TIAGO ZAMINELLI

EFEITO TIPO-ANTIDEPRESSIVO E ANTIOXIDANTE DO IBUPROFENO NO MODELO DA DOENÇA DE PARKINSON INDUZIDO POR ADMINISTRAÇÃO DE ROTENONA INTRAPERITONEAL EM RATOS

Curitiba
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Dissertação apresentada ao Programa de Pós Graduação em Farmacologia da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Farmacologia.

Orientadora: Profa. Dra. Maria Aparecida Barbato Frazão Vital

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Curitiba, 12 de Julho de 2013.

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Programa de Pós-Graduação em Farmacologia

1 **ATA DO JULGAMENTO DA 88ª DEFESA DE DISSERTAÇÃO DE MESTRADO**
2 Ao décimo segundo dia do mês de julho do ano de dois mil e treze, às quatorze
3 horas, no Anfiteatro nº 10 do Setor de Ciências Biológicas da Universidade Federal do
4 Paraná, reuniu-se a Comissão Examinadora da Dissertação de Mestrado de autoria do pós-
5 graduando **TIAGO ZAMINELLI**, intitulada "EFEITO TIPO-ANTIDEPRESSIVO E
6 ANTIOXIDANTE DO IBUPROFENO NO MODELO DA DOENÇA DE PARKINSON
7 INDUZIDO POR ADMINISTRAÇÃO DE ROTENONA INTRAPERITONEAL EM
8 RATOS", sob orientação da Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital e composta
9 pelos professores: Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Presidente -
10 Farmacologia - UFPR); Prof.ª Dr.ª Janaína Menezes Zanoveli (Farmacologia – UFPR) e
11 Prof.ª Dr.ª Estefânia Gastaldello Moreira (Ciências Fisiológicas - UEL). A Banca
12 Examinadora iniciou os trabalhos. O candidato teve quarenta e cinco minutos para expor
13 oralmente seu trabalho, sendo em seguida arguido durante trinta minutos por cada um dos
14 membros da Banca e tendo trinta minutos para responder a cada uma das arguições. No
15 final a Comissão Examinadora emitiu o seguinte parecer: APROVADO. De
16 acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, o pós-
17 graduando foi aprovado. Para a publicação, o trabalho deverá sofrer as modificações
18 sugeridas, que serão conferidas por sua orientadora. Nada mais havendo a tratar, a
19 Presidente deu por encerrada a sessão, da qual foi lavrada a presente ata, que será assinada
20 pela Presidente e pelos demais Membros da Banca Examinadora em Curitiba, 12 de julho
21 de 2013.

Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Presidente - Farmacologia - UFPR)

Prof.ª Dr.ª Janaína Menezes Zanoveli (Farmacologia – UFPR)

Prof.ª Dr.ª Estefânia Gastaldello Moreira (Ciências Fisiológicas - UEL)

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ZAMINELLI, Tiago. **Efeito tipo-antidepressivo e antioxidante do ibuprofeno no modelo da doença de Parkinson induzido por administração de rotenona intraperitoneal em ratos**. 2013. 68 páginas. Dissertação (Pós-graduação em Farmacologia) – Universidade Federal do Paraná, Curitiba, 2013.

RESUMO

A doença de Parkinson idiopática (DP) é uma doença neurodegenerativa, que afeta aproximadamente 1% da população mundial acima de 55 anos, manifestando-se através de sinais motores e sintomas não-motores, como a depressão, decorrentes principalmente da neurodegeneração dos neurônios dopaminérgicos na substância negra pars compacta (SNpc). Dentre os possíveis mecanismos envolvidos nesta patologia, destacam-se a disfunção mitocondrial, neuroinflamação e o estresse oxidativo. Em nosso estudo foi avaliado o efeito do antiinflamatório não esteróide (AINE) ibuprofeno, um inibidor não seletivo da enzima ciclooxigenase, sobre o prejuízo motor e comportamento tipo-depressivo induzidos pela administração intraperitoneal de rotenona em ratos. Nossos resultados demonstram que a administração de rotenona (2,5 mg/kg, por 10 dias, i.p.), foi capaz de reduzir a imunoreatividade da enzima tirosina-hidroxilase na SNpc, enquanto que o pós-tratamento com ibuprofeno (15 mg/kg, por 22 dias, p.o.) bloqueou esta redução. Também demonstramos que a rotenona foi capaz de induzir déficit motor (hipolocomoção) e comportamento tipo-depressivo, sendo ambos revertidos pelo pós-tratamento com ibuprofeno. Além destes parâmetros, foi avaliado o estresse oxidativo induzido por esta toxina. A administração de rotenona promoveu depleção nos níveis de GSH na região do hipocampo, e também reduziu a atividade da catalase em ambas as regiões, hipocampo e estriado. O pós-tratamento com ibuprofeno bloqueou a depleção do GSH induzida pela rotenona, bem como, aumentou os níveis basais deste antioxidante na região do estriado. O ibuprofeno também restaurou a atividade da catalase. Assim, acreditamos que o efeito neuroprotetor do ibuprofeno contra a toxicidade induzida pela rotenona em nosso estudo seja decorrente de suas propriedades antioxidante e inibidora da COX.

Palavras-chave: Rotenona. Deonça de Parkinson. Ibuprofeno. Estresse oxidativo.

ZAMINELLI, Tiago. **Antidepressant and antioxidative effect of ibuprofen in the intraperitoneal rotenone model of Parkinson's disease**. 2013. 68 pages. Dissertação (Pós-graduação em Farmacologia) – Universidade Federal do Paraná, Curitiba, 2013.

ABSTRACT

Idiopathic Parkinson's disease (PD) is a neurodegenerative disorder that affects approximately 1% of the population over 55 years of age. The disease manifests itself through motor and nonmotor symptoms induced mainly by the neurodegeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The possible mechanisms involved in this pathology include mitochondrial dysfunction, neuroinflammation, and oxidative stress. The present study evaluated the effects of the nonselective cyclooxygenase inhibitor ibuprofen on motor and depressive-like behavior induced by rotenone in rats. Rotenone (2.5 mg/kg, i.p., for 10 days) decreased tyrosine hydroxylase immunoreactivity in the SNpc, and ibuprofen treatment (15 mg/kg, p.o., for 22 days) blocked this impairment. We also found that rotenone induced motor deficits (hypolocomotion) and depressive-like behavior, and ibuprofen was able to reverse these deficits. In addition to motor and nonmotor behavior, we evaluated oxidative stress induced by rotenone. Rotenone administration depleted glutathione levels in the hippocampus and reduced catalase activity in both the hippocampus and striatum. Posttreatment with ibuprofen blocked the depletion of glutathione induced by rotenone and increased the basal levels of this antioxidant in the striatum. Ibuprofen also restored catalase activity. The neuroprotective effects of ibuprofen against toxicity induced by rotenone appear to be attributable to its antioxidant properties, in addition to cyclooxygenase inhibition.

Keywords: Rotenone, Parkinson's disease; Ibuprofen; Oxidative stress.

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LISTA DE ABREVIATURAS

6-OHDA	6- hidroxidopamina
AAS	Ácido acetilsalicílico
AINE	Antiinflamatório não esteroidal
AS	Ácido salicílico
CAT	Catalase
COX	Ciclooxigenase
DA	Dopamina
DOPAC	Ácido 3,4-dihidroxifenilacético
DP	Doença de Parkinson
EROs	Espécies reativas de oxigênio
GSH	Glutationa
HVA	Ácido homovanílico
IL-1	Interleucina-1
IL-6	Interleucina-6
MHC	Complexo maior de histocompatibilidade
MPP^+	1-metil-4-fenilpiridinio
MPTP	1-metill-4-fenil-1,2,3,6-tetrahidropiridina
NOS	Óxido nítrico sintase
SNpc	Substância negra parte compacta
SOD	Superóxido dismutase
SS	Salicilato de sódio
TH	Tirosina hidroxilase
TNF-α	Fator de necrose tumoral alfa

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1. INTRODUÇÃO

1.1 Doença de Parkinson

A doença de Parkinson (DP) foi primeiramente caracterizada no ano de 1817 pelo médico inglês James Parkinson, que inicialmente a denominou de ‘‘shaking palsy” ou “paralisia agitante” (Dauer & Przedborski, 2003). Atualmente, a DP, uma desordem neurodegenerativa progressiva e crônica, postula como a segunda doença neurodegenerativa mais comum na terceira idade, com prevalência de 1% em idosos acima de 55 anos (Blesa et al., 2012)

Patologicamente a DP é caracterizada por uma degeneração irreversível da Substância Negra parte compacta (SNpc), responsável por promover uma severa deficiência de dopamina (DA), e seus metabólitos, o ácido homovanílico (HVA) e o ácido 3,4-dihidroxifenilacético (DOPAC), na via nigroestriatal (Lang & Lozano, 1998; Dunnett & Bjorklund, 1999; Dauer & Przedborski, 2003), além da presença de inclusões intracitoplasmáticas de agregados protéicos, denominados de corpúsculos de Lewy, formados principalmente por α-sinucleína e ubiquitina (Figura 1) (Dauer & Przedborski, 2003).

