneiras pelo HAL nas áreas do córtex, estriado e SN, como também pelos tratamentos. De particular importância observou-se o aumento da imunorreatividade dos receptores NMDA em todas as regiões cerebrais sendo reduzidas pelo Mg ou NIF em todas as regiões analisadas. Esses achados nos permitem propor que o Mg pode ser útil para prevenir e minimizar distúrbios de movimento induzidos por antipsicóticos clássicos como o HAL, cujo mecanismo molecular parece estar envolvido com um significativo e possivelmente mais consistente bloqueio dos canais de cálcio, desde que o grupo de animais tratados com NIF apresentou respostas menos expressivas quando comparados ao grupo tratado com Mg. Em conjunto, os resultados apresentados nestes estudos indicam que a suplementação de Mg é capaz de prevenir ou amenizar distúrbios motores extrapiramidais, cujo mecanismo de ação parece estar envolvido no bloqueio de canais de cálcio. Estes distúrbios são frequentemente relacionados com o tratamento crônico antipsicótico, não existindo até o momento um tratamento preventivo eficaz. A suplementação de Mg, sendo um cátion bivalente de baixa toxicidade sistêmica e de baixo custo, pode significar uma alternativa acessível e eficaz para os pacientes sob tratamento neuroléptico.

Palavras-chave: discinesia orofacial; haloperidol; distúrbios do movimento; Mg^{2+}; Catalepsia; Dano oxidativo; nifedipina; cálcio; receptor NMDA.

ABSTRACT

BEHAVIORAL, BIOCHEMICAL AND MOLECULAR ASPECTS OF MAGNESIUM ROLE INVOLVED IN MOTOR DISORDERS EXPERIMENTALLY INDUCED IN RATS

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Advisor: Marilise Escobar Bürger

The chronic use of typical antipsychotics has been related to movement disorders development, which are manifested by repetitive, tremors and tardive dyskinesias, manifestations that can reach disabling levels and impair the pharmacological treatment of the original pathology. The aim of the present study was to evaluate magnesium supplementation (Mg; 40 mg / kg, oral, once daily) in prevention (28 days before) and reversion (12 days after), as well as in concomitant supplementation, on haloperidol-induced orofacial dyskinesia (OD) (12 mg / kg; im, once weekly for 4 weeks) in rats. The results showed that the Mg supplementation before, after and concomitant to HAL administration, prevented, reversed and protected from the high vacuous chewing movements frequency (VCM) and cataplepsy. Mg supplementation: i) prevented the generation of reactive species (RS) in cortex and *substantias nigra* (SN), and reduced the levels of carbonylated proteins (CP) in all evaluated brain areas; ii) reversed RE generation induced by HAL in the cortex and striatum and decreased CP levels in both SN and striatum; iii) prevented RS generation the cortex, striatum and SN, as well as the increased CP levels in SN. From these results, a comparative study between Mg supplementation and nifedipine administration (NIF-10mg / kg / ml, orally, once daily) on the movement disorders induced by HAL in rats was performed sequentially, in order to determine if Mg action is similar to NIF. Both Mg and NIF reduced HAL induced OD. Also, while HAL increased Ca^{2+}-ATPase activity in the striatum, Mg supplementation reversed it. In the cortex, both Mg and NIF reduced this activity. The immunoreactivity of dopaminergic D1 and D2 receptors and the glutamatergic receptor NMDA were modified in different ways by HAL in the cortex, striatum and SN areas, as well as by the treatments. Of particular importance, was observed the increased immunoreactivity of NMDA receptors in all brain areas being reduced by Mg or NIF in all brain areas analyzed. These findings allow us to propose that Mg may be useful in preventing and minimizing movement disorders induced by classical antipsychotics such as HAL, whose molecular mechanism seems to be involved with a significant and possibly more consistent calcium channel block activity, provided that the group of animals treated with NIF showed less expressive responses when compared to the group treated with Mg. Taken together, the results presented in these studies indicate that Mg supplementation is able to prevent or ameliorate extrapyramidal motor disorders whose mechanism of action seems to be involved in calcium channel blockade. These disorders are often related to chronic antipsychotic treatment, so far there is no effective preventative treatment. Mg supplementation, which is a bivalent cation with low systemic toxicity and low cost, may be an accessible and effective alternative for patients undergoing neuroleptic treatment.

Key words: orofacial dyskinesia; haloperidol; movement disorders; Mg^{2+}; catalepsy; oxidative damage; nifedipine; calcium; NMDA receptor.

LISTA DE ABREVIATURAS E SIGLAS

ADN	Ácido desoxirribonucleico
ARN	Ácido ribonucleico
D1	Receptor de dopamina tipo 1
D2/D2R	Receptor de dopamina tipo 2
DA	Dopamina
DO	Discinesia Orofacial
DT	Discinesia Tardia
EO	Estresse oxidativo
HAL	Halperidol
Mg	Magnésio
MMV	Movimentos de mascar no vazio
NIF	Nifedipina
NMDA	N-methyl-D-aspartate
NMDAR1	N-methyl-D-aspartate receptor type 1
RL	Radicais livres
SN	*Substantia nigra*
TBARS	Thiobarbituric acid reactive substances

SUMÁRIO

APRESENTAÇÃO

Esta tese apresenta os métodos e resultados embutidos em um artigo científico na seção **ARTIGO CIENTIFICO**, e em um manuscrito que se encontra na seção **MANUSCRITO CIENTÍFICO**, sob a formatação das revistas para os quais foram publicado e submetido para publicação, respectivamente.

Ao fim desta tese encontram-se os itens **DISCUSSÃO, CONCLUSÕES** e **PERSPECTIVAS** nos quais há conclusões gerais sobre o manuscrito científico contido neste estudo, bem como propostas para a continuidade do estudo.

As **REFERÊNCIAS** finais referem-se às citações que aparecem no item **INTRODUÇÃO** desta tese.

1 INTRODUÇÃO

O uso de medicamentos antipsicóticos no tratamento de psicoses como a esquizofrenia, implica em uma difícil contraposição entre o benefício de aliviar os sintomas psicóticos e o risco de efeitos adversos preocupantes, que por vezes, são incapacitantes e irreversíveis além de serem associados ao estigma social, deficiências físicas e uma pior qualidade de vida. Consequentemente, a não conformidade com estes efeitos colaterais dificulta a adesão ao tratamento, melhora clínica e o reengajamento na sociedade, resultando em recaída psicótica (CASEY, 2006; CHAPLIN; TIMEHIN, 2002; LAMBERT et al., 2004). Os antipsicóticos de primeira geração, tal como o haloperidol, são os mais suscetíveis de serem associados ao desenvolvimento de distúrbios motores extrapiramidais tais como parkinsonismo e discinesia tardia (DT). A esperança de que a nova geração de antipsicóticos atípicos não desenvolveria tais distúrbios parece improvável de ser cumprida (PELUSO et al., 2012; REMINGTON; HAHN, 2014). Estudos prospectivos encontraram prevalências de 13% com o uso de antipsicóticos de segunda geração e 32% com antipsicóticos de primeira geração (D'ABREU; AKBAR; FRIEDMAN, 2018). Além disso, o uso de antipsicóticos de primeira geração podem exacerbar os déficits cognitivos observados em pacientes esquizofrênicos (BABIN et al., 2011; SAEEDI; REMINGTON; CHRISTENSEN, 2006; WOODWARD et al., 2007).

A hipótese mais popular em vigor para a etiologia da DT é a da hipersensibilidade dopaminérgica, que propõe que o uso crônico de antagonistas dopaminérgicos, resulta em hipersensibilização gradual dos receptores de dopamina (CALABRESI et al., 1992; TEO; EDWARDS; BHATIA, 2012). O bloqueio dopaminérgico estriatal pode também aumentar os níveis extracelulares de glutamato, aumentando a excitotoxicidade por este neurotransmissor. A DO induzida por HAL também é associada ao dano oxidativo devido ao aumento da excitotoxicidade pela transmissão sináptica (ANDREASSEN; JØRGENSEN, 2000; BURGER et al., 2005a, 2005c; CASSANO T, PACE L, BEDSE G, LAVECCHIA AM, DE MARCO F, GAETANI S, 2016; LAU; TYMIANSKI, 2010; NAIDU; KULKARNI, 2001). Em conjunto, estas hipóteses vêm ao encontro de que a DT resulta de uma neurodegeneração de interneurônios estriatais pelo aumento da produção de radicais livres resultantes do aumento da liberação de dopamina e glutamato induzida pelo tratamento antipsicótico crônico (ANDREASSEN; JØRGENSEN, 2000; LOHR; KUCZENSKI; NICULESCU, 2003).

Devido à complexidade etiológica destes distúrbios, estratégias farmacológicas para reduzir esses efeitos têm sido aplicadas, como: fármacos antimuscarínicos (DESMARAIS; BEAUCLAIR; MARGOLESE, 2012), agonistas gabaérgicos (ALABED et al., 2011),

antagonista de receptores NMDA (PAPPA et al., 2010) e inibidores de transportador vesicular de monoaminas tipo 2 (VMAT2) (CORRELL; CARBON, 2017; SOLMI et al., 2018). Em contrapartida, tem se observado a melhora clínica de pacientes com DT que fazem uso de substâncias naturais (ANDERSON; MAES, 2012; LERNER et al., 2007; ZHANG et al., 2011) e em modelos animais a eficácia dessas substâncias também tem sido demonstrada tanto na prevenção, quanto na reversão de distúrbios motores extrapiramidais (BARCELOS et al., 2010, 2011; MACÊDO et al., 2011; PATIL; HIRAY; KASTURE, 2012; PEROZA et al., 2013; TREVIZOL et al., 2011).

Estudos sugerem que alterações de algumas funções cerebrais em condições normais e patológicas podem estar ligados a alterações na homeostase de magnésio (GOÑI-DE-CERIO et al., 2012; SARTORI et al., 2012; WANG et al., 2012). O magnésio (Mg) é um mineral essencial para os seres humanos, fundamental para regulação de uma grande variedade de sistemas enzimáticos (SABATIER et al., 2002; SARIS et al., 2000; WOLF; CITTADINI, 2003). Deste modo, o potencial terapêutico do magnésio tem sido empregado em diversas condições patológicas como eclampsia (EUSER; CIPOLLA, 2009), fibromialgia (PORTER et al., 2010), dor neuropática (PICKERING et al., 2011) e doenças cardiovasculares (MANRIQUE et al., 2010). Estudos demonstram que ao aumentar os níveis cerebrais de Mg, este apresenta papel importante na melhora da memória em modelos animais de doença de Alzheimer, tendo ações a nível de modulação de receptores e plasticidade sináptica (LI et al., 2014; SLUTSKY et al., 2010; XU et al., 2014). Recentemente em nosso grupo de pesquisa, foi observado a influência da suplementação de Mg em modelo animal de DO induzida por reserpina, mostrando que o Mg proteger do desenvolvimento de distúrbios motores, bem como sobre parâmetros oxidativos em regiões cerebrais responsáveis pelo controle motor (KRONBAUER et al., 2015).

2 REVISÃO DA LITERATURA

2.1 ESQUIZOFRENIA

As psicoses estão entre os distúrbios psiquiátricos mais graves, nos quais não somente há um marcante prejuízo do comportamento, mas também séria incapacidade de pensar de forma coerente, compreender a realidade ou adquirir percepção interior dessas anormalidades. A esquizofrenia, representante mais característica das psicoses, etimologicamente significa “mente dividida” ou “dissociada” e é uma doença ou um transtorno mental crônico, grave e incapacitante, caracterizada por uma alteração entre as funções do pensamento, da afetividade e do comportamento. Os transtornos esquizofrênicos são caracterizados por um conjunto diversificado de sintomas que podem ser divididos em sintomas positivos, negativo e cognitivos (MEYER; MACCABE, 2012; TANDON; NASRALLAH; KESHAVAN, 2010; VAN OS; KENIS; RUTTEN, 2010).

Os sintomas positivos geralmente são a primeira manifestação indicativa de esquizofrenia e referem-se a manifestações psicóticas tais como alucinações auditivas, sensoriais, delírios e alteração psicomotoras (GONZALEZ-BURGOS; FISH; LEWIS, 2011; LIEBERMAN, 1999). Os sintomas negativos são atribuídos a sintomas como pobreza de expressão, embotamento social, capacidade reduzida de sentir prazer (anedonia) e uma falta de motivação/desejo (apatia) (BRISCH, 2014; GONZALEZ-BURGOS; FISH; LEWIS, 2011). As deficiências cognitivas são menos explícitas, mas podem desempenhar um papel importante na incapacidade do paciente esquizofrênico. Os sintomas manifestam-se como déficits no processamento emocional, função executiva e de linguagem, processamento sensorial, atenção e memória de trabalho (GONZALEZ-BURGOS; FISH; LEWIS, 2011; MEYER; MACCABE, 2012).

A esquizofrenia afeta cerca de 24 milhões de pessoas em todo o mundo sendo considerada um problema mundial de saúde pública de primeira grandeza (WHITEFORD et al., 2016). Embora seja uma doença que afeta ambos os sexos com igual frequência, o distúrbio manifesta-se frequentemente mais cedo nos homens no início da adolescência e da fase adulta (ALEMAN; KAHN; SELTEN, 2003; SAHA et al., 2005). Duas hipóteses bioquímicas mais aceitas têm sido investigadas para elucidar a gênese da esquizofrenia, a hipótese dopaminérgica e a glutamatérgica (HOWES et al., 2013; HOWES; MCCUTCHEON; STONE, 2015).

Entretanto, a hipótese da hiperfunção dopaminérgica central é a mais investigada e mais aceita, apesar de se acreditar em um provável envolvimento simultâneo com outros sistemas de

neurotransmissores (LIEBERMAN et al., 1998). A hipótese dopaminérgica postula que os sintomas da esquizofrenia seriam secundários a uma hiperatividade dopaminérgica subcortical, mediada principalmente por receptores do tipo D2 nas vias mesolímbica (LARUELLE et al., 1999) e mesocortical (BOWIE; HARVEY, 2006; DAVIS et al., 1991). Tal hipótese foi fundamentada a partir da melhora dos sintomas com a utilização de antagonistas da neurotransmissão sináptica mediada por DA (CREESE; BURT; SNYDER, 1996; SEEMAN et al., 1975, 1976), bem como através da indução de surtos psicóticos em animais por intermédio de agonistas potentes dos receptores D2, como a apomorfina (VÕIKAR et al., 1999).

Estudos mais recentes têm fornecido mais detalhes sobre a natureza das mudanças dopamininérgicas pré e pós-sinápticas. Uma recente investigação demonstrou que a tirosina hidroxilase, enzima que limita a taxa de síntese de dopamina, é aumentada de forma significativa na *substantia nigra* (SN) de pacientes com esquizofrenia em comparação com pacientes saudáveis (HOWES et al., 2013), indicando que há um aumento da produção de dopamina no cérebro nos corpos neuronais dopaminérgicos, bem como em seus terminais no corpo estriado. Outro trabalho recente incluindo 176 amostras post-mortem de pacientes com esquizofrenia, mostraram a expressão do receptor D2 pré-sináptico foi aumentada, enquanto que a expressão de variantes predominantemente pós-sinápticas, foram reduzidas no córtex pré-frontal em comparação com os controles (KAALUND et al., 2014). A hipótese de que a receptores D2 estão de alguma forma alterados na esquizofrenia é apoiada por descobertas genéticas que têm mostrado uma clara associação entre o gene DRD2 e esquizofrenia (SCHIZOPHRENIA WORKING GROUP OF THE PSYCHIATRIC GENOMICS CONSORTIUM, 2014).

Outra hipótese que tem sido colocada como etiologia da esquizofrenia é a da hipofunção glutamatérgica. Um dos primeiros relatos envolvendo anormalidades glutamatérgicas em pacientes esquizofrênicos foi a constatação de que os níveis de glutamato encontravam-se reduzidos no líquido cefalorraquidiano (KIM et al., 1980). Entretanto, estes achados não foram reproduzíveis em estudos subsequentes e esta hipótese tem sido modificada com o passar dos anos (STONE; MORRISON; PILOWSKY, 2007). A disfunção do receptor NMDA surgiu a partir de observação de que antagonistas não competitivos desses receptores, incluindo a fenciclidina, dizocilpina (MK-801) e cetamina, levaram a efeitos psicológicos imediatos, que se assemelham estreitamente aos sintomas que ocorrem na esquizofrenia, incluindo sintomas positivos e negativos (JAVITT, 2007; KRYSTAL, J.H., KARPER, L.P., SEIBYL, J.P, FREEMAN, G.K, DELANEY, R., BREMNER, J.D, HENINGER, G.R, BOWERS JR, M.B., CHARNEY, 1994; MORGAN; CURRAN, 2006).

Em usuários crônicos de cetamina também são observados sintomas psicóticos (MORGAN; MUETZELFELDT; CURRAN, 2009; STONE et al., 2014). Evidências fornecidas por estudos post-mortem mostraram alterações no sistema glutamatérgico em esquizofrênico, tais como redução da densidade de subunidades NMDAR1 no córtex pré-frontal (SOKOLOV, 1998) e no córtex temporal superior (HUMPHRIES et al., 1996). No entanto, as conclusões gerais sobre a densidade de receptores NMDA têm sido inconsistentes (MCCULLUMSMITH et al., 2012). Supostamente, as anormalidades observadas na esquizofrenia estão relacionadas primariamente à localização de receptores glutamatérgicos em oposição ao déficit generalizado de glutamato, e estas anomalias podem surgir como resultado de alterações no trafego deste neurotransmissor (FUNK et al., 2009). Além disso, existem evidências para uma variedade de alterações funcionais que afetam os efeitos intracelulares do receptor de NMDA, que teriam um grande impacto na sinalização glutamatérgica (FUNK et al., 2012; PITCHER et al., 2011).

2.2 ANTIPSICÓTICOS

Os fármacos antipsicóticos utilizados no tratamento da esquizofrenia foram introduzidos na pratica clínica na década de 1950, com o desenvolvimento da clorpromazina (LÓPEZ-MUÑOZ; ALAMO, 2009). O objetivo do tratamento com antipsicóticos é a redução dos sintomas, a fim de melhorar a qualidade de vida das pessoas acometidas por esta doença, reduzindo a morbidade e a frequência e gravidade dos episódios psicóticos (TANDON; NASRALLAH; KESHAVAN, 2010).

Depois da descoberta da clorpromazina, protótipo no tratamento da esquizofrenia, outros fármacos com o mesmo perfil foram desenvolvidos. Estes medicamentos foram classificados como antipsicóticos de 1ª geração, clássicos ou típicos, devido às semelhanças no mecanismo de ação, eficácia e perfil de efeitos adversos (LEHMAN et al., 2004). Com o decorrer das décadas, novos fármacos passaram a ser desenvolvidas afim de reduzir os efeitos adversos e melhorar sua eficácia sobre os sintomas negativos da doença, passando a serem classificados como antipsicóticos de 2ª geração ou atípicos (ALEXANDER et al., 2011; GEDDES et al., 2000).