A etiologia da DP ainda permanece centrada em teorias, contudo, fortes evidências, obtidas a partir de estudos *post-mortem* em humanos e de dados experimentais provenientes de modelos animais, sugerem o envolvimento de disfunção mitocondrial, excitotoxicidade, toxinas ambientais e fatores genéticos como causa da DP (Dauer & Przedborski, 2003; Bové& Perier, 2012; Hirsch et al., 2013). Cabe ressaltar que o papel da neuroinflamação e do estresse oxidativo associados à DP têm sido uma das hipóteses mais estudadas nos últimos anos, com o intuito de melhor entender os mecanismos de morte neuronal presentes na DP (Mosley et al., 2012). Até o momento, evidências clínicas e experimentais corroboram o envolvimento da neuroinflamação na DP, sendo comum a observação de um aumento no número de células microgliais, citocinas pró-inflamatórias e ativação da ciclooxigenase (COX) em cérebros acometidos pela DP (McGeer et al., 1988; Knott et al., 2000; Nagatsu et al., 2000).

Em decorrência da progressão da neurodegeneração na SNpc, os pacientes parkinsonianos são acometidos por sinais motores, sinais cardinais da DP, como tremor muscular em repouso, rigidez muscular, bradicinesia e instabilidade postural, que se tornam evidentes e expressivos quando aproximadamente 70-80% dos neurônios dopaminérgicos na

SNpc já estão irreversivelmente degenerados (Dauer & Przedborski, 2003; Hirsch et al., 2013).

Além destes sinais motores, um número expressivo de pacientes parkinsonianos são acometidos por sintomas não-motores associados à DP. Dentre os principais, distúrbios neuropsiquiátricos, como a depressão, ansiedade, apatia e psicose. Contribuem para a redução na qualidade de vida destes pacientes, além dos sinais e sintomas já mencionados, distúrbios do sono, déficit cognitivo e do sistema nervoso autônomo (Hou & Lai, 2007).

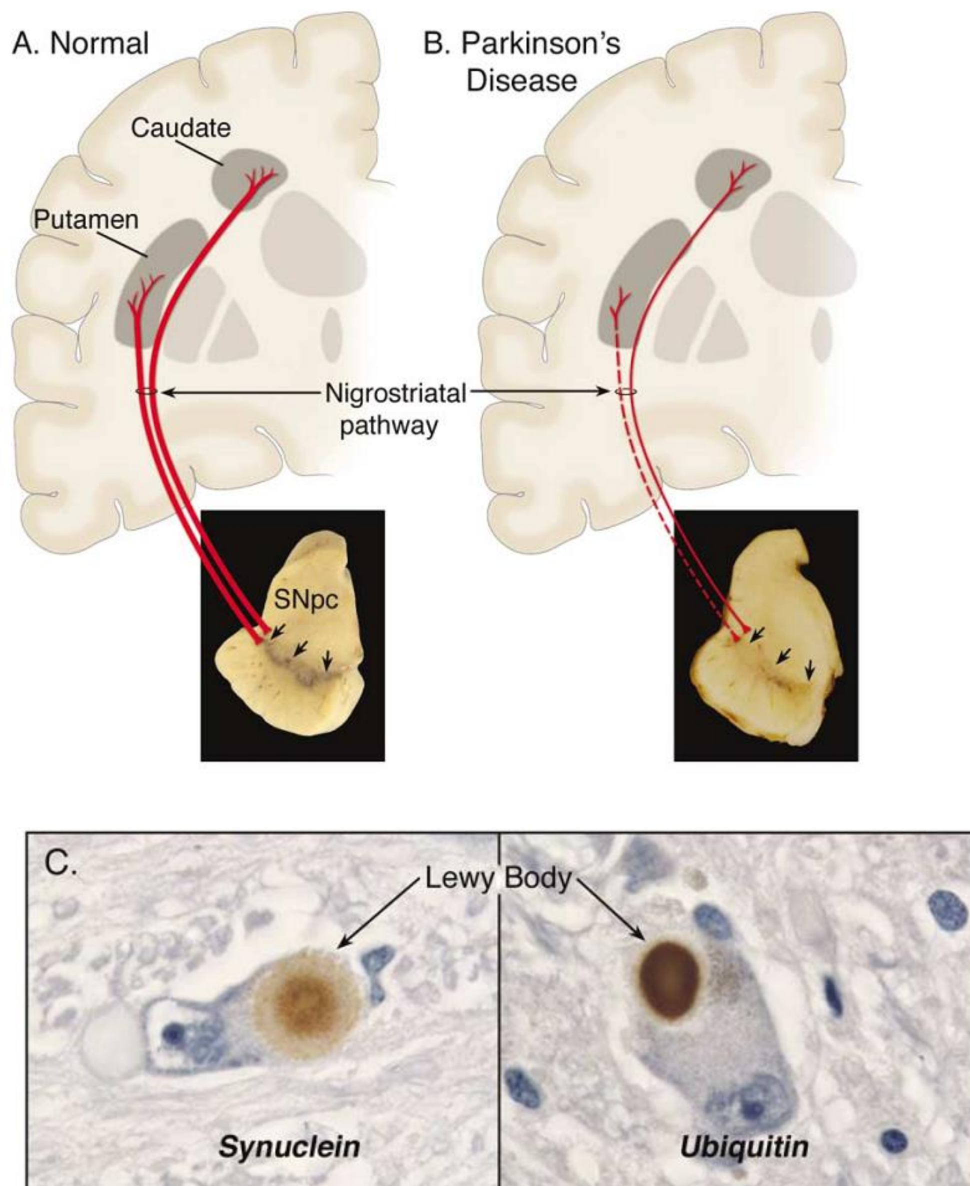

Figura 1. Principais mecanismos patológicos na DP: (B) neurodegeneração da via nigroestriatal e (C) presença de corpúsculos de Lewy intracitoplasmáticos. Adaptado de Dauer & Przedborski, 2003.

1.2 Depressão associada à DP

A prevalência da depressão em pacientes com DP é relativamente alta (Tabela 1), estimada em aproximadamente 25-40% (Tandberg et al., 1997; Poewe et al., 2007), sendo que em muitos destes pacientes (~25%) a depressão antecede a manifestação dos sinais motores (Silberman et al., 2004; Althaus et al., 2008) denotando uma correlação entre o quadro depressivo e o risco de desenvolver DP (Braak et al., 2004).

Embora a depressão seja um sintoma comum à outras patologias, bem como em populações não diagnosticadas com DP, evidências sugerem que este distúrbio psiquiátrico seja mais frequente em pacientes parkinsonianos do que em outros pacientes na mesma faixa etária, ou que sofrem de outras doenças crônicas, como osteoartrite (Aarsland et al., 2012).

A depressão associada à DP é determinante para a redução na qualidade de vida destes pacientes (Behari et al., 2005), estando associada com uma redução na funcionalidade, aumento no estresse e, sem dúvida, aumento no prejuízo cognitivo presente nestes indivíduos (Camargos et al., 2004; Silberman et al., 2004; Aarsland et al., 2012). Quanto à etiologia da depressão nos pacientes parkinsonianos, discute-se tanto a hipótese psicogênica como consequência das limitações motoras (Santiago et al., 2010), quanto a hipótese da neurodegeneração crônica, presente nos pacientes parkinsonianos, como promotoras do quadro depressivo em pacientes com DP (Camargos et al., 2004; Okun & Watts, 2002; Wolters, 2008, Frisina et al., 2009).

Além destas hipóteses, novas evidências sugerem o envolvimento de hormônios ligados ao estresse, mediadores inflamatórios e fatores neurotróficos no desenvolvimento da depressão associada a DP. De fato, níveis aumentados de mediadores inflamatórios, como o fator de necrose tumoral alfa (TNF-α), são observados em pacientes com DP e depressão, e estão relacionados não somente ao quadro depressivo, mas também aos prejuízos cognitivos e desordens do sono, comuns nestes indivíduos (Aarsland et al., 2012).

Ademais, acredita-se que de modo geral tanto a deficiência de DA em regiões frontais e subcorticais, quanto o prejuízo das vias noradrenérgicas e serotoninérgicas, aliados ao comprometimento do processo de neurogênese, estejam associados ao desenvolvimento de depressão em pacientes parkinsonianos (Santiago et al., 2010; Aarsland et al., 2012).

Dentre as principais estratégias farmacológicas para tratar a depressão associada à DP, o uso de agonistas dopaminérgicos têm se mostrado eficaz em casos de depressão moderada. Da mesma forma, o uso de antidepressivos que atuam sobre o sistema serotonérgico e

noradrenérgico, também demonstram eficácia no tratamento da depressão associada à DP (Aarsland et al., 2012).

Entretanto, poucos estudos até o momento foram direcionados para avaliar o efeito antidepressivo de drogas capazes de modular tanto fatores inflamatórios quanto fatores neurotróficos, em pacientes com DP e depressão.