Os antipsicóticos de 1ª geração, dentre os quais estão a clorpromazina, a flufenazina e o haloperidol, atuam bloqueando principalmente receptores dopaminérgicos D2 (CREESE; BURT; SNYDER, 1996; SUGAWARA; NIKAIDO, 2014) nas vias que estão mais relacionadas com a fisiopatologia da esquizofrenia. Entretanto, podem antagonizar vias dopaminérgicas que se projetam da SN para o estriado dorsal (núcleos da base) (HEIMER, 2003) conferindo um risco aumentado de efeitos colaterais extrapiramidais, tais como distonia, sintomas

parkinsonianos (rigidez, bradicinesia, tremor), acatisia e também a síndrome da discinesia tardia (DT).

Já os antipsicóticos de 2ª geração, clozapina, olanzapina, risperidona, entre outros, possuem a capacidade de bloquear tanto os receptores da serotonina (5-HT) quanto os dopaminérgicos (SUGAWARA; NIKAIDO, 2014). Esse grupo de antipsicóticos demonstrou, em contraste aos antipsicóticos típicos, respostas mais satisfatórias em pacientes acometidos por sintomas negativos da esquizofrenia bem como menores efeitos colaterais extrapiramidais, porém ainda bastante presentes (DIVAC et al., 2014; PELUSO et al., 2012; RUMMEL-KLUGE et al., 2012; WOODS et al., 2010). Por outro lado, apesar de serem menos neurotóxicos, são considerados desreguladores metabólicos, implicados com o desenvolvimento de diabetes melittus, pancreatite, ganho de peso, além de desencadear discrasias sanguíneas graves (NEWCOMER, 2005; WESTON-GREEN; HUANG; DENG, 2013). Contudo, pelo fato dos antipsicóticos atípicos apresentarem uma menor eficácia em relação aos sintomas positivos e também possuírem um maior custo, antipsicóticos típicos, em especial o HAL, continuam sendo amplamente empregados no tratamento farmacológico da esquizofrenia (PONTO et al., 2010).

2.3 HALOPERIDOL

O haloperidol é um antipsicótico pertencente à classe dos antipsicóticos clássicos ou típicos, que se destaca por sua potência, especificidade e longa duração de ação. (NAYAK; DOOSE; NAIR, 1987). Descoberto em 1958, pelo médico e farmacologista belga Paul Janssen, continua até hoje sendo um fármaco muito importante em todo o mundo, devido a sua ampla disponibilidade e baixo custo de aquisição (GRANGER; ALBU, 2005; PATEL et al., 2007; PONTO et al., 2010). Sua ação está envolvida principalmente no bloqueio dos receptores dopaminérgicos D2 na via mesolímbica (ANANTH; PARAMESWARAN; HARA, 2004; CREESE; BURT; SNYDER, 1996; TURRONE et al., 2003) sendo eficaz no controle dos sintomas positivos da esquizofrenia como transtornos afetivos, delírios, alucinações, confusão mental e psicoses agudas e crônicas que apresentem agitações psicomotoras (GRANGER; ALBU, 2005). No entanto, sua eficácia acaba comprometida devido aos efeitos colaterais extrapiramidais que podem ser causados pelo tratamento, incluindo acatisia, distonia, parkinsonismo e DT (ALVAREZ; SKOWRONSKI, 2003; ANANTH; PARAMESWARAN; HARA, 2004; GRANGER; ALBU, 2005; KNABLE et al., 1997; KURZ et al., 1995).

2.4 DISCINESIA TARDIA

A discinesia tardia (DT) é uma síndrome que pode ser desenvolvida após uso prolongado de antipsicóticos clássicos e caracteriza-se por movimentos involuntários, repetitivos e hipercinéticos da região orofacial, pescoço e tronco, podendo ser incapacitante e irreversível (JESTE; CALIGIURI, 2010; MARSÁLEK, 2000). A prevalência da DT é de cerca de 20 a 25% da população psiquiátrica que usa antipsicóticos, porém este índice pode ser de 50% com o aumento da idade (ANDREASSEN; JØRGENSEN, 2000). A DT apresenta uma incidência anual de novos casos de, aproximadamente, 3 a 5% e parece ocorrer de maneira irreversível, frequentemente cumulativa e incapacitante (KANE; WOERNER; LIEBERMAN, 1985; WOODS et al., 2010).

Como a DT é a manifestação mais grave dos sintomas extrapiramidais induzidos por antipsicóticos típicos, algumas hipóteses foram sugeridas para explicar esta condição. Dentre as quais, a hipersensibilidade dopaminérgica, (GORDON et al., 1987), relaciona o aumento do número e sensibilidade de receptores dopaminérgicos ao desenvolvimento da síndrome, em resposta ao bloqueio prolongado dos receptores de DA das membranas neuronais motoras. Embora a hipersensibilidade realmente ocorra, existem algumas contradições, sugerindo outras ideias relacionadas ao desenvolvimento de DT. Posteriormente, também foi considerada a ocorrência de um processo neurotóxico para explicar a irreversibilidade da síndrome em indivíduos tratados com antipsicóticos, propondo um mecanismo relacionado aos radicais livres (RL) e ao estresse oxidativo (EO). Esta linha de pensamento é conhecida como hipótese da degeneração neuronal para a DT (CADET; KAHLER, 1994; ELKASHEF; WYATT, 1999; LOHR et al., 1988; LOHR; KUCZENSKI; NICULESCU, 2003; SOARES-WEISER; MAAYAN; MCGRATH, 2011), a qual tem sido amplamente aceita, pois o bloqueio dopaminérgico pode causar uma elevação secundária na liberação e degradação da dopamina (DA), fatores que reconhecidamente elevam a geração de RL e a consequente neurodegeneração (LOHR, 1991; LOHR; KUCZENSKI; NICULESCU, 2003).

De acordo com isto, dados da literatura indicam que a administração de antipsicóticos causa um aumento na síntese de dopamina e consequentemente um aumento no seu metabolismo via aumento da atividade da enzima monoaminoxidase (MAO) (ANDREASSEN; JØRGENSEN, 2000; ZHU, 2004), que oxida a DA gerando o ácido 3,4-dihidroxifenilacetico (DOPAC) e produção de peróxido de hidrogênio (H_2O_2) que, ao reagir com metais de transição, forma RL via reação de Fenton. Além disso, a auto-oxidação da DA forma dopamino-quinonas (ASANUMA; MIYAZAKI; OGAWA, 2003; NAPOLITANO; MANINI; D'ISCHIA, 2011), que

também contribuem para a geração de RL. A geração de RL e o aumento dos produtos de lipoperoxidação podem resultar em EO em diferentes regiões cerebrais dopaminérgicas (BARCELOS et al., 2010; HASTINGS, 2009; MACÊDO et al., 2011; TREVIZOL et al., 2011), levando à morte celular. Ainda, o bloqueio dos receptores dopaminérgicos estriatais pode causar excitotoxicidade, através do aumento do glutamato extracelular, elevando assim os danos oxidativos (ALBENSI, 2007; BURGER et al., 2005a; COYLE; PUTTFARCKEN, 1993; TSAI et al., 1998; ZHURAVLIOVA et al., 2007). Tem sido relatado que o tratamento crônico com HAL diminui a expressão do transportador de glutamato GLT-1 nas sinapses glutamatérgicas do corpo estriado de ratos e também induzir efeitos secundários extrapiramidais (GOFF; COYLE, 2001; LIEBERMAN et al., 2008). Dados da literatura têm demonstrado que, em animais tratados com antipsicóticos, ocorre um aumento nos níveis de peroxidação lipídica e de carbonilação de proteínas, além de redução na atividade de enzimas antioxidantes, como a superóxido dismutase (SOD), a catalase (CAT) e a glutationa peroxidase (GPx), e também redução da glutationa reduzida (GSH) com um consequente aumento da glutationa oxidada (GSSH) (ABÍLIO et al., 2003; BURGER et al., 2005a, 2005b; NAIDU; SINGH; KULKARNI, 2003; PILLAI et al., 2007; POST; HOLSBOER; BEHL, 1998).

Efeitos colaterais extrapiramidais, incluindo a DT, têm sido implicados em outros comportamentos relacionados com o tratamento farmacológico como, a não aderência ao tratamento, a não-conformidade frente a estes efeitos adversos e consequente má adaptação social e aumento do risco de depressão e suicídio (BYERLY; NAKONEZNY; LESCOUFLAIR, 2007; KAROW et al., 2007; MORITZ et al., 2013). Como resultado da prevalência e da natureza debilitante destes distúrbios, estudos visando novas abordagens terapêuticas e tratamentos adjuvantes são de grande importância para a clínica.

2.4.1 Modelos animais de efeitos extrapiramidais

O estudo dos efeitos extrapiramidais induzidos por fármacos antipsicóticos tem sido bem descrito na literatura (ANANTH; PARAMESWARAN; HARA, 2004; MOORE et al., 1992; ROCHA et al., 1997; TADA et al., 2004; TURRONE et al., 2003). Estes modelos animais baseiam-se no aumento da atividade muscarínica estriatal, decorrente da hipofunção ou bloqueio exercido pelo antipsicótico nos receptores dopaminérgicos D2. A DT também tem sido estudada através de modelos animais de DO induzida por antipsicóticos em roedores, observada através do desenvolvimento de MMV, protrusão da língua e tremores faciais, que apesar de haverem controvérsias, são modelos validados por diversas evidencias cientificas como uma

representação adequada para o desenvolvimento de DT humana (BARCELOS et al., 2010; BUSANELLO et al., 2012; COLPO et al., 2007; KRONBAUER et al., 2015; NAIDU; SINGH; KULKARNI, 2003; PEROZA et al., 2013; TREVIZOL et al., 2011).

O modelo de MMV em ratos é caracterizado por contrações musculares espontâneas, orofaciais que causam a abertura da boca no plano vertical, com ou sem saliências de língua (TURRONE et al., 2003). A frequência de MMV serve como um indicador funcional para a gravidade da DT e também como um indicador da ação do fármaco (GUNNE; ANDRÉN, 1993). Estas atipias têm sido associados a alterações morfológicas bem como parâmetros oxidativos, dentre eles espécies reativas de oxigênio, TBARS e carbonilação de proteínas nas regiões cerebrais dos núcleos da base, como o corpo estriado e a região da *substantia nigra*. Estes estudos puderam observar resultados positivos na redução do desenvolvimento de distúrbios extrapiramidais relacionados à redução dos parâmetros oxidativos além de aumentar enzimas de defesa antioxidante (ANDERSON; MAES, 2012; BARCELOS et al., 2010, 2011; BUSANELLO et al., 2012; KRONBAUER et al., 2015; LERNER et al., 2007; LISTER et al., 2014, 2017; PATIL; HIRAY; KASTURE, 2012; PEROZA et al., 2013; TREVIZOL et al., 2011; ZHANG et al., 2011).

2.5 MAGNÉSIO

O magnésio (Mg) é o segundo cátion intracelular mais abundante e o quarto cátion mais abundante no organismo. Este íon é um importante cofator para mais de 300 enzimas e possui um papel fisiológico essencial em uma grande variedade de reações metabólicas essenciais, dentre elas a glicólise e o metabolismo proteico e lipídico (ANASTASSOPOULOU; THEOPHANIDES, 1995; ESKES et al., 1998; LEHNINGER; NELSON; COX, 2017; LUKASKI, 2000; RYAN, 1991). Durante a glicólise, 7 enzimas dependentes de Mg estão envolvidas, como por exemplo a hexoquinase, e no ciclo do ácido cítrico, a piruvato e isocitrato desidrogenase. Além disso, é essencial para a gliconeogênese a partir de piruvato, a via pentoses fosfato e no ciclo da ureia. No metabolismo lipídico, o Mg é necessário para a formação de fosfoglicerideos na síntese lipídica, e catalisação do primeiro passo da degradação de ácido graxos. Além disso, para a síntese de proteínas a partir de aminoácidos e ARN transportador nos ribossomos, o Mg é um requisito, e para a síntese de ADN. A formação de AMPc (monofosfato de adenosina cíclico), um segundo mensageiro intracelular, a partir da MgATPase, é catalisada pela adenilatociclase dependente de Mg. O AMPc ativa a proteínas quinases, controla a concentração intracelular de Ca^{2+} e liga-se a proteínas de repressão, regulando, assim, a transcrição de ácidos nucleicos (LEHNINGER; NELSON; COX, 2017).

O Mg forma complexos com os fosfolipídios das membranas celulares, o que contribui para a estrutura celular. Da mesma forma que os fosfolipídios, o Mg estabiliza o ARN e ADN pela sua alta afinidade pela ribose fosfato dos nucleotídeos e é, portanto, um componente essencial da integridade estrutural de cada célula. Os complexos Mg- fosfolipídios controlam os influxo de outros íons através da membrana celular, ao reduzir a fluidez da membrana e permeabilidade de cálcio e de sódio (DURLACH et al., 2004; MAGUIRE; COWAN, 2002; NAMDARI et al., 2011).

2.5.1 Fontes e distribuição do magnésio no organismo

O magnésio é amplamente distribuído em alimentos vegetais e animais bem como em bebidas. Vegetais de folhas verdes, como espinafre, legumes, nozes, sementes e cereais integrais, são boas fontes (INTAKES, 1997). Água de torneira, mineral ou engarrafadas também podem ser uma fonte de Mg, mas a quantidade varia de acordo com a fonte e a marca (variando de 1 mg / L a mais do que 120 mg / L) (AZOULAY; GARZON; EISENBERG, 2001). Aproximadamente 30% a 40% do Mg proveniente da dieta é normalmente absorvida pelo corpo

(FINE et al., 1991) e o Conselho de Alimentos e Nutrição dos Estados Unidos recomenda uma dose diária de 420 mg para homens e 320 mg para mulheres (INTAKES, 1997).

Suplementos de Mg estão disponíveis numa variedade de formas, incluindo o óxido, citrato, e cloreto de magnésio. A absorção de Mg a partir de diferentes tipos de suplementos de magnésio varia. Formas de Mg que se dissolvem bem em líquidos são mais completamente absorvidos no intestino do que as formas menos solúveis, Alguns estudos demonstraram que nas formas aspartato, citrato, lactato, e cloreto de Mg são absorvidas mais completamente e são mais biodisponíveis do que o óxido e sulfato de Mg (FIROZ; GRABER, 2001; LINDBERG et al., 1990; MÜHLBAUER et al., 1991; RANADE; SOMBERG, 2001; RYLANDER, 2014; WALKER et al., 2003).

Cerca de 21 a 28g de magnésio encontra-se amplamente distribuído no organismo de uma pessoa adulta. Aproximadamente 53% do total de reservas de magnésio encontram-se no compartimento ósseo, 27% no músculo, 19% nos tecidos moles, 0,5% nos eritrócitos e 0,3% no soro (SARIS et al., 2000). Dentro da célula 90% do íon está ligado, principalmente a ácidos nucleicos, ATP, fosfolipídios carregados negativamente e proteínas, e os 10% restantes encontram-se livres (WOLF; CITTADINI, 2003).

Embora pouco Mg esteja sob a forma ionizada, esta é a forma necessária para que haja a regulação da homeostase intracelular. A concentração sérica de magnésio é resultado de um balanço na ingestão/absorção do íon, biodistribuição e excreção. Os principais órgãos excretores envolvidos na homeostasia do Mg são os rins, que mediante uma baixa ingestão oral deste micronutriente, são capazes de reduzir a excreção diária do mesmo (MURPHY, 2000; SARIS et al., 2000). Os termos deficiência de Mg e hipomagnesemia são corriqueiramente utilizados como sinônimos. Contudo, a deficiência de Mg pode estar presente mesmo mediante concentrações normais de Mg sérico, que são comumente mantidas às custas de mobilização do íon a partir de outros compartimentos. Em contrapartida, o termo hipomagnesemia refere-se a baixas concentrações séricas de Mg (PHAM et al., 2007; SWAMINATHAN, 2003). A hipermagnesemia grave ou intoxicação por magnésio raramente manisfeta-se em doenças humanas. Tais condições só ocorrem em insuficiência renal grave (JAHNEN-DECHENT; KETTELER, 2012)

2.5.2 Magnésio e o sistema nervoso central

Muitas condições patológicas relevantes são associadas com a redução da disponibilidade de Mg e/ou o aumento da excreção, seja em nível sistêmico ou em tecidos específicos, tais como doenças cardiovasculares (ALTURA et al., 2009), diabetes mellitus (GUERRERO-ROMERO; RODRÍGUEZ-MORÁN, 2006), a síndrome metabólica (BARBAGALLO; DOMINGUEZ, 2007; BELIN; HE, 2007), citopatias mitocondriais (BARBIROLI et al., 1999) e distúrbios neuropsiquiátricos (CAMARDESE et al., 2012).

No sistema nervoso central (SNC) o papel crucial de cátions, incluindo o Mg, tornou-se evidente. Os mecanismos neurológicos deste íon são diversos e incluem interações com os neurotransmissores e receptores. O Mg facilita muitas comunicações neurotransmissor/receptor (POLESZAK et al., 2008; SZEWCZYK et al., 2008). É um co-factor para a biossíntese de dopamina e serotonina, os neurotransmissores responsáveis pelo humor, comportamento, apetite, função cognitiva, padrões de sono e respostas ao estresse (CARDOSO et al., 2009; SZEWCZYK et al., 2008). Pacientes com níveis adequados de magnésio tendem a exibir valores saudáveis destes mensageiros químicos no fluido cerebrospinal (BANKI et al., 1985). A suplementação tem representado um apoio significativo para o humor, sono e cognição, mesmo quando os níveis basais não estão disponíveis (SZEWCZYK et al., 2008).

O efeito benéfico da administração de Mg em condições patológicas do cérebro também tem sido investigado. Tem se estabelecido o papel importante do Mg no SNC devido a sua influência na estabilização elétrica das membranas celulares sendo essencial na condução sináptica (FREEDMAN et al., 1992), e na modulação dos receptores N-metil D-Aspartato (NMDA) (GATHWALA, 2001; GATHWALA et al., 2010; SOBOLEVSKII; KHODOROV, 2002), antagonizando assim as ações do Ca^{2+} pré- e pós-sinápticas (LÜSCHER; MALENKA, 2012). Vários estudos averiguaram a eficácia clínica da terapia de Mg em modelos animais de lesão cerebral traumática, mostrando que a sua administração pré- e pós- lesão favorecem a recuperação dos déficits cognitivos (HOANE, 2007; HOANE et al., 2008).