Tabela 1. Estudos de depressão em pacientes com doença de Parkinson

Estudo	Características dos pacientes	Escala	Duração da doença (anos)	% de pacientes com depressão
Kang et al., 2005	162 pacientes	GDS (score ≥7)	0.0–3.0	13.0
Martinez-Martin et al., 2007	545 pacientes, sem demência, idade média de 67.7 anos	NMSQ	7.0	50.1
Kulisevsky et al, 2008	1351 pacientes, sem demência, idade média de 70.6 anos	NPI	5.7	29.9
Aarsland et al., 2009	75 pacientes, sem demência, idade média de 67.8 anos	NPI	2.0	10.3
Barone et al., 2009	1072 pacientes, idade média 67.4 anos	NMSQ	5.1	22.5
Ravina et al., 2009	413 pacientes, idade média 61 anos	GDS-15 (score ≥5)	0.5	13.8
Negre-Pages et al., 2010	422 pacientes, sem demência, idade média 68.6 anos	HAM-D (score >7)	5.4	25.0
Riedel et al., 2010	1449 pacientes, idade média 70.7 anos	MADRS (score ≥14)	5.8	23.8
Vanderheyden et al., 2010	1086 pacientes	MINI	-	15.6
Brown et al., 2011	513 pacientes, idade média 67.9 anos	GMS, HADS	6.9	22.0

GDS, Geriatric Depression Scale; GMS, Geriatric Mental State; HADS, Hospital and Anxiety Depression Scale; HAM-D, Hamilton Depression Rating Scale; MADRS, Montgomery–Asberg Depression Rating Scale; MINI, Mini International Neuropsychiatric Interview; NMSQ, Nonmotor Symptoms Questionnaire; NPI, Neuropsychiatric Inventory. Adaptado de Aarsland et al., 2012.

1.3 Neuroinflamação e estresse oxidativo na DP

Crescentes evidências sugerem o envolvimento da neuroinflamação crônica nos mecanismos patológicos presentes na DP. Achados *post-mortem* em pacientes com DP (Tabela 2) indicam um aumento na ativação microglial, nos níveis de mediadores pró-inflamatórios como citocinas (TNF-α, IL-1b e IL-6) e de espécies reativas (de oxigênio e

nitrogênio) (Chen & Tansey, 2011; Collins et al., 2012). Um resumo destas vias na DP é apresentado na figura 2.

Diante de tais evidências, acredita-se que as células da glia, quando ativadas, passam a secretar altos níveis de mediadores pró-inflamatórios, que induziriam a morte dos neurônios dopaminérgicos e aumentariam ainda mais a ativação destas células, o que resultaria em um ciclo de promoção de inflamação e neurodegeneração (Glass et al., 2010; Chen & Tansey, 2011; Collins et al., 2012).

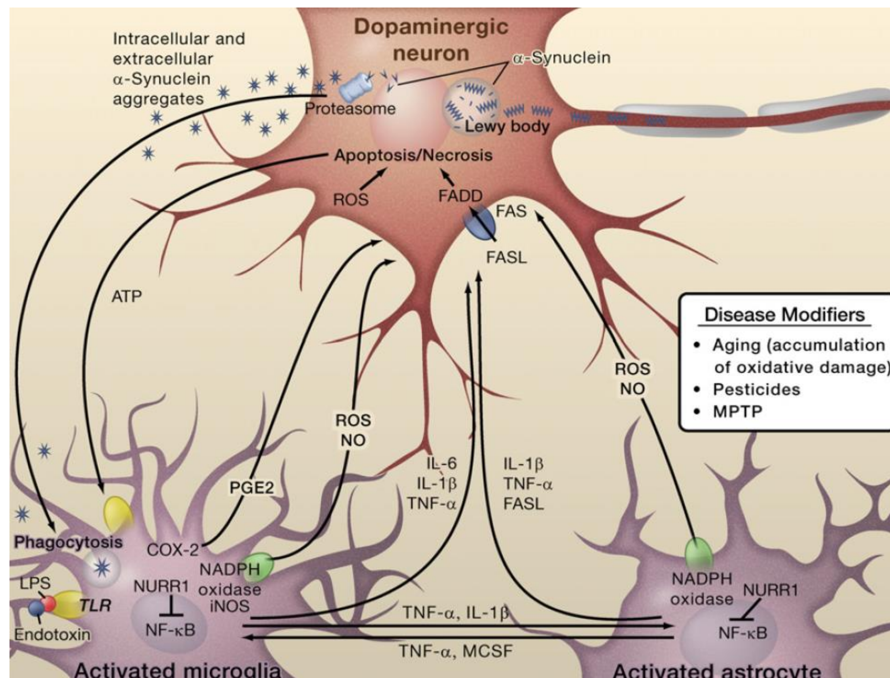

Figura 2. Principais vias inflamatórias e oxidativas envolvidas na fisiopatologia da DP: As principais características neuropatológicas da DP são a perda de neurônios dopaminérgicos na SNpc e presença de corpúsculos de Lewy. A liberação do conteúdo intraneuronal, como os corpúsculos de Lewy, é capaz de ativar as células da glia e promover a ativação de NF-kβ, com consequente síntese de espécies reativas (ROS, NO), além de mediadores pró-inflamatórios (prostaglandinas e citocinas). Esses fatores atuam diretamente sobre os neurônios dopaminérgicos na SNpc, amplificando a resposta inflamatória. Adaptado de Glass et al., 2010.

Da mesma forma, o processo de inflamação sistêmica é associado como fator relevante na neurodegeneração presente na DP, uma vez que tanto em modelos animais, quanto em pacientes com DP, é observado um aumento na infiltração linfocitária no sistema nervoso central (SNC), provenientes da periferia (Collins et al., 2012).

Além disso, como o cérebro é um tecido altamente rico em ácidos graxos poli-insaturados e pobre em defesas antioxidantes, este órgão é extremamente susceptível aos danos mediados por espécies reativas, tanto de oxigênio quanto de nitrogênio. De fato, uma das primeiras alterações observadas no cérebro de pacientes com DP é a depleção nos níveis de glutationa reduzida (GSH) na SNpc, relacionada principalmente ao decréscimo da

atividade do complexo I mitocondrial e da função mitocondrial (Valko et al., 2007; Dexter & Jenner, 2013; Sanders & Greenamyre, 2013).

Ademais, a exposição à toxinas ambientais que atuam induzindo estresse oxidativo, dentre estas a rotenona, é associada com um maior risco de desenvolvimento de DP em humanos. O desequilíbrio redox, proveniente tanto do processo inflamatório, quanto dos demais mecanismos fisiopatológicos envolvidos na DP, especialmente da disfunção mitocondrial, promove um quadro de estresse oxidativo, caracterizado por aumento na formação de espécies reativas e redução nos níveis de antioxidantes endógenos, como o GSH (Mosley et al., 2006; Dexter & Jenner, 2013; Sanders & Greenamyre, 2013).

Por fim, tanto o processo inflamatório quanto a formação de espécies reativas induzem danos às membranas lipídicas, proteínas e DNA, inibindo suas funções (Mosley et al., 2006; Valko et al., 2007; Dexter & Jenner, 2013; Sanders & Greenamyre, 2013) e contribuindo para a neurodegeneração presente na DP.

Tabela 2. Evidências de meuroinflamação em humanos com DP

Estudo	Evidências
McGeer et al., 1988	Supra regulação de moléculas de MHC no tecido cerebral.
Mogi et al., 1994a,b	Elevação dos níveis de TNF-α no estriado e fluído cérebro-espinhal.
Mogi et al., 1995	Elevação dos níveis de β2-microglobulina (cadeia leve do MHC) no estriado.
Blum-Degen et al., 1995	Elevação dos níveis de IL-1β e IL-6 no fluído cérebro-espinhal.
Langston et al., 1999	Presença de gliose e agrupamentos de micróglias ao redor de células nervosas em humanos expostos acidentalmente ao MPTP.
Mirza et al., 2000	Ausência de astrocitose reativa no processo inflamatório.
Knott et al., 2000	Supra regulação das enzimas NOS, COX-1 e COX-2.
McGeer et al., 2002	Associação entre polimorfismo da IL-1β e DP idiopática.
Imamura et al., 2003	Aumento do número de micróglia ativada, não somente na SNpc e putâmen, como também no hipocampo e córtex.
Ishida et al., 2006	Expressão aumentada de PAR-1 em astrócitos na SNpc de cérebros com DP.

COX (Ciclooxigenase); IL-1 (Interleucina-1); IL-6 (Interleucina-6); MHC (Complexo maior de histocompatibilidade); MPTP (1-metill-4-fenil-1,2,3,6-tetrahidropiridina); NOS (Óxido nítrico sintase); TNF- α (Fator de necrose tumoral alfa); Adaptado de Esposito et al., 2007

1.4 AINEs e neuroproteção na DP

Os antiinflamatórios não-esteroidais (AINEs) são um grupo heterogêneo de compostos que compartilham muitas propriedades farmacológicas, e também efeitos colaterais, sendo principalmente utilizados para promoção de analgesia e efeito antipirético, ou simplesmente, para diminuir os sintomas promovidos pelo processo inflamatório. Em contraste com os agentes antiinflamatórios esteroidais, que são inibidores da enzima fosfolipase A_2, e consequentemente bloqueadores da síntese de leucotrienos e prostaglandinas, os AINEs inibem apenas a atividade da enzima COX, levando à diminuição na produção de prostaglandinas, e, aumento compensatório na síntese de leucotrienos.

Desta forma, os efeitos farmacológicos dos AINEs são mediados por sua ação inibitória sobre a atividade da enzima COX, envolvida no metabolismo do ácido araquidônico e consequente formação de prostaglandinas além de outros produtos. A enzima COX é expressa sobre duas isoformas principais, a COX-1 e a COX-2, e até pouco tempo atribuía-se como principal papel fisiológico da enzima COX-1 (constitutiva) a citoproteção da mucosa gástrica, além de sua participação na agregação plaquetária e modulação da função renal, enquanto que a COX-2 era vista como a isoforma induzível e presente apenas em processos patológicos, como a inflamação. Entretanto, novos estudos sugerem que ambas as isoformas da COX estão distribuídas de forma heterogênea entre células do tecido nervoso central, sendo as isoformas COX-1 e COX-1b detectadas em células da microglia, enquanto a COX-2 é encontrada principalmente em células da glia, e em menor níveis (passíveis de supra regulação em pacientes com DP e em modelos de indução de parkinsonismo) nos neurônios dopaminérgicos da SNpc (Esposito et al., 2007).

Além disso, a COX-2 também está envolvida em processos de manutenção da homeostasia (Hinz & Brune, 2002), principalmente no SNC, onde a mesma atua sobre mecanismos envolvidos na excitabilidade da membrana, transmissão sináptica e consolidação da memória durante o sono REM (Cole-Edwards & Bazan, 2005; Chen & Bazan, 2005).

Assim, durante os últimos anos, o papel neuroprotetor dos AINEs na DP foi extensivamente estudado. Um breve resumo destes estudos é apresentado a seguir, nas tabelas 3 (estudos *in vitro*), 4 (estudos *in vivo*) e 5 (estudos epidemiológicos).

Tabela 3. Estudos *in vitro* avaliando o efeito neuroprotetor dos AINEs na doença de Parkinson

Referência	Modelo experimental	AINE	Resultados
(Grilli et al. 1996)	Cultura primária de células cerebelar de ratos	AAS (1-3 mM) SS (3-10 mM) Indometacina (1-20 mM)	Neuroproteção (↓ NF-κB) Neuroproteção (↓ NF-κB) Sem proteção
(Casper et al. 2000)	Cultura primária de neurônios mesencefálicos de ratos	AAS (1 mM) Acetaminofeno (1 mM) Ibuprofeno (0.1, 1 mM)	Todos os AINEs: ↓ redução na captação de DA induzida pelo glutamato ↑ 47% dos neurônios dopaminérgicos (0.1 mM ibuprofeno) ↓ 85% dos neurônios dopaminérgicos (1 mM ibuprofeno)
(Carrasco and Werner 2002)	Cultura primária de neurônios mesencefálicos de ratos incubados com 6-OHDA ou (5 µM) ou MPP^+ (5 µM)	AAS (1 mM)	↑ Sobrevivência dos neurônios dopaminérgicos via efeito antioxidante
(Morioka et al. 2004)	Células PC12 incubadas com MPP^+ (30 µM)	Indometacina (100 µM) Ibuprofeno (100 µM) Cetoprofeno (100 µM) Diclofenaco (100 µM) AAS (100µM)	↑ Neurotoxicidade ↑ Neurotoxicidade ↑ Neurotoxicidade ↑ Neurotoxicidade Sem proteção
(Wang et al. 2005)	Cultura primária de neurônios mesencefálicos e glia de camundongos incubado com MPP^+ (0.1-0.5 µM)	DuP697 (10 nM)	↓ Síntese de PGE_2 ↓ Ativação microglial
(Soliman et al. 2009)	Células N-2A de camundongos albinos incubadas com MPP^+ (500 µM)	AAS (0.001-1 mM) Ibuprofeno (0.005-5 mM) Piroxicam (0.005-5 mM)	Sem proteção Sem proteção Reversão parcial da depleção de ATP
(Hsieh et al. 2011)	Cultura primária de neurônios mesencefálicos de ratos incubados com MPP^+ (20 µM)	DFU (10,100 µM) Ibuprofeno (25, 250 µM)	Neuroproteção por "scavenging" de EROs ↓ Toxicidade induzida pelo MPP^+ ↓ Apoptose
(Tasaki et al. 2012)	Células de neuroblastoma humano (SH-SY5Y) incubadas com MPP^+ (5 mM)	AAS (1, 3, 10, 30 µM) Valdecoxibe (1, 3, 10, 30µM) Celecoxibe (1, 3, 10, 30 µM) Licofelone (1, 3, 10, 30 µM) Piroxicam (1, 3, 10, 30 µM) Tenoxicam (1, 3, 10, 30 µM) Meloxicam (1, 3, 10, 30 µM)	Sem proteção Sem proteção Sem proteção Sem proteção Neuroproteção (via cascata PI3K/Akt) Neuroproteção (via cascata PI3K/Akt) Neuroproteção (via cascata PI3K/Akt)

6-OHDA (6-hidroxidopamina); AAS (Ácido acetilsalicílico); AINE (Antiinflamatório não esteroidal); DA (Dopamina); EROs (Espécies reativas de oxigênio); MPP^+ (1-metil-4-fenilpiridinio); NF-kB (Fator nuclear kappa B); PGE_2 (Prostaglandina E_2); SS (Salicilato de sódio)

Tabela 4. Estudos avaliando o efeito neuroprotetor dos AINEs em modelos animais da doença de Parkinson

Referência	Animal	Modelo	AINE	Resultados
(Aubin et al. 1998)	Camundongo	MPTP (15 mg/kg, sc)	AAS (100 mg/kg, ip), aspegico (200 mg/kg, ip), AS (100 mg/kg, ip), paracetamol (100 mg/kg, ip), diclofenaco (100 mg/kg, ip), ibuprofeno (20 mg/kg, ip), indometacina (100 mg/kg, ip)	Neuroproteção por “scavenging” de EROs (AAS, AS, Aspergico) Sem proteção (outros AINEs)
(Ferger et al. 1999)	Camundongo	MPTP (30, 40 mg/kg, sc)	SS (50, 100 mg/kg, ip) antes do MPTP	Neuroproteção por “scavenging” de EROs ↓ Depleção de DA ↑ Imunoreatividade para tirosina hidroxilase
(Mohanakumar et al. 2000)	Camundongo	MPTP (30 mg/kg, ip, 2 vezes, 16 h intervalo)	AS (25, 50, 100 mg/kg, ip) antes do MPTP	Neuroproteção por “scavenging” de EROs ↓ Rigidez, depleção de GSH e DA
(Teismann and Ferger 2001)	Camundongo	MPTP (30 mg/kg, sc)	AAS (10, 50, 100 mg/kg, ip) ou meloxicam (2, 7.5, 50 mg/kg, ip) antes MPTP	Neuroproteção ↓ Depleção DA e prejuízo motor ↑ Imunoreatividade para tirosina hidroxilase
(Kurkowska-Jastrzębska et al. 2002)	Camundongo	MPTP (10 mg/kg, ip, 4 vezes, 1 h intervalo)	Indometacina (1, 2.5 mg/kg) antes do MPTP	Neuroproteção (somente dose de 1 mg/kg) ↓ Ativação microglial e infiltração linfocitária
(Sairam et al. 2003)	Rato	MPP^{+} (infusão intranigral)	AS (50, 100 mg/kg, ip), diclofenaco (5-100 mg/kg, ip), ou celecoxibe (2.5-50 mg/kg, ip) antes e depois do MPTP	Neuroproteção por “scavenging” de EROs (AS) Sem proteção (diclofenaco) ↑ Neurotoxicidade do MPP^{+} (celecoxibe)
(Maharaj et al. 2004)	Rato	MPP^{+} (infusão intranigral)	AAS (100 mg/kg, ip) ou acetaminofeno (100 mg/kg, ip), 4 vezes em 24 horas, antes do MPTP	Neuroproteção por “scavenging” de EROs (AAS) Neuroproteção parcial por “scavenging” de EROs (acetaminofeno)
(Przybyłkowski et al. 2004)	Camundongo	MPTP (15 mg/kg, ip, 4 vezes, 1 h intervalo)	Rofecoxibe (10 mg/kg, ip, por 13 dias) antes do MPTP	Sem proteção
(Sánchez-Pernaute et al. 2004)	Rato	6-OHDA (2.5 µl, infusão intranigral)	Celecoxibe (20 mg/kg, po) antes do MPTP, mais 21 dias após	Neuroproteção por inibição de COX-2 ↓ Ativação microglial ↑ Imunoreatividade para tirosina hidroxilase
(Di Matteo et al. 2006)	Rato	MPP^{+} ou 6-OHDA (infusão intranigral)	AAS (100 mg/kg, ip) ou meloxicam (50 mg/kg, ip) antes do MPP^{+} ou 6-OHDA	Neuroproteção por “scavenging” de EROs (no modelo de 6-OHDA) e mecanismo desconhecido no modelo do MPP^{+} (AAS) Sem proteção (meloxicam)
(Maharaj et al. 2006)	Rato	MPP^{+} (infusão intranigral)	AAS (100 mg/kg, ip), acetaminofeno (100 mg/kg, ip), or AAS + acetaminofeno (100 mg/kg, ip), 4 vezes após MPTP	Neuroproteção por “scavenging” de EROs e prevenção disfunção mitocondrial
(Reksidler et al. 2007)	Rato	MPTP (infusão intranigral)	Parecoxibe (2, 10 mg/kg, ip, por 21 dias)	Neuroproteção por inibição de COX-2 ↓ Prejuízo motor e cognitivo ↑ Imunoreatividade para tirosina hidroxilase