Em culturas de células neuronais, a depleção de Mg aumentou a morte celular gerada por dano oxidativo (ALTURA et al., 2009) enquanto que a sua elevação mostrou ação citoprotetora (REGAN et al., 1998). Ratos submetidos a uma baixa ingestão de magnésio apresentaram um aumento da susceptibilidade ao estresse oxidativo observando-se o aumento da lipoperoxidação lipídica e oxidação proteica (BOPARAI; KIRAN; BANSAL, 2007; KUZNIAR et al., 2003; RAYSSIGUIER et al., 1993). A atividade antioxidante indireta do Mg também está relacionada com a capacidade de bloquear receptores NMDA reduzindo consequentemente o influxo de Ca^{2+} (KUNER; SCHOEPFER, 1996; NIKOLAEV; MAGAZANIK; TIKHONOV, 2012; VARGAS-

CABALLERO; ROBINSON, 2004; WU; JOHNSON, 2009), e inibir a NADPH oxidase (BUSSIÈRE et al., 2002) culminado assim na redução de formação de radicais superóxido (AFANAS'EV et al., 1995). Além disso, pode também aumentar a GSH por possuir papel fundamental na sua síntese (BARBAGALLO et al., 1999; DJUKIĆ-COSIĆ et al., 2007; MILLS; LINDEMAN; LANG, 1986; REGAN; GUO, 2001).

Estudos evidenciaram que alterações na função cerebral em condições normais ou patológicas como, desordens psiquiátricas como depressão e esquizofrenia podem estar ligadas a alterações na concentração de magnésio (NECHIFOR, 2009; WIDMER et al., 1995), porém por vezes contraditórios quanto as concentrações intra e extracelulares. O tratamento com antipsicóticos demonstrou induzir um aumento na concentração intra-eritrocitária de Mg correlacionado com a melhora clínica em pacientes esquizofrênicos (NECHIFOR, 2008; RENN; YANG; CHOU, 2010), favorecendo a ideia de que o aumento da concentração de Mg é um elemento essencial para o mecanismo de ação das drogas antipsicóticas utilizadas no tratamento da esquizofrenia. Por outro lado, alguns autores têm demonstrado aumento das concentrações plasmáticas de Mg em pacientes esquizofrênicos.

Especificamente, pacientes esquizofrênicos apresentam aumento de concentrações plasmáticas de Mg, mas a administração do HAL reduz os níveis de Mg. Estes autores sugeriram também que a diminuição da concentração de Mg estaria envolvida na geração de efeitos secundários extrapiramidais induzidos pelo HAL (JABOTINSKY-RUBIN et al., 1993). Décadas antes, outro estudo propôs que os níveis baixos de Mg causados por antipsicóticos acarretam em um aumento da atividade colinérgica mediada pela acetilcolina (ALEXANDER; VAN KAMMEN; BUNNEY, 1979). Recentemente nosso grupo relacionou a influência da suplementação de Mg no desenvolvimento de distúrbios extrapiramidais em modelo agudo de discinesia orofacial induzido por reserpina. Neste estudo foi observado que a suplementação de Mg preveniu e também reverteu os distúrbios extrapiramidais, a geração de espécies reativas e carbonilação de proteínas (KRONBAUER et al., 2015).

2.6 BLOQUEADORES DE CANAIS DE CÁLCIO

Os antagonistas de canais de cálcio dependentes de voltagem vêm sendo empregados no tratamento da hipertensão arterial há várias décadas e constituem um grupo heterogêneo de drogas dentre as principais os derivados das diidropiridinas, como a nifedipina, felodipina e amlodipina, dos benzotiazepínicos, como o diltiazem, das fenilalquilaminas, como o verapamil (GROSSMAN; MESSERLI, 2004). Diferentes subtipos de canais de cálcio dependentes de

voltagem são conhecidos dentre eles os tipo L, tipo N, tipo P/Q e tipo R, todos similares, porém não estruturalmente idênticos, os quais se encontram largamente distribuídos no organismo, desempenhando importante papel, dependendo do tipo celular, como por exemplo, contração muscular, excitação de neurônios, regulação da expressão de genes, liberação de hormônios e neurotransmissores (AUGUSTINE; SANTAMARIA; TANAKA, 2003; BARBADO et al., 2009; CATTERALL, 2010; GRIENBERGER; KONNERTH, 2012; ROSENBERG; SPITZER, 2011; WEBB, 2017).

A homeostase do cálcio não é apenas crítica para a fisiologia e a saúde celular, mas também, quando desregulada, pode levar à neurodegeneração através de mecanismos complexos diversos, intimamente relacionadas desordens neurológicas (BRINI et al., 2014; GLEICHMANN; MATTSON, 2010; MARAMBAUD; DRESES-WERRINGLOER; VINGTDEUX, 2009). Frente a essas situações os bloqueadores dos canais de cálcio têm mostrado papel promissor no tratamento de disfunções do SNC (PETERS; BOOTH; PETERS, 2014; SAPOLSKY, 2000), mostrando melhora nos sintomas e apresentarem potencial terapêutico em estudos clínicos de doença de Parkinson e Alzheimer (ILIJIC; GUZMAN; SURMEIER, 2011; KALE et al., 2017; KOCHEGAROV, 2003; PERRET; LUO, 2009; SINGH et al., 2016). Há evidências do envolvimento de canais de calcio tipo L em doenças como doença de Parkinson, convulsões febris e distúrbios neuropsiquiátricos e sua inibição farmacologica possui importante valor terapeutico (ORTNER; STRIESSNIG, 2016). Bloqueadores desse subtipo de canal são utilizadas no tratamento da hipertensão, isquemia cardíaca e arritmias, como por exemplo a nifedipina. Particularmente, efeitos positivos da nifedipina sobre o desenvolvimento de DO em ratos já foram demonstrados (ABDEL-RAHEEM, 2010; BISHNOI; CHOPRA; KULKARNI, 2008), e desta forma ser o farmaco de escolha para o presente estudo.

3 JUSTIFICATIVA

O uso crônico de antipsicóticos típicos, como o haloperidol, está associado ao desenvolvimento de desordens motoras como tremores, acatisia e DT, os quais são efeitos adversos característicos desse grupo terapêutico. O magnésio é o segundo cátion intracelular mais abundante no organismo, onde desempenha importantes funções fisiológicas como cofator de enzimas, modulação das defesas antioxidantes. O tratamento antipsicótico clássico e o consequente desenvolvimento de desordens motoras extrapiramidais podem envolver interferências sobre os níveis normais de Mg no organismo, ou afetar suas funções fisiológicas no sistema

dopaminérgico nigro-estriatal. Deste modo, existe relevância estudar o papel deste mineral sobre tais distúrbios em modelos animais, visto que estas implicações ainda não foram cientificamente elucidadas.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Avaliar a influência da suplementação de magnésio como um agente de proteção e reversão do desenvolvimento de distúrbios extrapiramidais induzidos pela administração sub-crônica de haloperidol.

4.2 OBJETIVOS ESPECÍFICOS

- Avaliar os níveis plasmáticos do magnésio frente ao desenvolvimento de discinesia orofacial e correlacionar se esses níveis sofrem influência do tratamento com haloperidol;
- Avaliar o possível papel protetor do magnésio na prevenção dos distúrbios comportamentais extrapiramidais e parâmetros de estresse oxidativo cerebral induzidos por haloperidol;
- Avaliar a influência da suplementação de magnésio na reversão dos distúrbios comportamentais extrapiramidais e parâmetros de estresse oxidativo cerebral induzidos por haloperidol;
- Avaliar a influência da suplementação de magnésio concomitante a administração de haloperidol no desenvolvimento de distúrbios comportamentais extrapiramidais e parâmetros de estresse oxidativo cerebral induzidos por haloperidol;
- Avaliar comparativamente a influência da suplementação de magnésio e do fármaco nifedipina, concomitante ao tratamento sub-crônico com haloperidol sobre o desenvolvimento de distúrbios comportamentais extrapiramidais e parâmetros moleculares das vias dopaminérgica e glutamatérgica.

5 ARTIGO CIENTÍFICO

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Research report

Influence of magnesium supplementation on movement side effects related to typical antipsychotic treatment in rats

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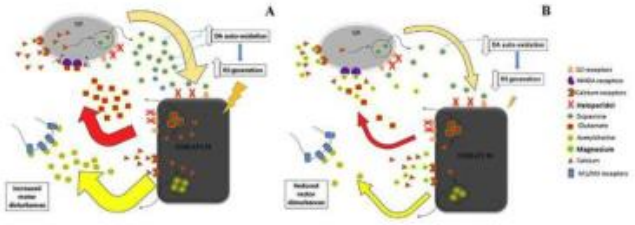
HIGHLIGHTS

- Extrapyramidal disturbances (ED) are side effects consequent to haloperidol treatment.
- ED occurs due to dopamine receptor blockade in the nigrostriatal system.
- ED are related to cholinergic activation and glutamatergic excitotoxicity.
- Mg_2^{++} supplementation inhibits Ca_2^{++} and blockade NMDA receptors.
- Mg_2^{++} supplementation protects against haloperidol-induced movement disturbances.

GRAPHICAL ABSTRACT



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ABSTRACT

Chronic use of typical antipsychotic haloperidolis related to movement disturbances such as parkinsonism, akathisia and tardive dyskinesia which have been related to excitotoxicity in extrapyramidal brain areas, requiring their prevention and treatment. In the current study we evaluated the influence of the magnesium on prevention (for 28 days before-), reversion (for 12 days after-) and concomitant supplementation on haloperidol-induced movement disorders in rats. Sub-chronic haloperidol was related to orofacial dyskinesia (OD) and catalepsy development, increased generation of reactive species (RS) and levels of protein carbonyl (PC) in cortex, striatum and *substantia nigra* (SN) in all experimental protocols. When provided preventatively, Mg reduced the increase of OD and catalepsy time 14 and 7 days after haloperidol administration, respectively. When supplemented after haloperidol-induced OD establishment, Mg reversed this behavior after 12 days, while catalepsy was reversed after 6 days of Mg supplementation.When Mg was concomitantly supplemented with haloperidol administration, OD and catalepsy were prevented. Moreover, Mg supplementation was able to prevent the RS generation in both cortex and SN, reducing PC levels in all brain areas evaluated. When supplemented after haloperidol, Mg reversed RS generation in cortex and striatum, decreasing PC levels in SN and striatum.The co-administration of haloperidol and Mg supplementation prevented RS generation in cortex, striatum and SN, and PC levels in the SN.These outcomes indicate that Mg supplementation may be a useful alternative to prevent movement disturbances resulting of classic antipsychotic pharmacotherapy as haloperidol.

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1. Introduction

Antipsychotic-induced extrapyramidal symptoms (EPS), such as acute dystonia, akathisia, parkinsonism, and tardive dyskinesia (TD), constitute a major problem for patients treated with antipsychotics and compromise treatment adherence and compliance [1–3]. TD is a potentially irreversible disorder associated with chronic first generation antipsychotic exposure and characterized by involuntary, stereotyped, and repetitive movements in different body parts [4]. Although the emerging use of second generation antipsychotics are related to lower risk to TD development, the expectation of being EPS-free antipsychotic drugs is not encouraging since most of the newer atypical antipsychotic agents may also induce varying degrees of EPS [5,6].

The pathophysiology of TD steel poorly understood but it has been suggested that the hypersensitivity of striatal post-synaptic dopamine receptors is implicated on it [7,8]. The exacerbation of dopamine metabolism causing by prolonged blockade of dopamine D2 receptors (D2R), lead to the generation of reactive species particularly in the basal ganglia consequently leading to neurodegeneration [9–11]. An enhancement of the glutamatergic transmission, in response of presynaptic dopamine receptors blockade, also participate on free-radicals formation, which has gained experimental support in the literature on involvement in the etiology of TD [9,12–15].

Magnesium (Mg) is the fourth most abundant mineral in the body and is recognized as a cofactor for more than 300 enzymatic reactions, including energy metabolism and nucleic acid synthesis and also essential for the regulation of blood pressure, insulin metabolism, cardiac excitability [16,17], neuromuscular conduction and neurotransmitter release [18]. In general, its actions are related to its physiological blockade of calcium channels [19–22]. Besides that, Mg plays a role on blocking N-methyl-D-aspartate (NMDA) receptor channels which is believed to have a great importance on physiological modulation of glutamate transmission involved in many neurological diseases, such as Alzheimer's disease, attention deficit hyperactivity disorder (ADHD) and Parkinson's disease [23–26].

Recently, a study from our group demonstrated a preventive and protect role of Mg supplementation in a reserpine-induced model of OD [27]. However, there is no more studies related withMg and the extrapyramidal effects.Therefore, the aim of this study is evaluate the influence of different periods of Mg supplementation in a sub-chronic haloperidol-induced model of OD.

2. Methods

2.1. Animals

Male *Wistar* rats weighing 250–320 g (about 3 months old) were used. Groups of three (±1) animals were kept in Plexiglas cages with free access to food (standard chow) and water in a room with controlled temperature (22–23 °C) and 12 h-light/dark cycle with lights on at 7:00 a.m. Animals were fed with standard chow *ad libitum* (PuroTrato®, RS, Brazil), which contains adequate levels of Mg following recommendations from the National Research Council (NRC, 1995), during all experiments. The experimental protocols were approved by the Animal Ethics Committee (Universidade Federal de Santa Maria – UFSM 064/2013), which is affiliated to the Council of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

2.2. Drugs

Haloperidol decanoate (Haloperidol decanoate – Janssen-Cilag) was dissolved in Tween® and diluted to a final concentration of 1% Tween® with distilled water. The vehicle consisted of a 1%Tween® solution. Magnesium aspartate (Fragon do Brasil Farmacêutica Ltda) was dissolved in deionized water.

2.3. Experimental procedure

2.3.1. Experiment 1: preventive influence of Mg supplementation on the development of sub-chronic haloperidol-inducedOD

Twenty-eight rats were randomly designated to two groups of fourteen animals each (n = 14) and orally supplemented (once a day, by gavage) with magnesium aspartate solution (40 mg/kg of body weight in 1 mL deionized water) (Mg – magnesium group) [27,28] or deionized water (C – control group). After 28 days of oral supplementation, basal behavioralassessments were performed and subsequently one-half of each experimental group was administrated once a week with haloperidol solution (12 mg/kg of body weight in 1 mL vehicle – 1% Tween, intramuscular-i.m.) or vehicle (1% Tween® solution) [29,30], for 4 weeks. As a result, 4 experimental groups were established: control group (C); haloperidol group (H); magnesium group (Mg) and magnesium + haloperidol (MgH). Before each haloperidol injection all animals were submitted to the behavioral evaluations. One week after the lasthaloperidol/vehicle administration all animals were submitted to last behavioral evaluations as described (Fig. 1A).

2.3.2. Experiment 2: influence of Mg supplementation on the reversal of sub-chronic haloperidol-induced OD

Twenty-eight rats were randomly designated to two groups of fourteen animals each (n = 14) and administrated with haloperidol solution (H group- 12 mg/Kg/mL; i.m.) or vehicle (C group), once a week, for 4 weeks. Seven days after the first H/vehicle administration, OD development and catalepsy time was quantified (basal) and subsequently, one-half of each experimental group was immediately supplemented once a day (by gavage) with magnesium aspartate (40 mg/Kg/mL) (Mg and MgH groups) or deionized water (C and H groups). OD and catalepsy time was quantified during the subsequent days (each 72 h). Mg supplementation was maintained throughout the behavioral assessment period until a significant difference between MgH and H groups was observed (15 consecutive days) (Fig. 1B).

2.3.3. Experiment 3: influence of Mg supplementation together with haloperidol administration on the development of OD

Twenty-four rats were randomly designated to four groups (n = 6): C (control), H (haloperidol), Mg (magnesium) and MgH (magnesium + haloperidol). All the animals were submitted to an evaluation of OD and immediately after administrated with haloperidol solution (H and MgH group – 12 mg/Kg/mL) or vehicle (C and Mg groups), once a week for four weeks. Concomitantly, animals were orally supplemented with magnesium aspartate (Mg and MgH groups – 40 mg/Kg/mL) or deionized water (C and H groups). OD was quantified previously to each haloperidol administration and catalepsy time one time seven days after de last haloperidol administration. Mg supplementation was maintained throughout the protocol (total of 28 days) (Fig. 1C).

2.4. Behavioral assessments

2.4.1. Orofacial dyskinesia (OD)

Rats were placed individually in cages (20 × 20 × 19 cm) containing one mirror under the floor and one behind the back wall of the cage to allow behavioral quantification when the animal

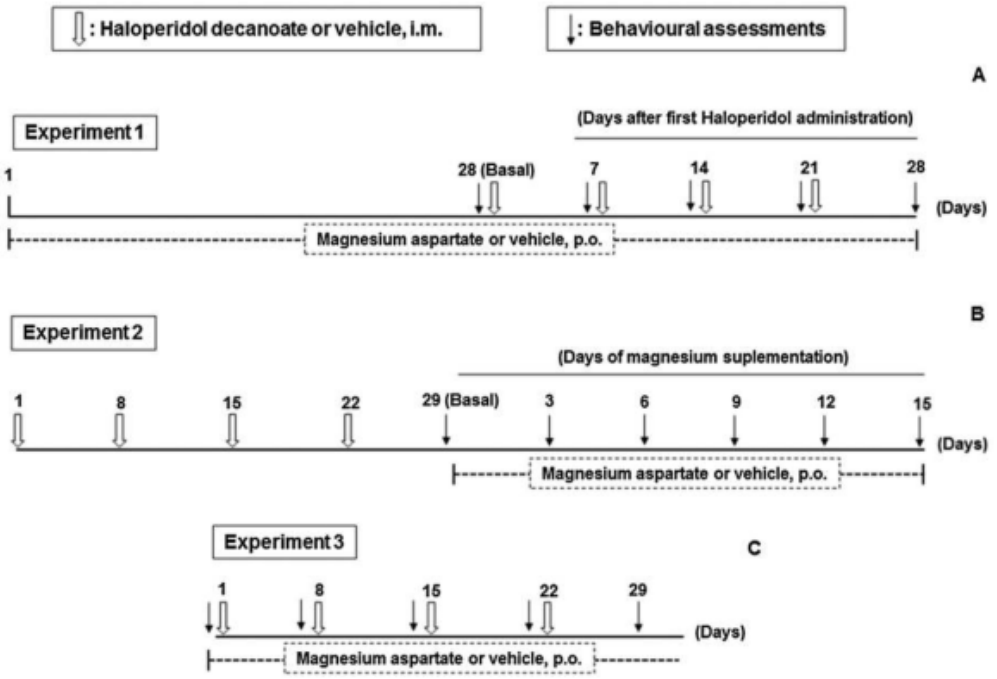


Fig. 1. Experimental designs. Preventive (A), reversal (B) and simultaneous (C) effects of Mg supplementation on the development of haloperidol-induced orofacial dyskinesia.

was facing away from the observer. To quantify the occurrence of OD, the frequency of vacuous chewing movements (VCM) was recorded for three sets of 6 min with intervals of 5 min, totaling 18 min of observation. VCM were referred to as single mouth opening in the vertical plane not directed towards physical material [31]. Observers were blind to the drug treatment. In a preliminary study (using 5 control and 10 haloperidol-injected rats) of inter-rater reliability, we found that the use of this method of observation and parameter definition usually results in >91% agreement between the three different observers.

2.4.2. Catalepsy time

Catalepsy was measured immediately after each OD observation on rats submitted to experiments 1 and 2, and only one time after last OD observation on experiment 3, using a wire grid (25 × 30 cm2) inclined 45° relative to the bench top. Each rat was placed with its forepaws near the edge of the grid and the amount of time spent in this atypical position (motionless) was recorded for three times, with a 5-min interval between them. All of the animals injected with haloperidol (H and Mg + H of all experiments) were individually placed on the inclined grid and observed for 60 s. At the end of the three replications, the mean time spent by the rat without moving was calculated for each test. This behavioral test was adapted from Rocha [32].

2.5. Biochemical assays

After behavioral evaluations, all animals were anesthetized with sodium thiopental (50 mg/kg body weight; ip) and euthanized by exsanguinations. The collected blood (collected by cardiac puncture in heparinized tubes) was centrifuged at 1300 × *g* for 15 min for plasma and used for plasma Mg levels.Brains were immediately removed and cut coronally at the caudal border of the olfactory tubercle. cortex, striatum and SNwere dissected according to Paxinos and Watson [33], and homogenized in 10 vol (w/v) of 10 mM Tris–HCl buffer (pH7.4) to determine reactive species (RS) generation and protein carbonyl (PC) levels.

2.5.1. Plasma Mg levels

Determinations of plasma Mg levels were performed by commercial kit (Labtest®). Mg ions react with the magon sulfonate (blue) in alkaline medium forming a pinkish color complex analyzed at 504 nm, which is proportional to the amount of Mg ions in the sample.

2.5.2. Reactive species (RS) generation with DCH (dichlorofluorescein-reactive species, DCH–RS)

RS levels were measured using the oxidant sensing fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCHF-DA) [34]. Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2′,7′-dichloro dihydrofluorescein diacetate, 5 (and 6) – carboxy-2′,7′-dichloro dihydrofluorescein diacetate, and dihydrorhodamine. The oxidation (DCHF-DA) to fluorescent dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. After homogenization of different brain areas (cortex, striatum and SN) in 10 vol (w/v) of 10 mM Tris-HCl buffer pH 7.4 and centrifuged (15 min, 3.500 rpm), 3 mL of the same buffer was added. After 10 s, 10 μM (DCHF-DA) (prepared in ethanol) was added to the mixture, and the fluorescence intensity from DCF was measured for 300 s and expressed as a percentage of the untreated control group. The protein content was normalized by quantification according to Lowry [35].

2.5.3. Protein carbonyl (PC) levels quantification

PC levels was quantified by the method of Levine [36], with some modifications. Soluble protein was mixed with 2,4-dinitrophenylhydrazine (DNPH; 10 mM in 2 M HCl) or HCl (2 M) and incubated at room temperature for 1 h. Denaturing buffer (150 mM

sodium phosphate buffer, pH 6.8, with 3% sodium dodecyl sulfate), ethanol (99.8%) and hexane (99.5%) were added, mixed by shaking and centrifuged. The protein isolated from the interface was washed twice with ethyl acetate/ethanol 1:1 (v/v) and suspended in denaturing buffer. Each DNPH sample was read at 370 nm in a spectrophotometer against the corresponding HCl sample (blank). Results were expressed as nmol carbonyl/g tissue.

2.6. Statistical analysis

In the experiment 1,Student's T-test was used in the basal assessment of OD (control and Mg groups), and catalepsy time (H and MgH groups), while subsequent evaluations were analyzed by three-way ANOVA (2 (control/Mg) × 2 (vehicle/H) × 4times of behavioral quantifications) for OD, and two-way ANOVA (2 (control/Mg) × 4 times of behavioral quantifications) for catalepsy. Behavioral quantifications were considered as a repeated measure followed by pairwise comparisons. In the experiment 2, Student's T-test was used in the basal assessment of OD (vehicle and H groups), which were quantified 7 days after the first haloperidol administration. From the 3rd day (when Mg supplementation was already initiated), three-way ANOVA was applied (2 (control/Mg) × 2 (vehicle/H) × 5times of behavioral quantifications). Catalepsy timewas analyzed by Students T-test on basal assessment (vehicle and H groups) and subsequently two-way ANOVA was applied (2 (control/Mg) × 5 times of behavioral quantifications), considering the behavioral quantifications as a repeated measure. In the experiment 3, two-way ANOVA was used in the basal assessment (2 (control/Mg) × 2 (vehicle/H)). One week after first haloperidol administration three-way ANOVA was applied (2 (control/Mg) × 2 (vehicle/H) × 4 times of behavioral quantifications). Student's T-test was applied to catalepsy time (H and MgH groups) (one assessment 7 days after the last haloperidol administration). Biochemical data from all experiments were analyzed by two-way ANOVA for each analyzed plasma and tissue (cortex, striatum, SN). All the comparisons were followed by Newman-Keuls multiple range test when appropriate (software Statistica10 for Windows was used). Values of $p < 0.05$ were considered as statistically significant for all comparisons made.

3. Results

3.1. Experiment 1: preventive influence of Mg supplementation on sub-chronic haloperidol-induced OD and catalepsy development

While Mg supplementation *per se* was not related to orofacial changes, haloperidol administration favored OD development, which was partially prevented by the Mg supplementation on days 14 to 28 (Fig. 2A). The same result is better observed on average VCM, obtained from VCM summed of all assessment days (Fig. 2B). Similar to VCM, previous supplementation of Mgprevented haloperidol-induced catalepsy until 21 days following the first haloperidol administration (Fig. 2C).

3.1.1. Preventive influence of Mg supplementation on reactive species generation and protein carbonil in cortex, striatum and substantia nigra

While Mg increased RS generation *per se* in both cortex (Fig. 6A) and striatum (Fig. 6B), haloperidol increased bothRS generation and PC levels in all evaluated brain areas (Fig. 6A-F). Previous administration of Mg exerted partial prevention on haloperidol-induced RS generation in both cortex (Fig. 6A) and SN (Fig. 6C), while increased PC level was completely prevented by Mg supplementation in cortex (Fig. 6D) and striatum (Fig. 6E), and partially prevented in SN (Fig. 6F).

3.2. Experiment 2: influence of Mg supplementation on the reversal of sub-chronic haloperidol-induced OD and catalepsy development

Sub-chronic administration of haloperidol increased VCM frequency in all assessments, while Mg supplementation reversed it partially on days 12–15. Control and Mg groups showed no significant changes in VCM frequency at all observations (Fig. 3A). This finding may also be observed on average number of VCM that Mg supplementation partially reversed the increasing of VCM frequency (Fig. 3B). Both H and MgH groups showed increased catalepsy time, while Mg supplementation from day 6 to day 15 progressively decreased the cataleptic behavior in MgH group when compared to H group (Fig. 3C).

3.2.1. Effects of Mg supplementation on increased generation of RS and PC levels haloperidol-induced in cortex, striatum, and substantia nigra

Haloperidol sub-chronic administration increased RS generation in all evaluated brain areas (Fig. 7A–C) and also increased PC levels in both striatum (Fig. 7E) and SN (Fig. 7F). When Mg supplementation occurred after haloperidol administration, RS generation decreased in both cortex (Fig. 7A) and striatum (Fig. 7B), and PC levels in both striatum (Fig. 7E) and SN (Fig. 7F) in comparison to haloperidol group.Mg supplementation decreased PC levels in cortex *per se* (Fig. 7D).

3.3. Experiment 3: influence of concomitant Mg supplementation on the sub-chronic haloperidol-induced OD and catalepsy development

Haloperidol sub-chronic administration increased VCM frequency in all assessments on haloperidol group, while Mg supplementation protected its development on week 2 to week 4 (Fig. 4A). *Per* se, Mg supplementation reduced VCM frequency on weeks 2 and 3 when compared to control group. Average number of VCM was reduced *per se* by Mg supplementation when compared to control group and also protected from increased VCM frequency when compared to haloperidol group (Fig. 4B). Mg supplementation also reduced catalepsy time when compared to haloperidol group (Fig. 4C).

3.3.1. Influence of Mg supplementation on increased generation of RS and PC levels haloperidol-induced in cortex, striatum, and substantia nigra

Haloperidol sub-chronic administration increased RS generation in all evaluated brain areas (Fig. 8A–C) and increased PC levels only in SN (Fig. 8F) when compared to control group. *Per se*, Mg supplementation increased RS generation in cortex (Fig. 8A) and decreased PC levels in SN (Fig. 8F). When compared haloperidol group, Mg supplementation protected from increased RS generation in all evaluated brain areas (Fig. 8A–C) and also from increase PC levels in SN (Fig. 8F).

3.4. Influence of Mg supplementation on plasma Mg levels

In the experiment 1, Mg supplementation prior to the haloperidol administration increased the Mg levels on plasma in both Mg and MgH groups. Haloperidol sub-chronic administration exerted no influence on plasma Mg levels (Fig. 5A). In the experiment 2, after haloperidol administration, Mg supplementation did not change the Mg levels on plasma in MgH group. *Per se*, haloperidol administration and Mg supplementation did not have any influence

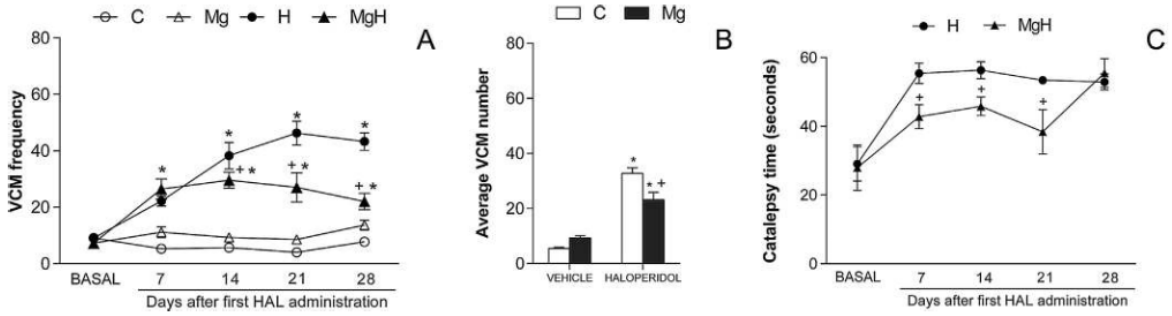


Fig. 2. Influence of Mg supplementation (40 mg/kg/1 mL or deionized water; orally, for 28 days; once a day)on the prevention of VCM (A–B) and catalepsy time (C) of rats subsequently treated with haloperidol (12 mg/kg/mL or vehicle; i.m., once a week, for four weeks) immediately after basal assessment. Data are expressed as mean ± S.E.M. Abbreviations: VCM: vacuous chewing movements; C: control, vehicle group; Mg: magnesium, vehicle group; H: haloperidol group; MgH: magnesium plus haloperidol group. *indicates significant difference from vehicle treated group in the same supplementation;+indicates significant difference between supplementations in the same treatment ($P < 0.05$).
Three-way ANOVA of VCM frequency revealed a significant main effect of drug [$F(1,24) = 98.04$; $p < 0.001$], time [$F(4108) = 18.90$; $p < 0.001$] and a significant drug × supplementation [$F(4108) = 17.77$; $p < 0.001$], time × drug [$F(4108) = 14.90$; $p < 0.001$], time × supplementation [$F(4108) = 9.52$; $p < 0.001$] and a significant time × supplementation × drug [$F(4108) = 3.59$; $p < 0.008$] interaction (A).Two-way ANOVA of average number of VCM revealed a significant main effect of drug [$F(1,24) = 140{,}02$, $p < 0{,}001$] and a supplementation x drug interaction [$F(1,24) = 14.76$, $p < 0.001$] (B). Two-way ANOVA of catalepsy time revealed a significant main effect of supplementation [$F(1,24) = 24.65$; $p < 0.001$], time [$F(4108) = 123.19$; $p < 0.001$], and a significant time × supplementation [$F(4108) = 17.69$; $p < 0.001$] interaction (C).

on plasma Mg levelswhen compared with control group (Fig. 5B). In the experiment 3, plasma Mg levels were not modified by Mg supplementation in relation to control group, but, when this supplementation was supplied together with haloperidol, plasma Mg level was lower than Mg group (Fig. 5C).

4. Discussion

The aim of this study was evaluate the influence of Mg supplementation on the development of OD and catalepsy in sub-chronic haloperidol-induced animal model. In addition, the influence of Mg supplementation on oxidative status in brain areas was also evaluated.

Typical antipsychotic treatment is frequently related to development of extrapyramidal side effects, including tardive dyskinesia (TD), which is a serious and sometimes, an irreversible condition [37,38]. Although the biological mechanisms underlying these adverse effects remain unclear, the involvement of excitotoxicity and particularly oxidative stress (OS) in haloperidol-induced motor disorders has been studied [31,39–43], in search of a better understanding about the pathophysiology of extrapyramidal disturbances caused by typical antipsychotics as haloperidol [9,44–46]. The motivation for the current study was subsequent to incredible turnouts in patients affected by movement disturbances, including tardive dyskinesia (TD), who were taking antipsychotic for over than 20 years, when they were supplemented with Mg incorporated into treatment protocols (Daniele Couturier, personal communication, January 4 2013).

Whereas typical antipsychotics continue to be widely used in psychiatric practice, with high frequency of severe motor disorders, the current study was developed, presenting the following findings: i) previous supplementation of Mg was able to partially prevent of haloperidol-induced OD and catalepsy; ii) OD and catalepsy haloperidol-induced were partially reversed by Mg since consolidation of movement disturbances until 15 days after to start the supplementation and lastly iii) Mg together with haloperidol partially avoided haloperidol-induced OD and catalepsy development.

Besides, the lower OD frequency and shorter catalepsy time observed in these three different experimental paradigms occurred together with reduced markers of OS, which were estimated by

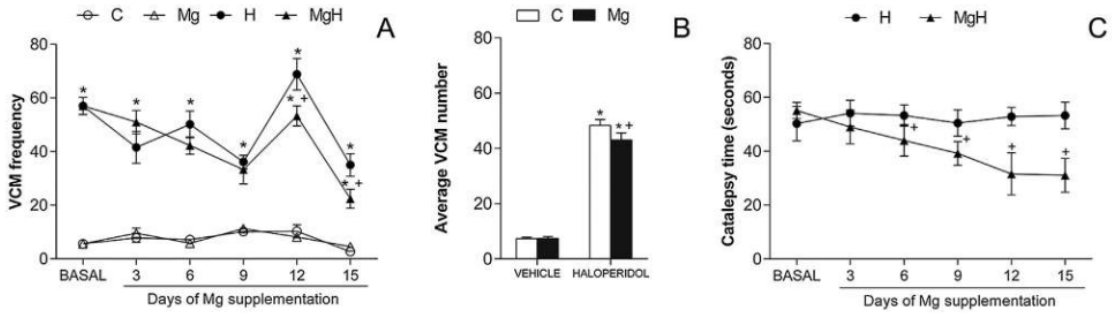


Fig. 3. Influence of Mg supplementation (40 mg/kg/1 mL or deionized water; orally,for 15 days; once a day) on the reversal of VCM (A-B) and catalepsy time (C) of rats previously treated with haloperidol (12 mg/kg/mL or vehicle; i.m., once a week, for four weeks) immediately after basal assessment.Data are expressed as mean ± S.E.M. Abbreviations: VCM: vacuous chewing movements; C: control vehicle group; Mg: magnesium vehicle group; H: haloperidol group; MgH: magnesium plus haloperidol group. *indicates significant difference from vehicle treated group in the same supplementation; +indicates significant difference between supplementations in the same treatment ($P < 0.05$).
Three-way ANOVA of VCM frequency revealed a significant main effect of drug [$F(1,24) = 490.01$; $p < 0.001$], time [$F(4108) = 17.99$; $p < 0.001$] and a significant time × drug [$F(4108) = 15.06$; $p < 0.001$] and time x supplementation [$F(4108) = 2.49$; $p < 0.034$] interaction (A). Two-way ANOVA of average number of VCM revealed a significant main effect of drug [$F(1,24) = 490.01$, $p < 0.001$] (B). Two-way ANOVA of catalepsy time revealed a significant main effect of supplementation [$F(1,24) = 28.99$; $p < 0.001$], time [$F(4108 = 17.36$; $p < 0.001$] and a significant time × supplementation interaction [$F(4108) = 21.19$; $p < 0.001$] (C).

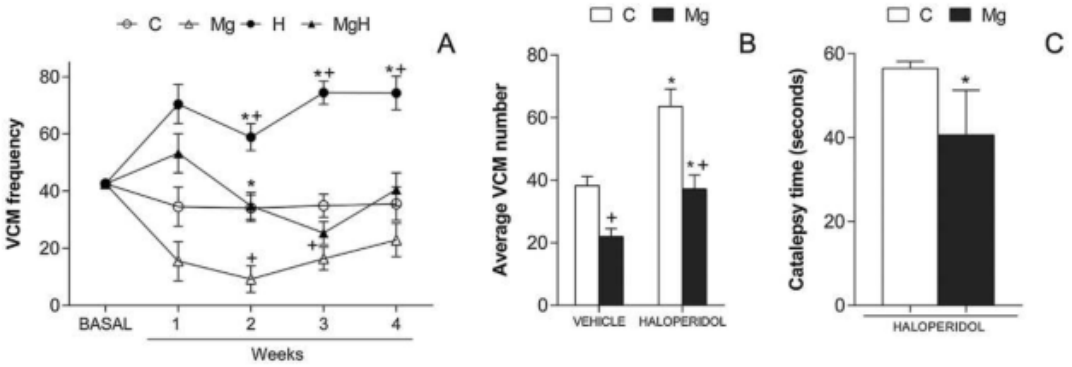


Fig. 4. Influence of Mg supplementation (40 mg/kg/1 mL or deionized water; orally, once a day) on the prevention of VCM (A-B) and catalepsy time (C) of rats, concomitant treated with haloperidol (12 mg/kg/mL or vehicle; i.m., once a week, for four weeks) immediately after basal assessment. Data are expressed as mean ± S.E.M. Abbreviations: VCM: vacuous chewing movements; C: control vehicle group; Mg: magnesium vehicle group; H: haloperidol group; MgH: magnesium plus haloperidol group. *indicates significant difference from vehicle treated group in the same supplementation; +indicates significant difference between supplementations in the same treatment ($P < 0.05$). Three-way ANOVA of VCM frequency revealed a significant main effect of drug [$F(1,20) = 36.03$; $p < 0.001$], supplementation [$F(1,20) = 39.76$; $p < 0.001$], time [$F(4,80) = 2,69$; $p < 0.036$], significant time × drug [$F(4,80) = 14,88$; $p < 0.001$] and a significant time x supplementation [$F(4,80) = 3,85$; $p < 0.006$] interaction (A). Two-way ANOVA of average number of VCM revealed a significant main effect of drug [$F(1,20) = 24.80$, $p < 0.001$] and a significant main effect of supplementation [$F(1,20) = 27.36$, $p < 0.001$] (B). Student's *t*-test of catalepsy time revealed a significant difference between Mg and haloperidol group $p < 0.001$ (C).