(Goren et al. 2009)	Rato	6-OHDA (infusão intranigral)	AAS (100 mg/kg, po) ou meloxicam (50 mg/kg, po) antes 6-OHDA	Sem proteção
(Gupta et al. 2009)	Rato	MPTP (infusão intranigral)	Rofecoxibe (2-8 mg/kg, po), celecoxibe (10-40 mg/kg, po), nimesulida (2.5-10 mg/kg, po), ou naproxeno (7-20 mg/kg, po) antes e após MPTP	Neuroproteção por “scavenging” de EROs ↓ Catatonia (todos os AINEs) ↓ Estresse oxidativo (rofecoxibe e celecoxibe) ↓ Prejuízo motor e depleção de GSH (rofecoxibe) ↓ Nível de nitrito (rofecoxibe)
(Soliman et al. 2009)	Camundongo	MPTP (40 mg/kg, ip)	Piroxicam (20 mg/kg, ip), antes e após MPTP	Neuroproteção ↓ Prejuízo motor e depleção de DA ↑ Imunoreatividade para tirosina hidroxilase
(Sui et al. 2009)	Rato	LPS (infusão intranigral)	Meloxicam (7.5 mg/kg, ip, por 15 dias) após LPS	Neuroproteção por inibição de COX-2 ↓ Ativação microglial ↑ Imunoreatividade para tirosina hidroxilase
(Gupta et al. 2010)	Rato	MPTP (infusão intranigral)	Nimesulida (5, 10 mg/kg, po) antes e após MPTP	Neuroproteção ↓ Prejuízo motor e estresse oxidativo ↑ Atividade complexo I mitocondrial
(Gupta et al. 2010b)	Camundongo	MPTP (10 mg/kg, ip, 4 vezes, intervalo de 1 h)	Licofelone (10 mg/kg, po) antes e após MPTP	Neuroproteção ↓ Prejuízo motor, catatonia e estresse oxidativo ↑ Atividade complexo I mitocondrial ↓ Apoptose por ↓ atividade da caspase-3
(Gupta et al. 2011)	Camundongo	MPTP (10 mg/kg, ip, 4 vezes, intervalo de 1 h)	Valdecoxibe (5, 10 mg/kg, po) ou NS-398 (5, 10 mg/kg, po) antes e após MPTP	Neuroproteção ↓ Prejuízo motor e catatonia ↑ Atividade complexo I mitocondrial ↓ Apoptose por ↓ atividade da caspase-3
(Tasaki et al. 2012)	Camundongo	MPTP (30 mg/kg, sc) por 5 dias	Meloxicam (10 mg/kg, ip, por 10 dias) antes MPTP	Neuroproteção por prevenir a redução da via pAkt ↓ Bradicinesia ↑ Imunoreatividade para tirosina hidroxilase
(Madathil et al. 2013)	Rato	Rotenona (infusão intranigral)	SS (50, 100 mg/kg, ip) antes e após rotenona	↓ Depleção de DA ↓ Perda de neurônios dopaminérgicos ↑ Atividade da SOD e complexo I mitocondrial
(Thakur and Nehru 2013)	Rato	Rotenona (2 mg/kg, sc) por 5 semanas	SS (100 mg/kg, ip) com rotenona	↑ Níveis de DA ↑ Imunoreatividade para tirosina hidroxilase ↓ Prejuízo motor e formação de EROs ↓ IL-6, IL-1β, TNF-α

6-OHDA (6-hidroxidopamina); AS (Ácido salicílico); AAS (Ácido acetilsalicílico); AINE (Antiinflamatório não esteroidal); DA (Dopamina); EROs (Espécies reativas de oxigênio); GSH (Glutationa); IL-1 (Interleucina-1); IL-6 (Interleucina-6); LPS (Lipopolisacarídeo); MPP^+ (1-metil-4-fenilpiridinio); MPTP (1-metill-4-fenil-1,2,3,6-tetrahidropiridina); SOD (Superóxido dismutase); SS (Salicilato de sódio); TNF- α (Fator de necrose tumoral alfa)

Tabela 5. Estudos clínicos e epidemiológicos avaliando o uso de AINEs, não derivados da aspirina, e o risco de desenvolver doença de Parkinson

Referência	Duração	População			Casos de Parkinson		Risco relativo	95% I.C.	Conclusão
		Total	Não usuários de AINEs	Usuários de AINEs	Não usuários de AINEs	Usuários de AINEs			
(Chen et al. 2003)	1980-2000	142,004	135,694	6,310	401	13	0.55	0.32-0.96	O uso de AINEs (não derivados da aspirina) atrasa ou previne contra o desenvolvimento da DP
(Hernán et al. 2006)	1995-2001	7,896	3,916	3,980	652	606	0.93	0.80-1.08	Os resultados sugerem que o uso de AINEs (não derivados da aspirina) reduz o risco de desenvolver DP em homens, mas não em mulheres
(Ton et al. 2006)	1977-1992 1993-2002	498 498	166 478	332 20	59 158	107 8	0.90 1.67	0.59-1.35 0.60-4.60	Os resultados não apoiam a hipótese de que AINEs (não derivados da aspirina) reduzem o risco de desenvolver DP
(Etminan et al. 2008)	1997-2003	697,078	445,355	251,723	3,283	1,727	0.84	0.81-1.09	Os resultados não apoiam a hipótese de que AINEs (não derivados da aspirina) reduzem o risco de desenvolver DP
(Becker et al. 2011)	1994-2009	19,995	8,208	11,787	1,591	2,435	1.07	0.99-1.16	Os resultados não apoiam a hipótese de que AINEs (não derivados da aspirina) reduzem o risco de desenvolver DP
(Driver et al. 2011)	1982-2008	3,696	2,647	1,049	417	199	1.28	1.05-1.56	Os resultados não apoiam a hipótese de que AINEs (não derivados da aspirina) reduzem o risco de desenvolver DP
(Manthripragada et al. 2011)	2001-2006	11,582	8,966	2,616	1,499	432	0.97	0.86-1.09	Os resultados não apoiam a hipótese de que AINEs (não derivados da aspirina) reduzem o risco de desenvolver DP

AINE (Antiinflamatório não esteroidal); DP (Doença de Parkinson)

1.4.1 Ibuprofeno e DP

O ibuprofeno foi o primeiro membro dos derivados do ácido propiônico apresentado como uma alternativa à aspirina, em 1969. O efeito antiinflamatório de ibuprofeno, assim como dos demais AINEs, é mediado pela sua capacidade em inibir não-seletivamente as isoformas da COX (Bushra & Aslam, 2010). Além disso, recentemente foi demonstrado em modelo animal da doença de Alzheimer que o ibuprofeno também atua como antioxidante. Dessa maneira, o ibuprofeno promoveu efeito neuroprotetor que se mostrou independente da inibição de COX (Wilkinson et al., 2012). Ao longo dos últimos anos, estudos avaliando o efeito neuroprotetor do ibuprofeno, especialmente em doenças neurodegenerativas, apresentaram resultados contraditórios e inconclusivos.

Estudos *in vitro* demonstraram que o efeito do ibuprofeno contra a toxicidade induzida pelo glutamato em neurônios dopaminérgicos é dependente da concentração deste AINE, ou seja, pode ocorrer aumento da sobrevivência e morte neuronal (Casper et al., 2000). Entretanto, foi relatado um aumento da toxicidade neuronal após tratamento com ibuprofeno e exposição de cultura primária de neurônios mesencefálicos ao 1-metil-4-fenilpiridino (MPP^+), uma neurotoxina capaz de induzir a morte dos neurônios dopaminérgicos (Morioka et al., 2004). Por sua vez, outro estudo falhou em demonstrar qualquer efeito do ibuprofeno frente à mesma toxina (Soliman et al., 2009). Por fim, recentemente Hsieh et al (2011) demonstraram, *in vitro*, efeito protetor do ibuprofeno contra a toxicidade mediada pelo MPP^+ em cultura primária de neurônios mesencefálicos.

Em modelos animais de DP, um dos poucos estudos que utilizaram ibuprofeno, também não foi capaz de demonstrar qualquer efeito neuroprotetor deste AINE frente à toxicidade induzida por 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP), uma neurotoxina capaz de induzir a morte dos neurônios dopaminérgicos, em ratos (Aubin et al., 1998). Contudo, em humanos, a utilização regular de ibuprofeno está associada à um menor risco em desenvolver DP (Chen et al., 2005; Gao et al., 2011).

1.5 Modelo de parkinsonismo induzido por rotenona

A rotenona é um praguicida altamente lipofílico, sendo portanto capaz de cruzar livremente as membranas celulares. Esta toxina apresenta alta afinidade pelo complexo I

mitocondrial, ocorrendo após esta interação a inibição deste complexo. Como consequência, há perda da função mitocondrial, além da formação deste complexo é inibida, induzindo além da perda da função mitocondrial, além da formação da espécies reativas, depleção de ATP e morte celular (Bové & Perier).