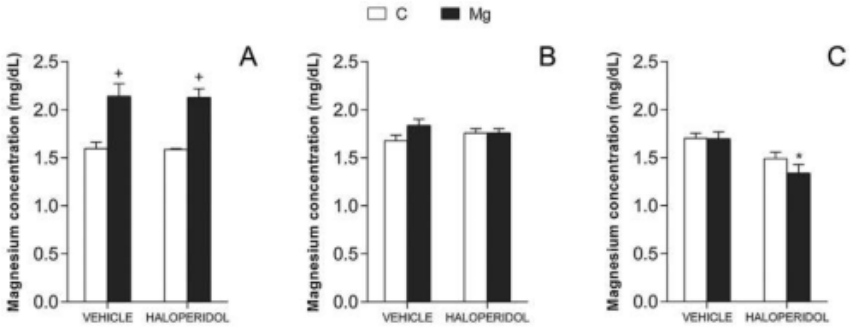


Fig. 5. Influence of daily Mg supplementation (40 mg/kg/1 mL or deionized water; orally) on the plasma Mg levelsin rats before (A), after (B) and concomitant with haloperidol treatment (C) (12 mg/kg/mL or vehicle; i.m., once a week for four weeks). Data are expressed as mean ± S.E.M. Abbreviations: C: control group; Mg: magnesium group. *indicates significant difference from control group; +indicates significant difference between the supplementations in the same treatment ($P < 0.05$).
Two-way ANOVA of plasma Mg levels before haloperidol treatment revealed a significant main effect of supplementation [$F(1,24) = 38.11$, $p < 0.001$] (A). Two-way ANOVA of plasma Mg levelsafter haloperidol treatment showed no significant main effects (B). Two-way ANOVA of plasma Mg levelsrevealed a significant main effect of drug[$F(1,20) = 15,26$, $p < 0.001$] (C).

RS generation and PC levels in brain areas related to movement control, such as cortex, striatum and SN. These findings corroborate with previous literature data, when different studies showed a close relationship between oxidative damages and haloperidol-induced motor disturbances [31,39,42,47,48]. More specifically, our current outcomes are showing that sub-chronic administration of haloperidol was related to extrapyramidal side effects development, as observed by increased VCM and catalepsy time in the animals, and that the Mg was able to minimize these movement disturbances when supplemented before, after and together with antipsychotic administration. Similar to a previous study from our laboratory, when Mg was able to minimize reserpine-induced movement disturbances [27], our current data confirms, through different experimental protocols, the protective influence of the Mg on these motor side effects.

Based on this, becomes important to return some basic functions of the nigrostriatal dopaminergic system, which is primarily involved in the haloperidol-induced extrapyramidal disorders development: i) firstly, in the dorsal striatum, medium spiny neurons (MSNs) are critical elements in the control of movement and motivation [49], and dopamine (DA) released from *substantia nigra* is a major modulator of MSN, activating D2-like receptors, which is related to decrease of both glutamate [50–52] and acetylcholine (ACh) output from caudate interneurons [49,53–55]; ii) when haloperidol is administrated, post-synaptic striatal D2-like receptors are blocked, affecting the inhibitory control of DA. This blockade activates glutamate neurotransmission in NMDA receptors, generating neurotoxicity in the nigrostriatal system [29,31,56], and also related to increased outflow of ACh from caudate nucleus, resulting in orofacial dyskinetic movements and oxidative damages [12,37,57–60]. In fact, glutamate neurotoxicity together with increased activity of ACh and OS generation have been described in the pathophysiology of haloperidol-induced extrapyramidal side effects, including TD; iii) haloperidol also blocks pre-synaptic D2R, in opposition to the negative feedback of DA release, inducing a secondary increase in DA synthesis and metabolism [61]. DA can also auto-oxidize themselves to dopamine-quinones, depleting glutathione and generating reactive species (RS) by mitochondrial dysfunction [37,62].

Taking together these considerations, we can highlight some neurochemical events that can be proposed as mechanism of action by Mg in this study, as follows: i) as calcium (Ca^{2+}) ions have

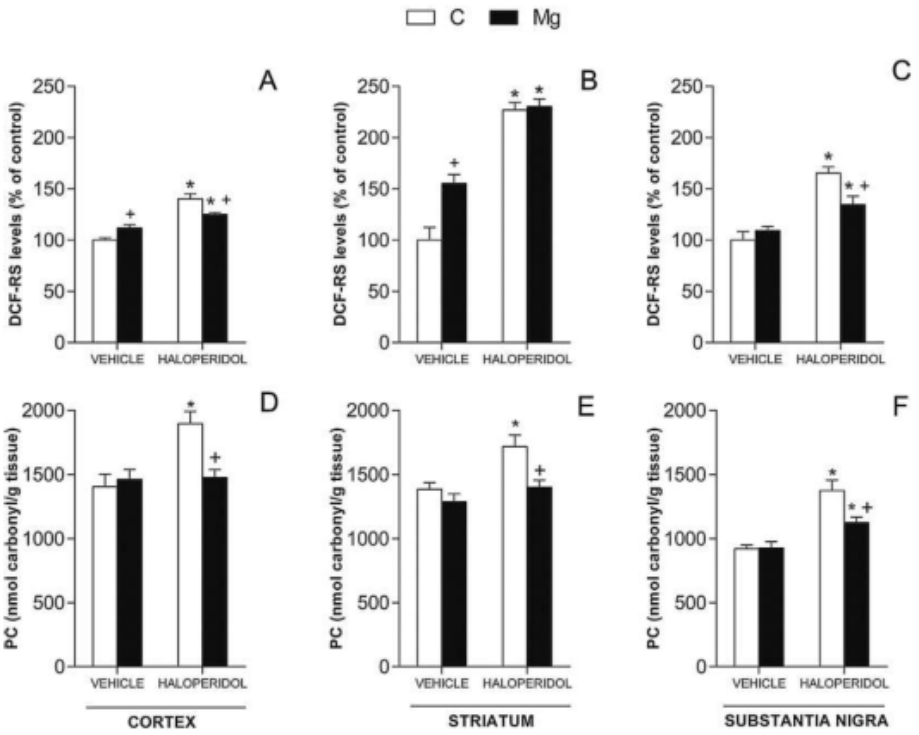


Fig. 6. Influence of Mg supplementation (40 mg/kg/1 mL or deionized water; orally, for 28 days; once a day) on RS generation (A–C) and PC levels (D–F) in cortex, striatum, and SN, respectively, in rats subsequently treated with haloperidol (12 mg/kg/mL or vehicle; i.m., once a week, for four weeks). Data are expressed as mean ± S.E.M. Abbreviations: C: control group; Mg: magnesium. *indicates significant difference from vehicletreated group in the same supplementation; +indicates significant difference between supplementations in the same treatment ($P < 0.05$).
Two-way ANOVA of RS generation revealed a significant main effect of supplementation in striatum [$F(1,24) = 10.18$; $p < 0.05$], a significant main effect of drug in cortex, striatum and SN [$F(1,24) = 75.40$; 120.79 and 42.95, $p < 0.001$, respectively] and a significant main effect of drug × supplementation in cortex, striatum and SN [$F(1,24) = 19.36$; $p < 0.001$; 7.96; $p < 0.009$; 8.38; $p < 0.007$, respectively] (A–C). Two way ANOVA of PC levels revealed a main effect of supplementation [($F(1,24) = 4.52$; $p < 0.043$, 9.79; $p < 0.004$ and 4.91; $p < 0.036$] and drug [$F(1,24) = 8.76$; $p < 0.006$, 11.56; $p < 0.002$, 35.98; $p < 0.001$] in cortex, striatum and SN, respectively, and a significant drug × supplementation interaction ($F(1,24) = 7.70$; $p < 0.01$, 5.57; $p < 0.026$] in cortex and SN, respectively (D–F)

been implicated in the regulation of vesicular neurotransmitters at voltage-dependent Ca^{2+} channels [63–65], and Mg is an ionic channel blocker [19], its supplementation favors a competitive interaction with Ca^{2+}, reducing neurotransmitter release and minimizing glutamate-mediated excitotoxic events, as also reducing ACh outflow, thus reducing impairments of voluntary movements consequent to cholinergic axis imbalance; ii) Mg reduces glutamate activation due to its role in blocking the NMDA receptors [66–68]. Indeed, hyperfunction of glutamatergic transmission leads to a high Ca^{2+} influx triggering pathological intracellular processes [69,70]; iii) as Mg inhibits events related to Ca^{2+}, such competition reduces mitochondrial calcium uptake, so reducing proapoptotic events, which are related to subsequent activation of caspases and reactive oxygen species (ROS) generation [71]; iv) by competing with Ca^{2+}, which is clearly related to increased synthesis and release of DA [72], Mg minimizes the extracellular level of this neurotransmitter, thus reducing its auto-oxidation and generation of free radicals. Taken together, our proposal of action mechanism involved in the beneficial influences of Mg supplementation on haloperidol-induced extrapyramidal side effects was summarized in the Scheme 1, which have support in the literature. In fact, since drugs acting as NMDA receptor antagonists could have beneficial effects in reducing extrapyramidal disorders [73]; Also, Mg could act as a modulator of glutamatergic transmission protecting against the damages caused by overproduction of ROS on neural cells. It is known that Ca^{2+} also plays an important role in the regulation of neurotransmitters release in central nervous system, including dopamine, which is in part responsible by generate ROS after a chronic block of dopamine receptors [69–72].

As commented above, together with decreased OD and catalepsy, a lower RS generation and protein oxidation were observed in dopaminergic brain areas of MgH group, independently of the period of supplementation, as follows: In the first experiment, Mg supplementation 28 days before haloperidol administration was able to prevent RS generation in both cortex and SN, thus decreasing haloperidol-induced PC levels in cortex, striatum and SN. In the experiment 2, after four weeks of haloperidol injection, Mg was able to reverse RS generation in both cortex and striatum, as well to minimize PC levels in both striatum and SN. Lastly, in the experiment 3, simultaneous Mg and haloperidol administration was related to decreased RS generation in cortex, striatum and SN, as well lower PC levels in SN only. Taken together, this protective influence of Mg shown here are in accordance with our previous study, when Mg exerted beneficial influence on movement disturbances and brain oxidative damages reserpine-induced, strengthening the relationship between OS in extrapyramidal brain areas and movement disturbances [27].

Of particular importance for our findings, the beneficial influence of Mg supplementation on oxidative damages in the SN is similar in the three experimental protocols performed here. Indeed, this brain area concentrates neuronal dopaminergic bodies, which send their projections to the striatum, who in turn

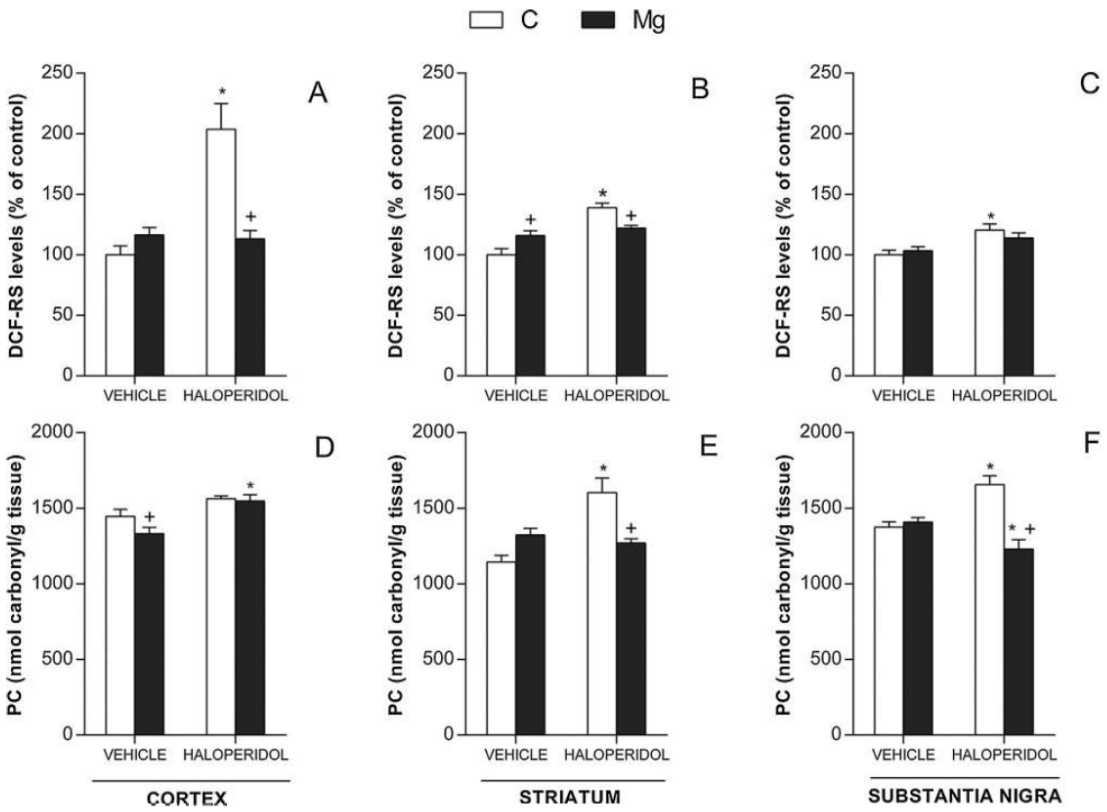


Fig. 7. Influence of Mg supplementation (40 mg/kg/1 mL or deionized water; orally, for 15 days; once a day) on RS generation (A–C) and PC levels (D–F) in cortex, striatum, and SN, respectively, in rats previously treated with haloperidol (12 mg/kg/mL or vehicle; i.m., once a week for four weeks). Data are expressed as mean ± S.E.M. Abbreviations: C: control group; Mg: magnesium group. *indicates significant difference from vehicle treated group in the same supplementation; +indicates significant difference between supplementations in the same treatment ($P < 0.05$).
Two-way ANOVA of RS generation revealed a significant main effect of drug in cortex, striatum, and SN [$F(1,24) = 19.54$, $p < 0.001$; 33.22, $p < 0.001$; and 14.12, $p < 0.001$, respectively], a significant main effect of supplementation in cortex [$F(1,24) = 12.91$; $p < 0.001$], and a significant drug × supplementation interaction in cortex and striatum [$F(1,24) = 19.89$, $p < 0.001$; 17.50, $p < 0.001$, respectively] (A–C). Two way ANOVA of PC levels revealed a significant main effect of drug in cortex and striatum [$F(1,24) = 18.82$, $p < 0.001$; 11.96, $p < 0.002$, respectively] and a significant drug × supplementation interaction in striatum and SN [$F(1,24) = 19.13$, $p < 0.001$; 23.12, $p < 0.001$, respectively] (D–F).

sends projections to other regions of basal ganglia involving multiple neurotransmitters, such as acetylcholine, glutamate and GABA. These observations make somewhat complex the understanding these findings, since our study was performed in brain isolated areas, while the "dopaminergic" imbalance is actually a dopamino-GABAergic, –glutamatergic and –cholinergic [49,76], involving the cortical nigrostriatal system together.

It is common consensus that until today, most diseases are not cured, but they can be controlled or minimized by medications. Unfortunately, the use of medicines is frequently associated with the development of serious side effects, as in the case of typical antipsychotics, which for its low cost and high clinical efficacy remain in psychiatric therapeutic arsenal. In this sense, is critical that the movement side effects consequent to antipsychotic treatment should be minimized, since the treatment of choice for medical treatment of psychotic conditions in many countries are typical antipsychotics, specifically with haloperidol [77].

Mg is an economical and simple element, which is essential in human physiologic processes, playing modulator role on the cell proliferation and metabolism [19,22]. Thus, a low Mg bioavailability has been related to occurrence of OS, while different experimental studies investigate the consequences of the hypomagnesaemia [78–81]. Moreover, induction of apoptosis mediated by oxidative processes related to Mg deprivation has been observed in additional tissues, including cardiovascular [82,83] and hepatic [84] system. In addition to increasing RS generation and to cause oxidative damages, a low Mg availability can trigger apoptotic processes, so affecting DNA structure, impairing its repair mechanisms [85]. Recent data from experimental and epidemiological studies suggest an important consequence of the magnesium deficiency and/or of disturbances of Mg metabolism in different situations [86], including cardiovascular diseases [87], obstetric conditions such as eclampsia [20,88], stroke [89] besides affective disorders [90–92].

Besides movement disturbances and oxidative damage markers, plasma magnesium levels were also observed in the current study, and these findings may be related to different supplementation times employed in the three experimental protocols. These outcomes lead us to hypothesize that the plasma level of Mg cannot be the central factor involved in its antidyskinetics effects, which were observed here. Indeed, our findings showed an increased Mg plasma level only when its supplementation was provided before haloperidol, whereas the Mg plasma level has not been modified after haloperidol, and it was decreased when Mg and

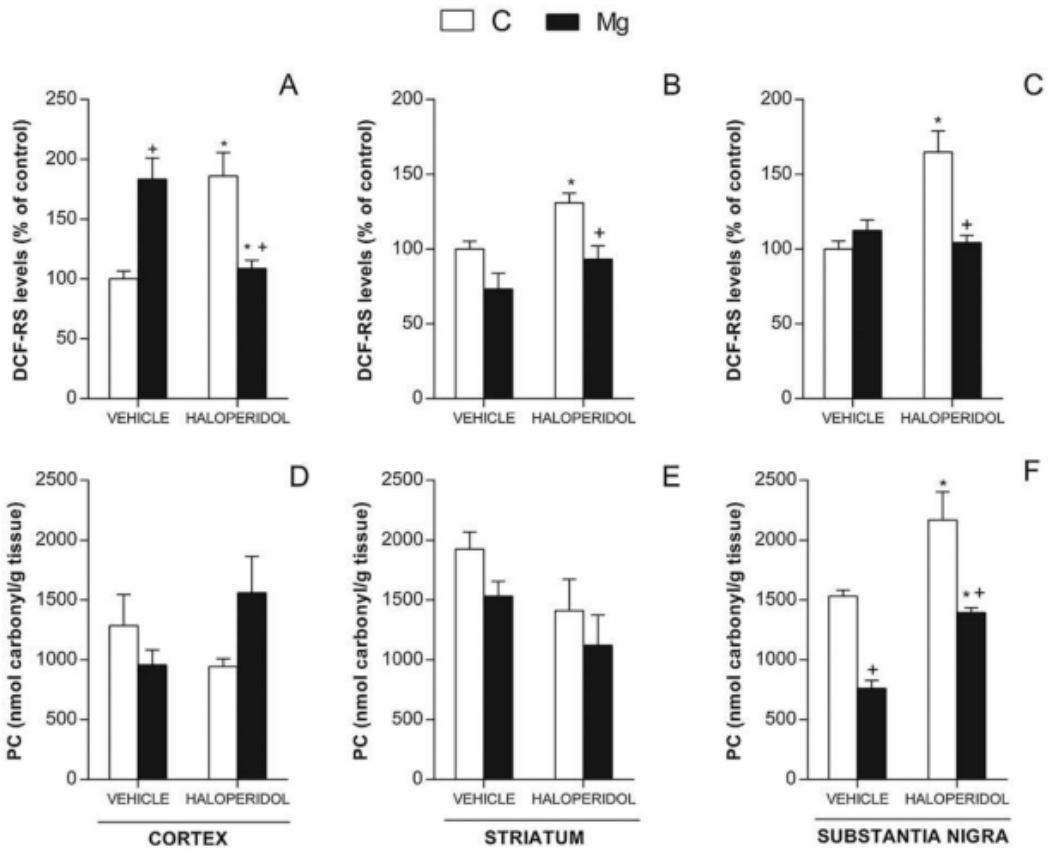
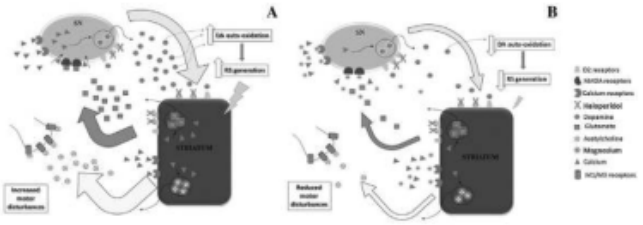


Fig. 8. Effect of Mg supplementation (40 mg/kg/1 mL or deionized water; orally, once a day) on RS generation (A–C) and PC levels (D–F) in cortex, striatum, and SN, respectively, in rats treated concomitantly with haloperidol (12 mg/kg/mL or vehicle; i.m., once a week for four weeks). Data are expressed as mean ± S.E.M. Abbreviations: C: control group; Mg: magnesium group. *indicates significant difference from vehicletreated group in the same supplementation; +indicates significant difference between supplementations in the same treatment ($P < 0.05$).
Two-way ANOVA of RS generation revealed a significant main effect of drug in striatum and SN [$F(1,20) = 9.79$, $p < 0.005$; 10.64, $p < 0.003$, respectively], a significant main effect of supplementation in striatum and SN [$F(1,20) = 15.78$, $p < 0.001$; 7.79, $p < 0.01$, respectively], and a significant drug × supplementation interaction in cortex and SN [$F(1,20) = 32.67$, $p < 0.001$; 17.22, $p < 0.001$, respectively] (A-C). Two way ANOVA of PC levels revealed a significant main effect of drug in striatum and SN [$F(1,20) = 5.06$, $p < 0.035$; 24.93, $p < 0.001$, respectively], a significant main effect of supplementation in SN [$F(1,20) = 36.90$, $p < 0.001$] and a significant drug × supplementation interaction in cortex [$F(1,20) = 4.93$, $p < 0.037$] (D–F).



Scheme 1. Proposed mechanism of action for the beneficial influence of Mg supplementation. (A) Deleterious events triggered after D2R blockade by haloperidol:increase of DA release, RS generation, cholinergichyperfunction and increase of excitotoxicity by high glutamate release in basal ganglia; (B) Protective influence of Mg supplementation on extrapyramidal events triggered by haloperidol: Mg blocks calcium channels reducing DA (decreased free radicals generation), acetylcholine (reduced M1/M3 receptor activation) and glutamate release; Mg also blocks NMDA receptors reducing glutamatergic activation, which is related to excitotoxicity.

haloperidol were offered in set. Observing OD development in the animals exposed to our second experimental protocol, beneficial influence of the Mg supplementation seems to have weaker effect to reduce OD previously established by the haloperidol, possibly because the orofacial disturbance was already consolidated before Mg supplementation be initiated. Interestingly, plasma Mg level was not changed in this experimental group, leading us to think that haloperidol could modify Mg absorption, but at this time we do not know about the brain level of Mg, which appears to have been enough to reverse part the motor disturbances, as observed here. Inversely, when haloperidol was administered together with Mg supplementation, plasma Mg level was decreased, agreeing with

our hypothesis about some interaction between haloperidol or its metabolite with Mg. However, as in this experimental group the OD was not yet consolidated, Mg may have reached sufficient brain level to exert its beneficial effects, as also observed in our findings.

Literature shows some controversial data about a possible interaction between haloperidol treatment and plasma Mg levels, since clinical studies involving therapeutic doses of antipsychotics in schizophrenic patients showed increased Mg levels in erythrocytes, but not in plasma [93–95]. In contrast, Jabotinski-Rubin et al. [99] reported that some schizophrenic patients that are exposed to haloperidol treatment show hypomagnesaemia. This phenomenon was related to generation of extrapyramidal side effects induced by haloperidol [96–98].

5. Conclusion

The present study indicates the beneficial influence of Mg supplementation on haloperidol-induced OD and catalepsy, which are related to oxidative damages and neurotoxicity in brain areas involved in the extrapyramidal system. These findings shows the beneficial influence of Mg supplementation on movement disturbances that occur during haloperidol treatment. After decades of the beginning of neuroleptic treatment to schizophrenia patients, this problem still stays in psychiatric clinic, implicating in a no adherence to pharmacologic treatment. In this sense, interventions that can minimize these disorders are important.

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6 MANUSCRITO CIENTÍFICO

Influence of magnesium supplementation and L-type calcium channel blocker on haloperidol-induced movement disturbances

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Abstract

Haloperidol (HAL) is an antipsychotic related to movement disorders. Magnesium (Mg) showed benefits on orofacial dyskinesia (OD), suggesting its involvement with N-methyl-d-aspartate receptors (NMDAR) since it acts blocking calcium channels. Comparisons between nifedipine (NIF; a calcium channel blocker) and Mg were performed to establish the Mg mechanism. Male rats concomitantly received haloperidol (HAL) and Mg or NIF for 28 days, and OD behaviors were weekly assessed. Both Mg and NIF decreased HAL-induced OD. HAL increased Ca^{2+}-ATPase activity in the striatum, and Mg reversed it. In the cortex, both Mg and NIF decreased such activity. Dopaminergic and glutamatergic immunoreactivity were modified by HAL and treatments: i) in the cortex: HAL reduced D1R and D2R, increasing NMDAR immunoreactivity. Mg and NIF reversed this HAL influence on D1R and NMDAR, while only Mg reversed HAL effects on D2R levels; ii) in the striatum: HAL decreased D2R and increased NMDAR, while Mg and NIF decreased D1R and reversed the HAL-induced decreasing D2R levels. Only Mg reversed the HAL-induced increasing NMDAR levels; iii) in the *substantia nigra* (SN): while HAL increased D1R, D2R, and NMDAR, both Mg and NIF reversed this influence on D2R, but only Mg reversed the HAL-influence on D1R levels. Only NIF reversed the HAL effects on NMDAR immunoreactivity. These findings allow us to propose that Mg may be useful to minimize HAL-induced movement disturbances. Mg molecular mechanism seems to be involved with a calcium channel blocker because the NIF group showed less expressive effects than the Mg group.

Keywords: Nifedipine; dopaminergic system; NMDA receptor; typical antipsychotic.

1 Introduction

The chronic use of antipsychotics for the treatment of schizophrenia is characterized by severe, disabling and irreversible adverse effects, being frequently manifested by bucco-lingual-masticator involuntary movements that may expand to the neck, trunk and extremities, called tardive dyskinesia (TD) [1–3]. The promising reduction of these effects given by the advent of second-generation antipsychotics did not eliminate the high prevalence risk of developing such effects [4–6]. The TD pathophysiology is complex and poorly understood, and it is directly related to the prolonged blockade of D2 receptors leading to a dopaminergic hypersensitivity, increasing the dopamine metabolism and the glutamatergic transmission that ends up in the nigrostriatal region neurodegeneration [7]. Many pharmacological and non-pharmacological strategies have been tested to treat the TD or attenuate its symptoms [8–12]; however, typical neuroleptic drugs remain being prescribed, especially haloperidol.

Recent studies of our research group have shown the beneficial influences of magnesium supplementation on an animal model of orofacial dyskinesia (OD) [13,14]. Magnesium is an essential mineral for the organism, playing a role in homeostasis as a cofactor of more than 300 biochemical reactions [15,16], neurotransmitter release [17], modulation of calcium channels and blocking N-methyl-d-aspartate (NMDA) receptors [18–21], besides controlling synapses density and plasticity [22]. For this reason, magnesium deficiency can facilitate different pathologies, including neurological diseases like Alzheimer's, attention deficit hyperactivity disorder (ADHD), Parkinson's, depression and anxiety [23–26], among others.

L-type voltage-gated calcium channels (LTCCs) are a subfamily of voltage-gated Ca^{2+} channels that participate in critical neurological functions like synaptic plasticity and regulate neurotransmitter release from some neuron types [27–31]. As the LTCCs have been linked to essential roles in neurobiological functions, they became a therapeutic alternative to treat some neurological diseases like age-dependent memory deficits, Alzheimer's disease (AD) and Parkinson's disease (PD) [32–35]. The use of calcium channel blockers to treat TD in schizophrenic patients have been experimentally described [36], while some evidence has shown that LTCCs blockers can neuroprotect the haloperidol-induced OD [37,38].

In our last study, we suggested that magnesium supplementation could exert positive influence against haloperidol-induced orofacial dyskinesia through blocking calcium channels consequently preventing excitatory effects generated by this bivalent cation. Based on this, our current study was performed to comparatively evaluate the possible beneficial influence of magnesium and a calcium channel blocker (nifedipine) on haloperidol-induced orofacial dyskinesia, accessing molecular targets related to the dopaminergic and glutamatergic pathways.

2 Material and methods

2.1 Animals

Three-month-old male *Wistar* rats weighing about 250-300g were used. Three animals per group (±1) were kept in Plexiglas cages with free access to food (standard chow) and water in a room with controlled temperature (22-23°C) and 12h-light/dark cycle with lights on at 7:00 a.m. Animals were fed with standard chow *ad libitum* (PuroTrato®, RS, Brazil), which contains adequate levels of Mg during all experiments. This procedure follows the recommendations of the National Research Council (NRC, 1995). The experimental protocols were approved by the Animal Ethics Committee (Universidade Federal de Santa Maria – UFSM #1235290115), which is affiliated to the Council of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

2.2 Drugs

Haloperidol decanoate (Haloperidol decanoate - Janssen-Cilag) was dissolved in Tween® and diluted to a final concentration of 1% Tween® with distilled water. The vehicle consisted of a 1% Tween® solution in sesame oil. Magnesium aspartate (Fragon do Brasil Farmacêutica Ltda) was dissolved in deionized water. Nifedipine (Fragon do Brasil Farmacêutica Ltda) was dissolved in Tween® solution.

2.3 Experimental procedures

Twenty-eight rats were submitted to an OD basal evaluation and immediately after, they were randomly designated to four experimental groups (n=7). The groups were: C (control), HAL (haloperidol), Mg (magnesium + haloperidol) and NIF (nifedipine +

haloperidol). These three last experimental groups received haloperidol administration

(HAL, Mg and NIF groups – 12mg/Kg/mL, i.m.), and the control group was injected with the vehicle solution, once a week for four weeks. Concomitantly, animals were orally treated with magnesium aspartate (Mg group - 40mg/Kg/mL), nifedipine (NIF group – 10mg/Kg/mL) or vehicle (C and H groups). OD was previously quantified to each HAL administration on the 7th day after the last HAL administration. Mg and NIF treatments were maintained throughout the protocol (total of 28 days) (Figure 1).

2.3.1 Behavioral assessments

2.3.1.1 Orofacial dyskinesia (OD)

Rats were individually placed in glass cages (20x20x19 cm) containing one mirror under the floor, which can be adapted to allow behavioral quantification when the animal was facing away from the observer. The frequency of vacuous chewing movements (VCM) referred to a single mouth opening in the vertical plane not directed towards physical material, was recorded for three sets of 6 min with intervals of 5 min [13,39]. Animals were first submitted to a basal test. It was excluded from the experiment all rats showing a VCM score higher than 40 [12]. Observers were blind to the drug treatment.

2.3.2 Samples collection

After behavioral evaluations, animals were anesthetized with sodium thiopental (50mg/Kg body weight; i.p.) and euthanized by exsanguination. The collected blood (collected by cardiac puncture in heparinized tubes) was centrifuged for plasma and used for plasma Mg levels. Brains were immediately removed for cortex, striatum and *substantia nigra* dissection according to Paxinos and Watson [40].

2.3.2.1 Plasma Mg levels

Mg plasma levels determination was assessed by a commercial kit (Labtest®) [13,14]

2.3.2.2 Ca^{2+}-ATPase activity

The Ca^{2+}-ATPase activity was measured as previously described by Rohn et al., 1993 [41] with minor modifications [42]. The enzymatic activity was expressed as nmol Pi/min/mg protein.

2.3.2.3 Protein determination

Protein concentration was measured according to Bradford (1976) [43].

2.3.2.4 Immunobloting

Molecular analyses in the cortex, striatum and *substantia nigra* were performed by western blot, as previously described [44]. Membranes were rinsed in buffer (0.05% Tween-20 in TBS) and then incubated with primary antibodies: anti-β actin (1:500, Santa Cruz Biotechnology), anti-NMDAR (1:3000, Santa Cruz Biotechnology), anti-D1R (1:700; Santa Cruz Biotechnology) and anti-D2 (1:700, Santa Cruz Biotechnology), followed by anti-rabbit (1:40.000, Santa Cruz Biotechnology,) or anti-goat IgG horseradish peroxidase conjugate (1:20.000, Santa Cruz Biotechnology). After being rinsed with buffer, immune complexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., NJ, USA), and band intensities were quantified with ImageJ software (NIH). Actin was used as an internal control for Western blot, and such that data were standardized according to actin values.

2.4 Statistical analysis

Student's t-test was applied to compare control and haloperidol (C and HAL groups). One-way ANOVA was used to compare all haloperidol-treated groups (HAL, Mg and NIF groups), followed by Newman-Keuls multiple range test when appropriate (software Statistica10 for Windows was used). Values of $p<0.05$ were considered as statistically significant for all comparisons. Graphics were made using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA.

3 Results

3.1 Influence of magnesium and nifedipine treatment on haloperidol-induced orofacial dyskinesia development (Figure 2)

Student's t-test showed that sub-chronic HAL increased VCM frequency, as shown in Figure 2, in comparison to the control group from day 7 to 28 after the first HAL administration (*p<0.0001*). Newman-Keuls test showed that Mg (*p<0.05*) and NIF (*p<0.01*) (Fig. 2A, 2C) on day 14, and Mg (*p<0.005*) and NIF (*p<0.005*) on day 28

(Figure 2B, 2C) significantly reduced the VCM frequency in relation to HAL group. Student's t-test showed that HAL administration increased the average VCM number of all assessments in relation to the control group. *Post hoc* test revealed a significant reduction of average VCM in Mg (*p<0.024*) and NIF (*p<0.004*) treated animals (Figure 2D).

3.2 Magnesium plasma levels determination (Figure 3)

Newman-Keuls test showed that Mg supplementation increased the plasma level of this bivalent cation in comparison to other experimental groups (HAL and NIF, p<0.001, for both), while an increasing trend was observed in the HAL group (Fig. 3).

3.3 Influence of magnesium and nifedipine treatment on the Ca^{2+}-ATPase activity in the cortex, striatum, and *substantia nigra* (Figure 4)

In the cortex, Student t-test showed that HAL exerted no influence on the Ca^{2+}-ATPase activity. Newman-Keuls test showed that both Mg (*p*<0.001) and NIF (*p*<0.001) decreased the activity of this enzyme in comparison to HAL group (Fig. 4A). In the striatum, while HAL increased the Ca^{2+}-ATPase activity *per se*, only the Mg was able to reverse this increasing (*p<0.01*), and its activity (*p*<0.001) was lower than that observed in the NIF-treated group (Fig. 4B). Inversely to what was observed in the striatum, HAL decreased the Ca^{2+}-ATPase activity in the *substantia nigra* (*p<0.0005*), which was not reversed by any of the treatments. NIF further decreased Ca^{2+}-ATPase activity, thus intensifying the HAL influence (Fig. 4C).

3.4 Influence of magnesium and nifedipine treatment on dopaminergic markers and NMDAR immunoreactivity in the cortex, striatum, and *substantia nigra* (Figure 5-7)

In the cortex, HAL reduced *per se* D1R (*p<0.002*) and D2R (*p<0.001*), thus increasing NMDAR (*p<0.001*) immunofluorescence in relation to the control group (Fig. 5A-C). Newman-Keuls test showed that Mg and NIF treatments reversed D1R (*p*<0.001, for both) and NMDAR (*p*<0.05, for both) changes haloperidol-induced (Fig. 5A, 5C), but they were ineffective to reverse D2R (Fig. 5B).

In the striatum, HAL increased *per se* NMDAR (*p<0.009*) (Fig. 6C), thus decreasing D2R immunoreactivity (*p<0.0004*) (Fig. 6B), without changing D1R (Fig. 6A). Mg and NIF decreased *per se* D1R ($p<0.01$ for both, Fig.6A), and also intensified the HAL-induced D2R decrease (Fig. 6B) ($p<0.001$ and $p<0.01$, respectively). Also, only Mg reversed the increase of the HAL-induced NMDAR (Fig. 6C), which level was smaller than that observed in the NIF group ($p<0.05$).

In the *substantia nigra,* HAL increased *per se* the immunoreactivity of all quantified molecular markers: D1R (*p<0.02*), D2R (*p<0.003*) and NMDAR (*p<0.004*), when compared to the control group (Fig. 7A-C). While only the Mg treatment reversed the increase in the HAL-induced D1R levels ($p<0.05$, Fig.7A), only the NIF treatment reversed the increase in the HAL-induced NMDAR ($p<0.05$, Fig.7C). Besides, Mg and NIF reversed the HAL influence on D2R ($p<0.001$ and $p<0.01$, respectively, Fig. 7B).

4 Discussion

Our research group has shown magnesium (Mg) beneficial influences when it was supplemented to animals submitted to both reserpine- and haloperidol-induced orofacial dyskinesia (OD). Considering the postulated hypotheses, the main one was that magnesium could act as a calcium ion antagonist by directly blocking calcium channels as well as NMDA receptors. The NMDA receptors activation is related to progressive pathological processes, including oxidative stress and excitotoxicity, being directly related to OD development [13]. In this sense, our aim was to comparatively evaluate the magnesium supplementation with an L-type calcium channel blocker (nifedipine-NIF) to determine if the Mg acts through this hypothesis.

Our findings showed favorable Mg plasma levels, and the quantification was carried out to monitor its biological effects. As expected, our current findings showed that HAL-injected animals presented increased VCM, the Mg in minor proportion and the NIF prevented the OD development. In addition, HAL modified both dopaminergic and glutamatergic molecular markers in the cortico-nigro-striatal brain area, controlled by the Mg and NIF treatment in a different way.