Além de sua ação sobre o complexo I da cadeia respiratória, a rotenona atua inibindo a formação de microtúbulos de tubulina, favorecendo o acúmulo de tubulina intracelular que é altamente tóxico para os neurônios dopaminérgicos (Figura 3) (Bové & Perier, 2012). Um breve resumo dos estudos utilizando esta toxina para induzir parkinsonismo em animais é apresentado na tabela 5.

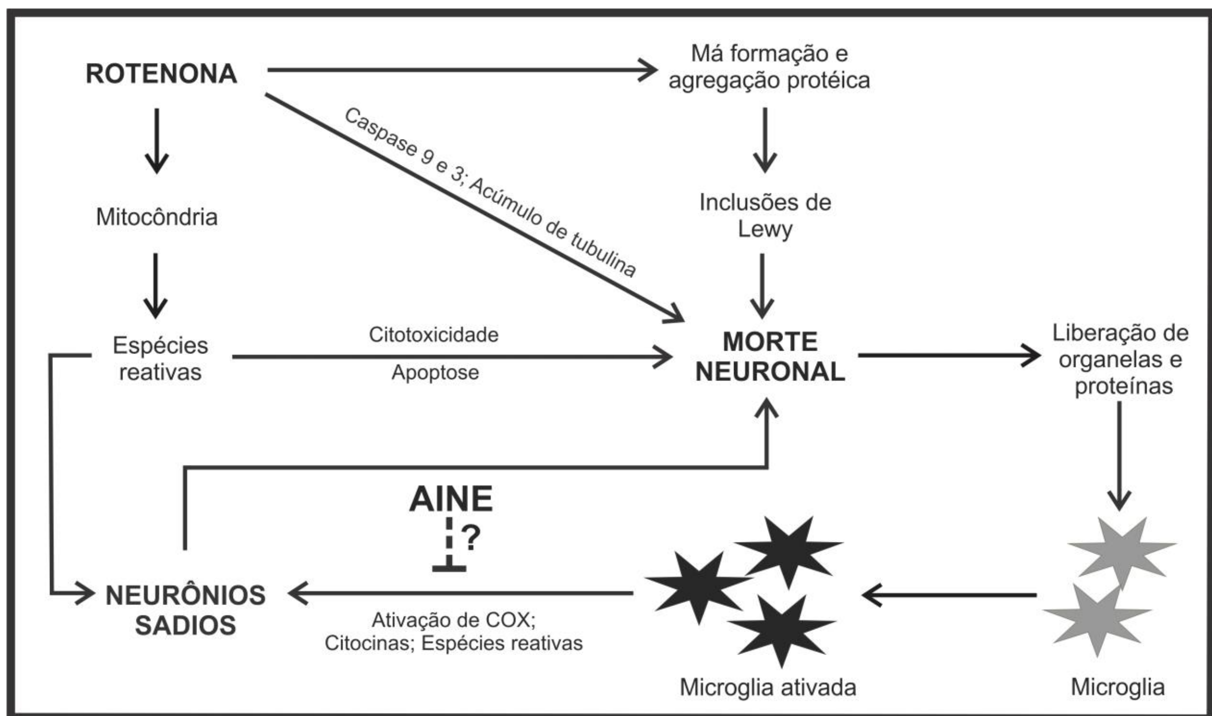

Figura 3. Mecanismos de toxicidade induzidos pela rotenona em neurônios dopaminérgicos. Através da inibição do complexo I mitocondrial, a rotenona induz a formação de espécies reativas de oxigênio e nitrogênio, contribuindo para a morte neuronal. Além disso, a rotenona é capaz de induzir a ativação de mecanismos de apoptose, além de formação de corpúsculos de Lewy. A morte dos neurônios dopaminérgicos promove a ativação de células gliais, que passam a sintetizar e secretar mediadores inflamatórios e espécies reativas, induzindo a morte de neurônios dopaminérgicos sadios.

Considerando que a SNpc é uma região extremamente suscetível a citotoxicidade, uma vez que possui baixos níveis de defesas antioxidantes naturais, além de níveis elevados de radicais livres, neste trabalho avaliamos o potencial efeito neuroprotetor do ibuprofeno, sobre o prejuízo motor, comportamento tipo-depressivo e estresse oxidativo induzidos pela administração de rotenona em ratos em áreas do encéfalo relacionadas à DP e depressão.

Tabela 6. Estudos utilizando a rotenona como modelo de indução de parkinsonismo

Administração	Espécie	Via administração	Estriado		SNpc		Prejuízo motor	Toxicidade sistêmica	Referência
			DA	TH	DA	TH			
Intracranial	Rato	3 µg/MFB	▼▼				Sim		Alam et al., 2004
	Rato	2 µg/MFB	▼▼▼		▼▼		Sim		Antkiewicz-Michaluk et al., 2004
	Rato	2-12µg/SNPc	▼	▼		▼	Sim		Saravanan et al., 2005
	Rato	12µg/SNPc ou MFB	▼▼▼			▼	Sim		Sindhu et al., 2005
	Rato	12µg/MFB	▼▼▼				Sim		Sindhu et al., 2006
	Rato	6µg/SNPc	▼▼▼			▼▼	Sim		Saravanan et al., 2006
Intravenosa	Rato	-	▼			▼	Sim	Sim	Betarbet et al., 2000
	Rato	2.5 mg/kg/day/28d		▼▼▼		▼▼	Sim	Sim	Hoglinger et al., 2003
	Rato	2.0-3.5 mg/kg/day/21d		▼			Sim	Sim	Fleming et al., 2004
	Rato	-				▼	Não		Garcia-Garcia et al., 2005
	Rato	2.0-3.0 mg/kg/day/14-28d	▼	▼					Milusheva et al., 2005
Subcutânea	Rato	2.0-3.0 mg/kg/day/7-32d		▼			Sim	Sim	Sherer et al., 2003
	Rato	10 mg/kg/day/7d	=						Antkiewicz-Michaluk et al., 2004
	Rato	2.0-3.5 mg/kg/day/21d		▼			Sim	Sim	Fleming et al., 2004
	Rato	2.5 mg/kg/day/3-20d		▼		=	Sim	Sim	Lapointe et al., 2004
	Rato	2 mg/kg/day/21d		▼▼▼		=		Sim	Zhu et al., 2004
	Rato	1.25 mg/kg/day/14d	▼▼			▼▼			Ling et al., 2004
	Rato	2.5 mg/kg/day/28d	▼▼▼		▼▼		Sim	Sim	Yang et al., 2005
	Rato	-					Sim	Sim	Pasha et al., 2005
	Rato	2.2-2.5 mg/kg/day/28d		▼▼▼		▼	Sim	Sim	Hoglinger et al., 2005
	Rato	2.5 mg/kg/day/28d	▼▼▼				Sim		Yang et al., 2006
	Rato	90 mg/kg/day/1d	▼			▼	Sim	Sim	Huang et al., 2006
	Rato	3.0 mg/kg/day/35d		▼	=				Betarbet et al., 2006
	Rato	1.5 mg/kg/day/28d		=		▼		Não	Phinney et al., 2006
	Rato	2.5 mg/kg/day/28d	▼	▲		▼▼▼	Sim	Sim	Luo et al., 2007
	Camundongo	2.5-5 mg/kg/day/30-45d		=		=	Sim	Sim	Richter et al., 2007
	Rato	2.0 mg/kg/day/35d	▼▼			▼▼	Sim	Sim	Thakur & Nehru, 2013
Intraperitoneal	Rato	6.0 mg/kg/day/40d	▼▼			▼▼			He et al., 2003
	Rato	2.75-3.0 mg/kg/day/21d	▼▼	▼		▼▼	Sim	Não	Cannon et al., 2009
	Rato	2.5 mg/kg/day/10d	▼▼			▼	Sim		Morais et al., 2012
Oral	Camundongo	0.25-30 mg/kg/day/28d	▼	▼		▼			Inden et al., 2007
Nasal	Camundongo	2.5 mg/kg/day/30d	=	=		=	Não		Rojo et al., 2007

▼ % de redução desconhecida; ▼ redução ≤25%; ▼▼ redução 25-50%; ▼▼▼ redução 50-75%; = sem alteração; ▲ % de aumento desconhecida; DA (Dopamina); MFB (Mesencéfalo medial); SNPc (Substancia negra parte compacta); TH (Tirosina hidroxilase); Adaptado de Cicchetti et al., 2009.

2. OBJETIVOS

2.1 Objetivo geral

Investigar o efeito neuroprotetor e tipo antidepressivo do ibuprofeno em modelo animal de parkinsonismo induzido por administração de rotenona em ratos.

2.2 Objetivos específicos

1. Determinar através de curva dose resposta a dose de ibuprofeno a ser utilizada neste estudo;

2. Avaliar o efeito do ibuprofeno nos parâmetros comportamentais: teste do campo aberto; teste do nado forçado modificado;

3. Avaliar o efeito do ibuprofeno nos parâmetros neurobioquímicos: imunohistoquímica para TH e estresse oxidativo no estriado e hipocampo.