Moreover, the HAL showed no influence on the Ca^{2+}-ATPase activity in the cortex whereas decreased this enzyme activity in the *substantia nigra* (SN), and

inversely increased its activity in the striatum, which receives dopaminergic enervations from SN. This finding is in agreement with a previous study that showed an inhibitory influence of the HAL on the Ca^{2+}-ATPase activity in rabbits sarcoplasmic membrane fragments, suggesting that HAL interacts with the catalytic site of the Ca^{2+}-ATPase (TAKARA; ALONSO, 1996). Regarding this, we believe that the reduced activity of the Ca^{2+}-ATPase observed in the SN is due to its dopaminergic constitution, which concentrates neuronal bodies, being more susceptible to the haloperidol deleterious effects [45,46]. Extrapyramidal side effects like tardive dyskinesia, parkinsonism, and even Parkinson's disease have been related to neuronal death consequent to damages in the dopaminergic neurons, especially in the SN. Thus, the increased Ca^{2+}-ATPase activity in the striatum consequent to the chronic HAL administration could be a compensatory mechanism due to the excitotoxic events related to increased NMDAR levels, as observed in this study, and its activation may be linked to the increased calcium entry, generating oxidative events.

Excitotoxicity and oxidative stress have been linked to typical antipsychotic-induced extrapyramidal adverse effects, manifested by involuntary movement disorders, often disabling ones [47–54]. The findings reported are in agreement with these observations, due to the increased HAL-induced NMDAR immunoreactivity in all brain areas assessed in this study. The haloperidol-induced blockade of post-synaptic D2R hinders the inhibitory self-control of the DA, resulting in increased glutamatergic neurotransmission, which can generate neurotoxicity in the nigrostriatal area of the motor control. Both neurotoxicity and oxidative damages are a consequence of an excessive calcium influx, responsible for activating several pathological processes [55,56]. Of particular importance, our outcomes showed a more significant beneficial reduction of the Mg in the NMDAR levels, since its protective action was observed in the cortex and striatum. These brain areas are closely related to HAL-induced extrapyramidal effects development, as observed by animals increased VCM frequency.

Mg has played a significant role in the NMDAR, considering Mg antagonism and its physiologic deficiency have been related to CNS disorders, including movement disorders [57–59]. Regarding this, the current findings are in agreement with previous studies from our laboratory, leading us to propose a mechanistic

hypothesis for the beneficial influence of Mg supplementation [13,14]. NMDA receptors can trigger the opening of L-type Ca^{2+} channels that open during strong depolarization [60–62] and thus potentiate the calcium influx in cells. Our study shows that NIF administration was able to prevent the increase of NMDAR in both cortex and SN. Several studies have already demonstrated the neuroprotective role of LTCC blockers [34,63,64], including their protective role on the extrapyramidal adverse effects induced by haloperidol in animal models of OD [37,38].

Besides antagonizing NMDA receptors, magnesium can compete with calcium in voltage-dependent channels acting as an antagonist *per se* [65–67]. This allows inferring that NIF and Mg may be acting by a similar mechanism, having the Mg a discrete advantage. Haloperidol-induced chronic dopamine D2 antagonism results in hypersensitivity of the indirect basal ganglia output pathway. The overactivity of glutamatergic projections from the striatum to the *susbstantia nigra pars compacta* (SNc) is thought to cause excitotoxic damages on dopaminergic neurons of SNc. Also, increased activity of glutamatergic projections from the striatum to *substantia nigra pars reticulata* might increase the inhibition of SNc. Both changes would reduce DA outflow from the SNc to caudate-putamen, which further increases the activity of the indirect pathway [52]. In the current study, HAL decreased D2R in the striatum, increasing it in the SN, clearly indicating a protective adaptation, since, in this last brain area, D2R exerts a negative feedback function on the generation and DA release [68].

Furthermore, the striatum is where classical antipsychotics, such as HAL, can exert an important blockage because it is a pivotal brain area involved in the motor control and has high D2R density. This imbalance is related to involuntary movements development induced by haloperidol but is unlikely the sole explanation [69–71]. Interestingly, both Mg and NIF reversed the effects of HAL on the D2R levels only in the SN, thus intensifying the decrease of D2R levels in the striatum. Moreover, HAL increased D1R in SN, exerting no effects in the striatum. Mg was able to protect the increased D1R in SN, and both Mg and NIF decreased it in the striatum. A recent hypothesis to explain the pathophysiology of the TD is related to D2 hypersensitivity and neurodegeneration due to oxidative events, leading to maladaptive synaptic plasticity and imbalance between direct and indirect pathway in the basal ganglia. Taken together, these events in cascade result in secondary

effects affecting GABAergic, glutamatergic and cholinergic circuits [69,70,72,73]. In this scenario, our current outcomes are in accordance with our previous studies, when Mg supplementation was able to revert and prevent reserpine- [14] and HAL-induced [13] movement disorders, closely related to oxidative damages. In fact, in our current findings, Mg supplementation showed to be effective on the NMDAR as well, which appears to be its central mechanism of action, since this bivalent cation had shown a critical role in other studies [74,75]. Comparatively, NIF also shows a neuroprotective role by protecting from oxidative stress, resulted from calcium overload or neuroinflammatory events [63,76,77].

From the presented outcomes, we are proposing a molecular mechanism of action to explain the protective effects of Mg supplementation over haloperidol-induced movement disorders such as TD. To the best of our knowledge, we are proposing for the first time a molecular mechanism to try to explain the Mg beneficial effects on movement disorders, especially, haloperidol-induced orofacial dyskinesia when compared to nifedipine. Nifedipine is clinically used for cardiovascular purposes and has been prescribed for the treatment of antipsychotic-related motor disturbances [36]. Mg supplementation shows remarkable actions over nifedipine itself.

5 Conclusion

In summary, we propose that like nifedipine, magnesium exerts its protective activity on movement disorders induced by haloperidol through the blockade of voltage-dependent calcium channels and the blockade of glutamatergic NMDA receptors. This study contributes to a better understanding of magnesium supplementation concerning movement disorders induced by antipsychotics. This work also contributes to new researches aiming at comprehending and determining the pathophysiology of this syndrome what can bring a positive impact on the patients' quality of life.

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Statement of Interest

Authors declare that there was no conflict of interest.

Figure captions

Figure 1. Experimental design. After the basal assessment, animals were divided into 4 groups: control, haloperidol (HAL), magnesium + haloperidol (Mg), nifedipine + haloperidol (NIF). HAL (12mg/kg/mL, i.m.) or vehicle, which was immediately administered after basal assessment and immediately after each following behavioral assessment on days 7, 14, 21 and 28. Concomitantly, animals received magnesium aspartate (40mg/kg/mL), NIF (10mg/Kg/mL) or vehicle by gavage during all protocol.

Figure 2. Influence of supplementation, after basal assessment (**A**), of magnesium (40mg/Kg/mL) and NIF treatment (10mg/Kg/mL) on the prevention of OD induced by HAL administration (12mg/Kg/mL, i.m. once a week for four weeks) (**B-D**). Influence of Mg and NIF on average VCM number of all assessment days (**E**). Data are expressed as mean ± S.E.M ($p<0.05$). Abbreviations: VCM: vacuous chewing movements. Mg: magnesium; NIF: nifedipine; *indicates significant differences from the control group. # indicates significant difference from the haloperidol vehicle group. Different lowercases indicate significant difference among treatments in haloperidol administered rats.

Figure 3. Influence of Mg (40mg/Kg/mL) and NIF (10mg/Kg/mL) on the plasma magnesium concentration. Data are expressed as mean ± S.E.M ($p<0.05$). Abbreviations: Mg: magnesium; NIF: nifedipine; *indicates significant differences from the control group. # indicates significant difference from the haloperidol vehicle

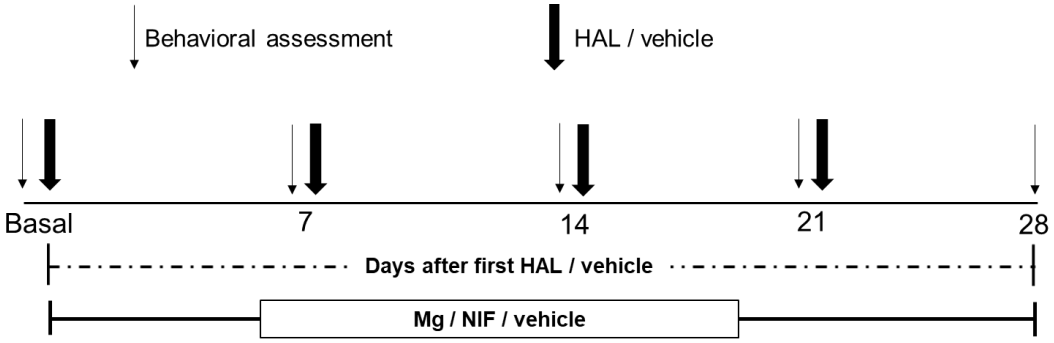
group. Different lowercases indicate significant difference among supplementation in haloperidol administered rats.

Figure 4. Influence of Mg (40mg/Kg/mL) and NIF (10mg/Kg/mL) on the Ca^{2+}-ATPase activity in cortex (**A**), striatum (**B**) and *substantia nigra* (**C**). Data are expressed as mean ± S.E.M (*p<0.05*). Abbreviations: Mg: magnesium; NIF: nifedipine; *indicates significant differences from the control group. # indicates significant difference from the haloperidol vehicle group. Different lowercases indicate significant difference among the treatments in haloperidol administered rats.

Figure 5. Influence of Mg (40mg/Kg/mL) and NIF (10mg/Kg/mL) on the D1R **(A)**, D2R **(B)** and NMDAR (**C**) immunoreactivity in the cortex. Data are expressed as mean ± S.E.M (*p<0.05*). Abbreviations: Mg: magnesium; NIF: nifedipine; *indicates significant differences from the control group. # indicates significant difference from the haloperidol vehicle group. Different lowercases indicate significant difference among the treatments in haloperidol administered rats.

Figure 6. Influence of Mg (40mg/Kg/mL) and NIF (10mg/Kg/mL) on the D1R **(A)**, D2R **(B)** and NMDAR (**C**) immunoreactivity in the striatum. Data are expressed as mean ± S.E.M (*p<0.05*). Abbreviations: Mg: magnesium; NIF: nifedipine; *indicates significant differences from the control group. # indicates significant difference from the haloperidol vehicle group. Different lowercases indicate significant difference among the treatments in haloperidol administered rats.

Figure 7. Influence of Mg (40mg/Kg/mL) and NIF (10mg/Kg/mL) on the D1R **(A)**, D2R **(B)** and NMDAR (**C**) immunoreactivity in the *substantia nigra*. Data are expressed as mean ± S.E.M (*p<0.05*). Abbreviations: Mg: magnesium; NIF: nifedipine; *indicates significant differences from the control group. # indicates significant difference from the haloperidol vehicle group. Different lowercases indicate significant difference among the treatments in haloperidol administered rats.



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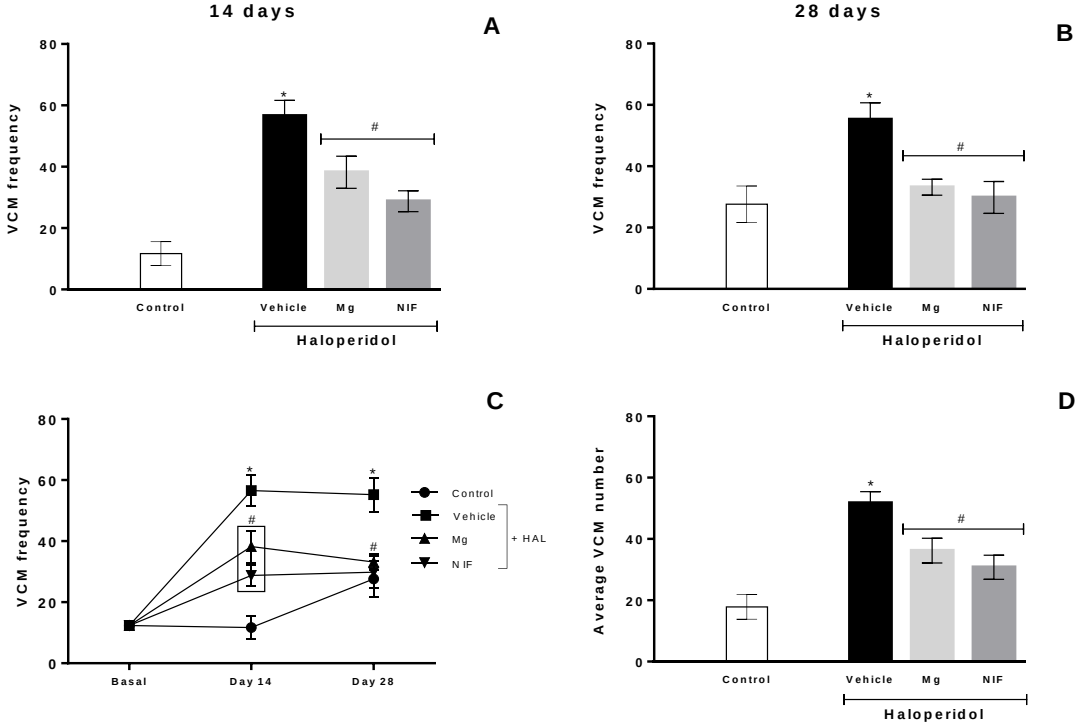

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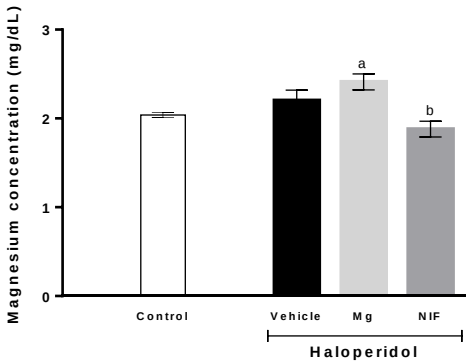

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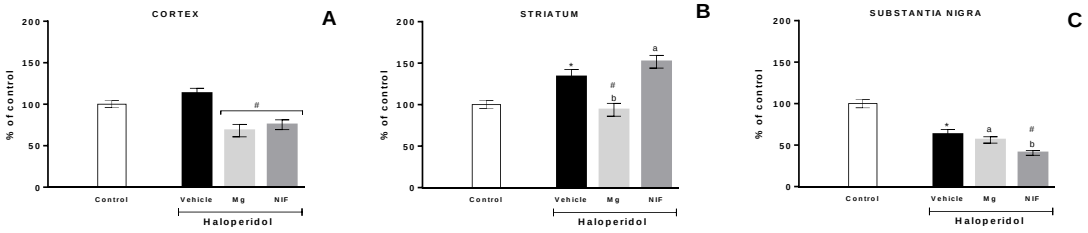

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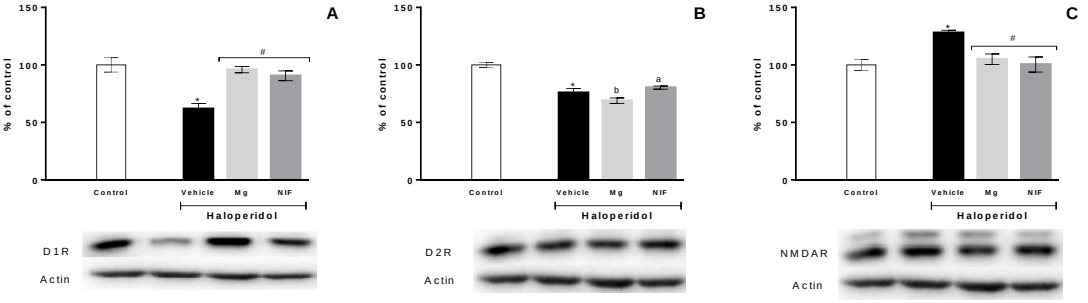

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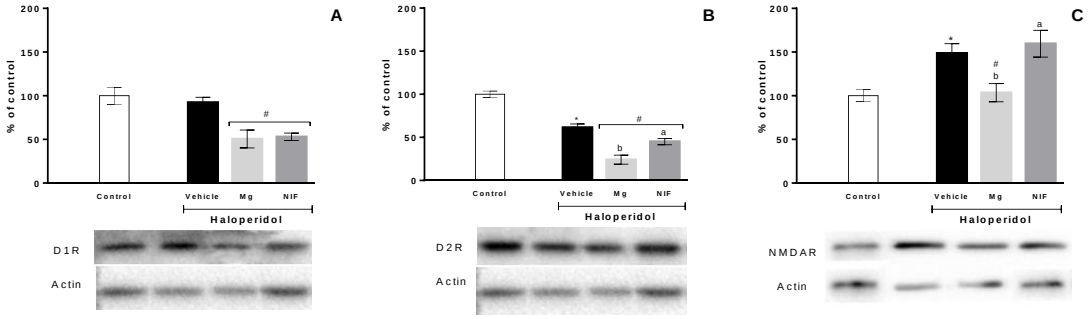

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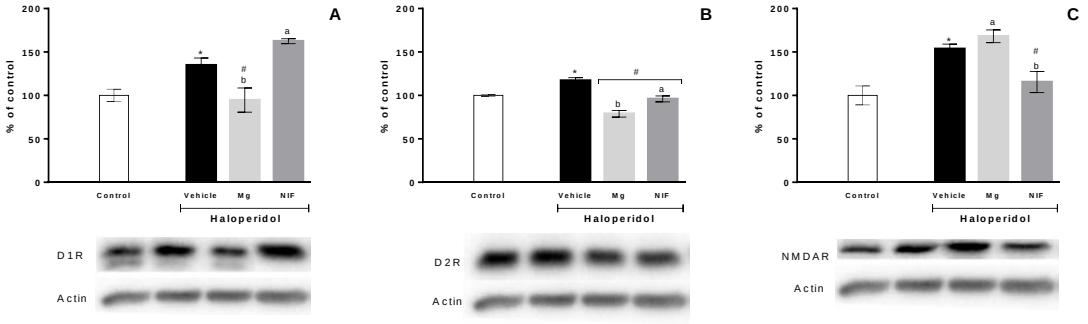

Figure 7.

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7 DISCUSSÃO

Modelos animais envolvendo distúrbios do movimento utilizados para mimetizar efeitos adversos relacionados ao uso crônico de antipsicóticos típicos como o haloperidol, são amplamente empregados afim de melhor compreender a fisiopatologia destes distúrbios, a qual ainda é pouco compreendida. A hipótese mais relacionada está no desenvolvimento de excitotoxicidade e estresse oxidativo, resultado da geração de espécies reativas, as quais consequentemente culminam em processos oxidativos e apoptose. Substâncias com propriedades antioxidantes, capazes de reduzir os danos oxidativos associados ao uso de antipsicóticos, têm sido empregados no intuito de prevenir ou reduzir os efeitos colaterais extrapiramidais (BARCELOS et al., 2011; BUSANELLO et al., 2012; MACÊDO et al., 2011; PEROZA et al., 2013; TEIXEIRA et al., 2012; THAKUR et al., 2015).