3. ARTIGO

Antidepressant and antioxidative effect of ibuprofen in the intraperitoneal rotenone model of Parkinson's disease

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Abstract

Idiopathic Parkinson's disease (PD) is a neurodegenerative disorder that affects approximately 1% of the population over 55 years of age. The disease manifests itself through motor and nonmotor symptoms induced mainly by the neurodegeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The possible mechanisms involved in this pathology include mitochondrial dysfunction, neuroinflammation, and oxidative stress. The present study evaluated the effects of the nonselective cyclooxygenase inhibitor ibuprofen on motor and depressive-like behavior induced by rotenone in rats. Rotenone (2.5 mg/kg, i.p., for 10 days) decreased tyrosine hydroxylase immunoreactivity in the SNpc, and ibuprofen treatment (15 mg/kg, p.o., for 22 days) blocked this impairment. We also found that rotenone induced motor deficits (hypolocomotion) and depressive-like behavior, and ibuprofen was able to reverse these deficits. In addition to motor and nonmotor behavior, we evaluated oxidative stress induced by rotenone. Rotenone administration depleted glutathione levels in the hippocampus and reduced catalase activity in both the hippocampus and striatum. Posttreatment with ibuprofen blocked the depletion of glutathione induced by rotenone and increased the basal levels of this antioxidant in the striatum. Ibuprofen also restored catalase activity. The neuroprotective effects of ibuprofen against toxicity induced by rotenone appear to be attributable to its antioxidant properties, in addition to cyclooxygenase inhibition.

Keywords: Rotenone, Parkinson's disease; Ibuprofen; Oxidative stress.

INTRODUCTION

Parkinson's disease (PD) was first described by James Parkinson in 1817. It is a chronic, progressive neurodegenerative disorder and the second most common neurodegenerative disorder that affects patients usually older than 55 years of age. The disease appears to have a slightly higher prevalence in men than in women (Hou and Lai 2007).

The pathological process present in parkinsonian patients leads to a substantial decrease in dopamine (DA) levels that is responsible for the motor symptoms of PD, such as resting tremor, bradykinesia, rigidity, and gait dysfunction (Dauer and Przedborski 2003). Nonmotor symptoms are also common and can significantly debilitate a patient's activities and quality of life. Depression associated with PD is a very common comorbidity, present in approximately 35% of patients (Aarsland et al. 2012). Other disorders, such as anxiety, apathy, and psychosis, are estimated to be present in 16-70% of these patients (Hou and Lai 2007).

Pathologically, PD is characterized by the cytoplasmic accumulation of proteinaceous aggregates (i.e., Lewy bodies, α-synuclein, and ubiquitin; Mosley et al. 2012), resulting in a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and their projections to the caudate nucleus (Dauer and Przedborski 2003). The mechanism by which neurons degenerate in PD is not fully understood, but it appears to involve several factors, including neuroinflammation, oxidative stress, deficiency in mitochondrial complex I activity, apoptosis, and excitotoxicity (Hirsch et al. 2012; Thakur and Nehru 2013).

The use of the rotenone-induced PD model in animals is extreme valuable. Rotenone is both an herbicide and pesticide extracted from *Leguminosa* plants. It is highly lipophilic and readily crosses the blood-brain barrier. It has been used extensively as a prototypical

mitochondrial toxin in cell cultures (Blesa et al. 2012; Bové and Perier 2012), apart from to be linked to a higher risk of developing PD in humans exposed to it (Dhillon et al. 2008; Tanner et al. 2011). This toxin impairs oxidative phosphorylation in mitochondria by inhibiting reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase or complex I of the electron transport chain (Bové and Perier 2012). Rotenone also inhibits the formation of microtubules from tubulin (Choi et al. 2011). The oxidative impairment of mitochondrial function induced by rotenone causes bioenergetic failure through the production of oxidative molecules, leading to cell death accompanied by microglial activation and neuroinflammation (Cabezas et al. 2012).

Ibuprofen, a nonsteroidal antiinflammatory drug (NSAID), was the first member of the propionic acid derivatives to be presented as an aspirin alternative in 1969. The antiinflammatory effect of ibuprofen is mediated by its ability to nonselectively inhibit the isoforms of cyclooxygenase (COX). A recent study that used an animal model of Alzheimer's disease found that ibuprofen acted as an antioxidant, promoting neuroprotection through alternative mechanism that are independent of COX inhibition (Wilkinson et al. 2012).

Over the past few years, the potential neuroprotective effects of ibuprofen, especially in neurodegenerative diseases, has presented conflicting results. *In vitro* studies demonstrated that the effects of ibuprofen against glutamate-induced toxicity on dopaminergic neurons are concentration-dependent, inducing increases in both neuronal survival and death (Casper et al. 2000). An increase in neuronal toxicity upon exposure to 1-methyl-4-phenylpyridinium (MPP^+) was also reported (Morioka et al. 2004), in addition to the absence of any effect against the same neurotoxin (Soliman et al. 2009). In contrast to the *in vitro* results, Hsieh et al. (2011) demonstrated that ibuprofen can protect against toxicity mediated by MPP^+.

In animal models of PD, one of the few studies that investigated ibuprofen did not demonstrate a neuroprotective effect against toxicity induced by 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine in rats (Aubin et al. 1998). However, in humans, the use of ibuprofen has been shown to be associated with a decreased risk of developing PD (Chen et al. 2005; Gao et al. 2011).

Considering that the SNpc is extremely susceptible to cytotoxicity because it has lower amounts of natural antioxidant defenses and higher levels of free, in the present study we evaluated the potential neuroprotective effects of ibuprofen on motor and depressive-like behavior in a model of parkinsonism induced by short-term rotenone administration in rats.

METHODS

Animals

Male Wistar rats from our breeding colony, weighing 290-330 g at the beginning of the experiments, were used. The animals were randomly housed in groups of five in polypropylene cages with wood shavings as bedding and maintained in a temperature-controlled room (22 ± 2ºC) on a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The animals had free access to water and food throughout the experiment. The studies were performed in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals, United States National Institutes of Health. The protocol complied with the recommendations of the Federal University of Paraná and was approved by the University Ethics Committee (protocol no. 589).

Behavioral analysis

Open-field test (OFT). The apparatus consisted of a circular box (97 × 32.5 cm) with the floor divided into 19 squares around a central circle (Broadhurst 1960). The animals were

gently placed in the right center of the open field and allowed to freely explore the area for 5 min. Two motor parameters were quantified in this test: locomotion frequency (i.e., the number of crossings from one quadrant to another) and rearing frequency (i.e., the number of times the animals stood on their hind paws). The open field was cleaned with a 5% water-ethanol solution before behavioral testing to eliminate possible bias caused by odors left by previous rats.

Modified forced swim test (FST). This procedure was a modification of the methods proposed by Lucki (1997), Porsolt et al (1978), and Reneric et al (2002). The test was conducted in two sessions. First, in the training session, the rats were placed in a tank (25 cm diameter, 60 cm height) that contained water at a temperature of 23 ± 1ºC at a depth of 25 cm for 15 min. Twenty-four hours after the training session, the rats were subjected to the FST for 5 min, which was videotaped for the subsequent quantification of the following parameters: immobility (i.e., the lack of motion of the whole body, consisting only of small movements necessary to keep the animal's head above the water), climbing (i.e., vigorous movements with forepaws in and out of the water, usually directed against the wall of the tank), and swimming (i.e., large forepaw movements that displaced water and moved the body around the cylinder, more than necessary to merely keep the head above the water). The water was changed after each animal to avoid possible bias.

Neurochemical analysis

Preparation of subcellular fractions of brain. The striatum and hippocampus were homogenized with 200 mM potassium phosphate buffer, pH 6.5, and the homogenate was used to determine reduced glutathione (GSH) and lipid hydroperoxide (LOOH) levels,

followed by centrifugation at 9,000 × *g* for 20 min. The supernatant was used for the determination of superoxide dismutase (SOD) and catalase (CAT).

Protein assay. Protein concentrations were determined using the Bradford method according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA), with bovine serum albumin as the standard.

Determination of LOOH content. The levels of LOOH in the striatum and hippocampus were determined using the Ferrous Oxidation-Xylenol Orange (FOX2) method as described by Jiang et al. (1992). Briefly, 10 μl of 90% methanol was added to 100 μl of the homogenate, sonicated, and centrifuged at 9,000 × *g* for 20 min at 4°C. The supernatant was mixed with FOX2 reagent and incubated for 30 min at room temperature. Absorbance was determined at 560 nm in a microplate reader, and the results are expressed as mmol/mg of tissue.

Determination of GSH levels. Reduced glutathione levels in the striatum and hippocampus were determined using the method of Sedlak and Lindsay (1968). Aliquots of tissue homogenate were mixed with 12.5% trichloroacetic acid, vortexed for 10 min, and centrifuged at 900 × *g* for 15 min. The supernatant was then mixed with 0.4 M TRIS buffer (pH 8.9) and 0.01 M 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). Absorbance was measured by spectrophotometry at 415 nm with a microplate reader. The procedures were performed at 4ºC, and the individual values were interpolated into a standard curve for GSH (0.375-3 μg) and are expressed as μg/g of tissue.