Nesse sentido, em estudo prévio do nosso grupo de pesquisas mostrou a influência da suplementação de magnésio frente ao desenvolvimento de distúrbios do movimento em um modelo animal de DO induzido por reserpina (KRONBAUER et al., 2015). Neste estudo foi possível observar o efeito benéfico do Mg, o qual exerceu resposta preventiva e de reversão sobre tais distúrbios, os quais estão intimamente relacionados ao desenvolvimento de danos oxidativos no sistema dopaminérgico nigro-estriatal. De fato, o fator que nos impulsionou a realizar este experimento, partiu de alguns relatos de casos comunicados ao nosso grupo de pesquisa, quando um médico austríaco relatou inúmeras melhoras clínicas de transtornos do movimento revelados pelos seus pacientes, os quais estavam sob tratamento com haloperidol e outros antipsicóticos, cuja suplementação de Mg foi incluída em seus protocolos de tratamento (Daniele Couturier, comunicação pessoal, 2013).

É bem sabido sobre a importância da suplementação de Mg em várias patologias, desde metabólicas, cardiovasculares e neurológicas (CAMARDESE et al., 2012; HOUSTON, 2011; VORMANN, 2003). As ações benéficas do Mg sobre processos oxidativos, observadas em nosso primeiro estudo, também foram observadas previamente em outros estudos (BLACHE et al., 2006; BUHA et al., 2012; OLATUNJI; SOLADOYE, 2007; REGAN et al., 2002; REGAN; GUO, 2001). Além disso, uma carência nutricional deste cátion está associada ao desenvolvimento de patologias inclusive relacionadas ao controle do movimento (OYANAGI; HASHIMOTO, 2011; TANIGUCHI et al., 2013).

Sendo assim, dando seguimento ao nosso estudo, avaliamos o papel da suplementação de Mg em três protocolos experimentais de DO induzidos por HAL: suplementação de Mg antes, após e concomitante à administração do antipsicótico, avaliando também parâmetros de

estresse oxidativo em áreas cerebrais relacionadas ao controle motor. Nossos resultados mostraram que a suplementação de Mg antes da administração de HAL foi suficiente para atenuar os distúrbios do movimento, observados pela menor frequência dos MMV e menor tempo de catalepsia. Acompanhado a isso, observou-se a prevenção da geração de ER nas regiões do córtex e SN e a oxidação de proteínas, no córtex, estriado e SN, regiões envolvidas no controle motor. As concentrações plasmáticas de Mg foram aumentadas com a administração de HAL. A suplementação após a administração de HAL minimizou a consequente elevação da frequência dos MMV e o tempo de catalepsia, reduziu a geração de ER no córtex e estriado, como também os níveis de carbonilação proteica no estriado e na SN, os quais foram aumentados pela administração de HAL. As concentrações plasmáticas deste de Mg mantiveram-se inalteradas com a administração de HAL. Concomitantemente, a suplementação de Mg preveniu o aumento dos MMV e catalepsia induzidos pelo HAL, prevenir a geração de ER nas regiões do córtex, estriado e SN e a oxidação de proteínas na SN. A suplementação *per se* não alterou as concentrações plasmáticas de Mg, a qual foi reduzida pela administração de HAL. Tomados em conjunto, os resultados observados neste estudo mostraram que a administração de HAL induziu DO o que corrobora com estudos anteriores e mostra a viabilidade do protocolo. Além disso, a suplementação de Mg mostrou-se mais uma vez benéfica frente ao desenvolvimento de DO, desta vez induzida por HAL. Da mesma maneira, estes efeitos foram novamente relacionados aos efeitos antioxidantes nas áreas cerebrais responsáveis pelo controle motor.

Fisiologicamente, o Mg desempenha importante papel em diversos processos celulares. Além de ser importante cofator enzimático, o Mg é considerado um antagonista natural de canais de cálcio (ISERI; FRENCH, 1984) bem como de receptores NMDA (CLARKE; GLASGOW; JOHNSON, 2013), também permeáveis à este cátion. Levando isso em consideração, frente aos resultados encontrados no estudo, nós podemos supor alguns mecanismos que poderiam estar envolvidos nos efeitos observados do Mg frente ao desenvolvimento de DO: 1) Ao inibir a entrada de Ca^{2+} e a liberação de acetilcolina, reduzir a hiperfunção colinérgica consequente do bloqueio do circuito inibitório dopamina-acetilcolina no estriado, resultado do bloqueio dos receptores dopaminérgico D2 neurotransmissores; 2) A suplementação de Mg provavelmente pode, ao bloquear receptores NMDA, reduzir a excitotoxicidade glutamatérgica gerada pelo bloqueio de receptores dopaminérgicos D2 estriatais, impedindo o influxo exacerbado de Ca^{2+}, promotor do aumento de liberação de neurotransmissores e desencadeador de processos oxidativo/apoptóticos; 3) A suplementação de Mg provavelmente pode inibir a entrada de Ca^{2+} em neurônios dopaminérgicos da SN, e

impedir a liberação exacerbada de dopamina consequente do bloqueio de receptores dopaminérgicos pré-sinápticos.

Frente a estas hipóteses, observando um elo comum envolvendo Ca^{2+}, propomos avaliar um possível mecanismo de ação do Mg ao compararmos com a utilização concomitante de NIF, um bloqueador de canais de cálcio, em modelo animal de DO induzido por HAL. Os parâmetros moleculares analisados foram a atividade da Ca^{2+}ATPase, receptores da via dopaminérgica D1 e D2 e receptor glutamatérgico NMDA.

Como observado em estudos anteriores (KRONBAUER et al., 2015, 2017), a suplementação de Mg preveniu o aumento dos MMV induzidos pelo HAL. De maneira levemente superior quando comparado ao Mg, a NIF também demonstrou efeito benéfico prevenindo o aumento de MMV. O uso de bloqueadores de cálcio como a NIF já demonstrou efeitos positivos em modelo animal de DO induzida por HAL (ABDEL-RAHEEM, 2010; BISHNOI; CHOPRA; KULKARNI, 2008) e o possível mecanismo relacionado foi de que o bloqueio dos canais de cálcio poderia amenizar os efeitos deletérios deste cátion em processos oxidativos, os quais estão intimamente relacionados a etiologia dos distúrbios do movimentos desenvolvidos pelo uso de antipsicóticos. O envolvimento de concentrações intracelulares elevadas de cálcio no desenvolvimento de patologias é bastante estudado e a etiologia de várias desordens neurológicas também está relacionada (BOJARSKI; DEBOWSKA; WOJDA, 2010; CASSANO T, PACE L, BEDSE G, LAVECCHIA AM, DE MARCO F, GAETANI S, 2016; MARAMBAUD; DRESES-WERRINGLOER; VINGTDEUX, 2009; PIVOVAROVA et al., 2004)

Um mecanismo fisiológico de controle dos níveis de cálcio intracelular é a Ca^{2+}ATPase, a qual é capaz de equilibrar concentrações intra e extracelulares, sendo importante em processos patológicos (BRUCE, 2018). Neste estudo observamos que o HAL apresentou diferentes influencias sobre a atividade da Ca^{2+}ATPase. A SN por sofrer mais os efeitos deletérios do HAL pode explicar a redução da atividade da Ca^{2+}ATPase e consequentemente, um efeito compensatório aumentaria a atividade na região do estriado, em resposta a eventos excitotóxicos relacionados ao aumento da entrada de cálcio nos neurônios. Em relação aos resultados observados nos tratamentos com Mg e NIF, pouco pode ser suposto. O Mg mostrou ser um cofator importante nos estados de transição ativos e inativos da enzima Ca^{2+}ATPase (ECHARTE; ROSSI; ROSSI, 2007; PICARD et al., 2007) e bloqueadores de canais de cálcio, incluindo NIF, também demonstraram modular sua atividade (CHURCH et al., 1994; KOH et al., 1987).

A administração de HAL aumentou a imunorreatividade dos recepetores de NMDA em todas as áreas cerebrais analisadas neste estudo. Este resultado concorda com a hipótese de que as desordens motoras relacionadas ao uso de antipsicóticos estão associadas ao estresse oxidativo gerado por excitotoxicidade, consequente do aumento da neurotransmissão glutamatérgica a qual também é exacerbada pelo bloqueio dos receptores D2, que são inibitórios (ZHURAVLIOVA et al., 2007). A suplementação de Mg mostrou resultados benéficos ao reduzir o NMDA nas regiões do córtex e estriado. Conhecido por antagonizar receptores NMDA, foi de grande importância o resultado benéfico do Mg observado na redução significativa destes receptores nas regiões do córtex e estriado. Somado ao aumento de influxo de cálcio gerado pelo aumento da neurotransmissão glutamatérgica, canais de cálcio do tipo L podem ser ativados após despolarização aumentada potencializando o influxo deste cátion e eventos oxidativos (KIMURA; KATAYAMA; NISHIZAWA, 1999; RAJADHYAKSHA et al., 1999; TSIEN et al., 1988). Nosso estudo demonstrou que NIF pode ser capaz de prevenir o aumento de receptores NMDA nas regiões do córtex e SN, efeito este que pode estar associado ao bloqueio direto de canais de cálcio. Os bloqueadores de canais de cálcio tipo L tem conhecida atividade neuroprotetora em modelo *in vitro* de doença de Alzheimer (ANEKONDA et al., 2011) e modelo animal de doença de Parkinson (SINGH et al., 2016), bem como modelo de DO induzida por haloperidol (ABDEL-RAHEEM, 2010; BISHNOI; CHOPRA; KULKARNI, 2008). Somados as ações antagonistas de receptores NMDA e de canais de cálcio já conhecidas e os resultados encontrados neste estudo, podemos inferir que Mg e NIF possivelmente podem estar atuando de maneira semelhante ou compartilhando de uma mesma rota de ações, observando uma discreta vantagem do Mg. Isto nos abre novos questionamentos para futuros afim de melhor compreender e elucidar de que maneira o Mg exerce tais efeitos benéficos.

Neste estudo observamos que a administração de HAL diminuiu os receptores D2 no estriado e aumentou na SN. Considerando que a SN é uma área cerebral onde os receptores D2 exercem uma função de *feedback* negativo na liberação de DA (ANZALONE et al., 2012), pode-se observar claramente uma adaptação protetora frente aos efeitos do HAL. A região do estriado possui alta densidade de receptores D2, e o bloqueio pelo HAL pode ser bastante expressivo, já que esta área cerebral também é importante para o controle motor. Tanto Mg quanto NIF reverteram os efeitos do HAL nos receptores D2 somente na SN, intensificando a diminuição dos níveis de D2R no estriado. Além disso, o HAL aumentou os receptores D1 na SN, não exercendo efeitos no estriado. O Mg preveniu o aumento dos receptores D1 na SN, e ambos Mg e NIF diminuíram no estriado. Uma hipótese recente para explicar a patofisiologia

da TD está relacionada com a hipersensibilidade D2 e neurodegeneração devido a eventos oxidativos, levando à desadaptação da plasticidade e desequilíbrio entre a via direta e indireta nos gânglios basais. Em conjunto, esses eventos em cascata resultam em efeitos secundários que afetam os circuitos GABAérgicos, glutamatérgicos e colinérgicos (BORDIA et al., 2016; EROSA-RIVERO et al., 2014; GITTIS; KREITZER, 2012; TEO; EDWARDS; BHATIA, 2012). Sendo assim pouco pode-se aferir a respeito destes resultados observados apenas nas vias dopaminérgicas sem consideram a vasta gama de circuitos nervosos que estão relacionados com o controle motor nos gânglios basais.

Tomados em conjunto, os resultados observados nos mostram que a suplementação Mg é capaz de prevenir e reverter os distúrbios extrapiramidais em modelos animais de DO induzidos por HAL, concordando com estudo anterior onde pode-se observar estes efeitos em modelo animal induzido por reserpina. Como primeiros resultados, podemos constatar que o Mg exerceu uma ação antioxidante, modulando parâmetros de estresse oxidativos analisados em áreas cerebrais responsáveis pelo controle motor. Isto corrobora com a principal hipótese relacionada ao desenvolvimento de distúrbios do movimento induzidos por antipsicóticos típicos, como o HAL, a qual envolvem excitotoxidade e danos oxidativos que culminam com morte neuronal. Considerando que muitos eventos oxidativos envolvem cálcio como fator determinante, e que os efeitos fisiológicos do Mg estão intimamente ligados a estes processos, como bloqueio fisiológico de canais de cálcio e antagonismo de receptores NMDA, os quais são permeáveis ao cálcio, os resultados aqui apresentados, ao compararmos a suplementação de Mg com um bloqueador especifico de canais de cálcio do tipo L, mostram semelhança de ação, em alguns casos com melhores proporções para o Mg. Até o momento o conjunto de resultados observados neste estudo nos direciona mais profundamente na busca pela elucidação dos efeitos benéficos relacionado à suplementação de Mg frente a distúrbios do movimento induzidos por antipsicóticos típicos.

A DT ainda é considera um grave agravante no tratamento de pessoas com esquizofrenia. Neste sentido, cabe a importância de prover alternativas terapêuticas que possam minimizar esse desconforto e proporcionar uma melhor qualidade de vida. A suplementação com Mg pode ser uma ótima alternativa, visto que é de fácil acesso e de baixo custo monetário.

8 CONCLUSÕES

A partir do presente estudo pode-se propor as seguintes conclusões:

1. A suplementação de Mg antes, concomitante e após a administração de haloperidol foi suficiente para prevenir e reduzir os distúrbios do movimento, como observado através da menor frequência dos MMV e menor tempo de catalepsia;
2. A suplementação de Mg antes da administração de haloperidol preveniu a geração de ER nas regiões do córtex e *substantia nigra*, reduzindo também a oxidação de proteínas no córtex, estriado e *substantia nigra*, as quais constituem áreas envolvidas no controle do movimento;
3. A suplementação de Mg antes da administração de haloperidol aumentou a concentração plasmática deste íon, indicando que o antipsicótico não interfere em sua absorção a partir de alimentos ou suplementação;
4. A suplementação de Mg após administração de haloperidol reduziu a geração de ER no córtex e estriado, como também os níveis de proteínas carboniladas no estriado e *substantia nigra*, os quais foram aumentados pela administração de haloperidol;
5. A suplementação de Mg concomitante a administração de haloperidol preveniu a geração de ER nas regiões do córtex, estriado e *substantia nigra* e a oxidação de proteínas na *substantia nigra*;
6. A suplementação de Mg e o tratamento com nifedipina, concomitante à administração de haloperidol foram suficientes para prevenir o desenvolvimento dos distúrbios do movimento, como observado através da menor frequência dos MMV;
7. A suplementação de Mg, comparado ao tratamento com nifedipina, mostrou resposta semelhantes e até superior, sobre a prevenção dos distúrbios do movimento e sobre modificações moleculares induzidos pelo haloperidol, caracterizando uma rota de ação comum, permitindo novas abordagens em busca de um mecanismo de ação relacionado a suplementação de Mg frente ao desenvolvimento de disutrbios extrapiramidais induzidos por antipsicóticos.

9 PERSPECTIVAS

Novos estudos relacionados ao benefício da suplementação de Mg sobre o desenvolvimento de distúrbios do movimento estão em fase de desenvolvimento, visando avaliar:

- Investigar a concentração de Mg em regiões cerebrais relacionadas ao modelo de DO;
- Investigar a influência da suplementação de Mg nos déficits cognitivos induzidos por haloperidol;
- Investigar a influência da suplementação de Mg nos níveis dos neurotransmissores dopamina, acetilcolina e glutamato em tecido cerebral por Cromatografia Líquida de Alta Eficiência;
- Determinar concentrações teciduais de Ca^{2+} em regiões relacionadas ao modelo de DO;

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ANEXO A

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que o Projeto intitulado "Papel do magnésio na prevenção e reversão de distúrbios motores e cognitivos induzidos experimentalmente em ratos.", protocolado sob o CEUA nº 1235290115, sob a responsabilidade de **Marilise Escobar Bürger** *e equipe; Maikel Kronbauer; Higor Zuquetto Rosa; Vinicia Garzella Metz* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei 11.794, de 8 de outubro de 2008, com o Decreto 6.899, de 15 de julho de 2009, com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovado** pela Comissão de Ética no Uso de Animais Universidade Federal de Santa Maria (CEUA/UFSM) em reunião de 05/05/2015.

We certify that the proposal "Role of magnesium in the prevention and reversal of motor and cognitive disorders experimentally induced in rats.", utilizing 160 Heterogenics rats (160 males), protocol number CEUA 1235290115, under the responsibility of **Marilise Escobar Bürger** *and team; Maikel Kronbauer; Higor Zuquetto Rosa; Vinicia Garzella Metz* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes (or teaching) - it's in accordance with Law 11.794, of October 8 2008, Decree 6899, of July 15, 2009, with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 05/05/2015.

Vigência da Proposta: de 07/2015 a 12/2018 Laboratório: Programa De Pós-graduação Em Farmacologia

Procedência:	Biotério Central UFSM				
Espécie:	Ratos heterogênicos	Gênero: Machos	idade:	2-3 meses	N: 160
Linhagem:	Wistar		Peso:	250-300g	

Nota: O uso de antipsicóticos típicos está associado ao desenvolvimento de problemas de memória e cognição, assim como ao desenvolvimento de desordens motoras como tremores, acatisia e discinesia tardia (DT). O Haloperidol é um antipsicótico amplamente empregado no controle das doenças mentais e também associado aos efeitos adversos característicos desse grupo terapêutico. Modelos animais de discinesia orofacial têm sido empregados em busca de prevenção e/ou reversão dos distúrbios do movimento decorrentes do tratamento antipsicótico. Neste sentido, sabe-se que o magnésio é o segundo cátion intracelular mais abundante e é um importante cofator para mais de 300 enzimas de diversas reações metabólicas responsáveis pela homeostase do organismo. O efeito benéfico da administração de Mg2+ em condições patológicas do cérebro tem sido investigado. Neste estudo, o possível papel de prevenção e/ou reversão do Mg2+ será avaliado em modelo animal de discinesia tardia, discinesia orofacial sub-crônica induzida por haloperidol, bem como a susceptibilidade ao desenvolvimento da discinesia orofacial em animais submetidos previamente a uma dieta restrita de Mg2+.

Santa Maria, 17 de junho de 2015

Profa. Dra. Daniela Bitencourt Rosa Leal
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

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Horário de atendimento: das 8:30 às 11:30 e 14:00 às 16:30hs : e-mail: ceua.ufsm@gmail.com
CEUA Nº 1235290115

ANEXO B

Title: Influence of magnesium supplementation on movement side effects related to typical antipsychotic treatment in rats

Author: Maikel Kronbauer,Vinicia Garzela Metz,Karine Roversi,Veronica Tironi Dias,Caren Tatiane de David Antoniazzi,Raquel Cristine da Silva Barcelos,Marilise E. Burger

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