Determination of SOD activity. Superoxide dismutase activity in the striatum and hippocampus was determined based on the capacity of SOD to inhibit pyrogallol autoxidation (Marklund and Marklund 1974; Gao et al. 1998). Pyrogallol (1 mM) was added to the buffer solution (200 mM Tris HCl–ethylenediaminetetraacetic acid [EDTA], pH 8.5) and supernatant aliquots and then vortexed for 1 min. The reaction was incubated for 20 min at

room temperature, stopped with the addition of 1N HCl, and centrifuged at 18,700 × *g* for 4 min. The absorbance of the resulting supernatant was measured at 405 nm using a microplate reader. The amount of SOD that inhibited the oxidation of pyrogallol by 50% relative to the control was defined as one unit of SOD activity.

Determination of CAT activity. CAT activity in the striatum and hippocampus was determined according to the method of Aebi (1984). Briefly, 10 µl aliquots of the supernatant was added to 990 µl of the reaction buffer that contained 1 mM Tris, 5 mM EDTA, and 30% H_2O_2 (pH 8.5) and vortexed for 1 min. The decrease in the optical density caused by the decomposition of H_2O_2 was measured at 240 nm and recorded to calculate CAT activity. Catalase activity was defined as the amount of enzyme required to decompose 1 nM of H_2O_2 per minute at 25°C. The results are expressed as millimole per minute per milligram of protein (mmol•min^{-1}/mg of protein).

Tyrosine hydroxylase immunohistochemistry. For the histological and immunohistochemical study, the rats were deeply anesthetized with thiopental and intracardially perfused with saline, followed by 4% of the fixative solution (formaldehyde in 0.1 M phosphate buffer, pH 7.4). The brain was then placed in a 30% sucrose solution for 48 h before sectioning. The brains were then stored in a freezer (-80°C). Afterward, they were fixed in a cryostat at -20°C and cut. From the corpus callosum, 180 cuts were counted to reach the substantia nigra, according to Paxinos and Watson (1998). We collected twelve 40-µm sections from the substantia nigra, which were placed on plates that contained an antifreeze substance. The tissue sections were incubated with primary anti-tyrosine hydroxylase (TH) antibody raised in mouse and diluted in phosphate-buffered saline (PBS) that contained 0.3% Triton X-100 (1:500; cat no. AB152, Chemicon, Temecula, CA, USA) overnight at room temperature. The slides were then incubated with biotin-conjugated secondary antibody (1:200; cat no. S-1000, Vector Laboratories, Burlingame, CA, USA) for 2

h at room temperature. After several washes in PBS, the antibody complex was detected using a modification of the ABC system (Vectastain ABC Elite kit, cat no. PK6101, Vector Laboratories), followed by reacting with 3,3'-diaminobenzidine. The slides were then dehydrated in an ascending series of ethanol concentrations, cleared in xylene, and coverslipped. To perform readings of the slices, we took photographs using a microscope (LEICA DM 2500) at 10× magnification and transferred the pictures to a computer. The optical density of each slice was determined using ImageJ software. To estimate the extent of neuronal loss in the midbrain caused by rotenone, we assessed the dopaminergic cell group in the SNpc in control animals, which showed a preserved cytoarchitecture and normal TH immunostaining. We then compared these observations to the same parts of the dopaminergic cell groups in the rotenone and other groups.

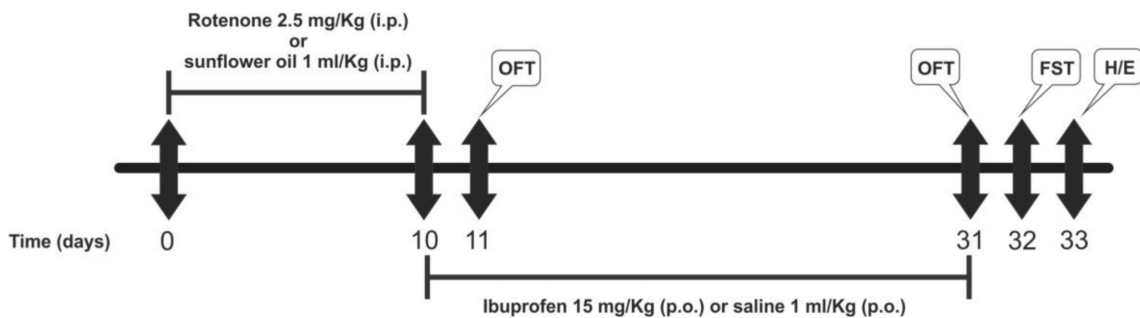
Experiment design

Dose-response curve. The rats were randomly distributed into the following groups: saline, 5 mg/kg ibuprofen, 15 mg/kg ibuprofen, 30 mg/kg ibuprofen, and 50 mg/kg ibuprofen. Ibuprofen (Sigma-Aldrich, Germany) and saline were administered by gavage 24, 8, and 1 h before locomotor activity and depressive-like behavior were measured in the open-field test (OFT) and modified forced swim test (FST), respectively.

Rotenone and ibuprofen treatment. The rats were randomly distributed into two groups: vehicle and 2.5 mg/kg rotenone. Rotenone (Sigma-Aldrich, Germany) or vehicle (sunflower oil) was injected intraperitoneally daily for 10 days, from 10:00 AM to 11:00 AM. On day 10, after rotenone administration, the animals were randomly redistributed into four subgroups: vehicle+saline, vehicle+ibuprofen, rotenone+saline, and rotenone+ibuprofen. On the same day, treatment began with 15 mg/kg ibuprofen (selection based on the dose-response

curve) or saline by gavage, which was conducted once daily for 21 days. All of the animals were weighed daily.

The open-field test was conducted on days 11 and 31. The FST was conducted on day 31 (training) and day 32 (test). Subsequently, on day 33, all of the animals were decapitated, followed by dissection of the striatum and hippocampus for the neurochemical assays.



OFT (open field test); FST (forced swim test); H/E (dissection of the striatum and hippocampus).

Statistical analysis

The Kolmogorov-Smirnov test was used to determine the normal distribution of the data ($p > 0.05$). All of the data were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey *post hoc* test. The results are expressed as mean ± standard error of the mean (SEM). Statistically significant differences were set at $p < 0.05$.

RESULTS

Dose-Response Curve

The open-field test results (Table 1) showed no difference between groups in locomotion frequency ($F_{4,38} = 0.3461$, $p = 0.8451$) and rearing frequency ($F_{4,38} = 0.9881$, $p = 0.4257$). However, in the FST, the animals treated with ibuprofen (5, 15, and 30 mg/kg)

exhibited a decrease in immobility time compared with the saline group ($F_{4,57}$ = 11.17, p = 0.0001). The analysis of swimming time ($F_{4,57}$ = 1.119, p = 0.3567) and climbing time ($F_{4,57}$ = 0.5187, p = 0.7223) revealed no differences between groups (Table 1).

Behavioral Analysis

Assessment of motor activity

In the OFT performed on day 11, A reduction of motor activity was observed in the animals treated with rotenone compared with the vehicle group ($F_{3,66}$ = 11.51, p = 0.0001). In the second trial, performed on day 31, the animals treated with rotenone and ibuprofen exhibited an increase in locomotion frequency compared with the rotenone group ($F_{3,66}$ = 4.316, p = 0.0077; Fig. 1).

Assessment of depressive-like behavior

In the FST, we observed a significant decrease in swimming time ($F_{3,43}$ = 5.638, p = 0.0024) and an increase in immobility time ($F_{3,43}$ = 6.562, p = 0.0009) in the animals treated with rotenone compared with the vehicle group. Ibuprofen treatment reverses these impairments. No differences in climbing time were observed between groups ($F_{3,43}$ = 0.6222, p = 0.6045; Fig. 2).

Neurochemical Analysis

Tyrosine hydroxylase immunohistochemistry

The immunohistochemical analysis (Fig. 3 and 4) revealed a significant reduction (~13%) of TH immunoreactivity in the SNpc in the animals treated with rotenone compared with the vehicle group ($F_{3,67}$ = 9.620, p = 0.0001). Ibuprofen treatment protected animals from dopaminergic neuron loss (Fig. 3, 4).

Quantification of LOOH levels in the hippocampus and striatum

The levels of LOOH in the hippocampus were not different between groups ($F_{3,36}$ = 2.445, p = 0.0797). In the striatum, however, the animals treated with rotenone and ibuprofen exhibited a decrease in LOOH compared with the vehicle group ($F_{3,39}$ = 5.256, p = 0.0038; Table 2).

Quantification of GSH levels in the hippocampus and striatum

The levels of GSH in the hippocampus in the animals treated with rotenone were reduced compared with the vehicle group, and ibuprofen treatment reversed this depletion ($F_{3,23}$ = 8.034, p = 0.0008). Ibuprofen treatment increased the basal levels of GSH in the striatum ($F_{3,29}$ = 11.67, p = 0.0001; Table 2).

Quantification of SOD activity in the hippocampus and striatum

The activity of SOD in the hippocampus ($F_{3,38}$ = 2.842, p = 0.0506) and striatum ($F_{3,39}$ = 3.486, p = 0.0246) in the animals treated with rotenone and ibuprofen was significantly reduced compared with the vehicle group (Table 2).

Quantification of CAT activity in the hippocampus and striatum

The activity of CAT in the hippocampus ($F_{3,21}$ = 10.76, p = 0.0002) and striatum ($F_{3,16}$ = 21.92, p = 0.0001) in the animals treated with rotenone was significantly reduced compared with the vehicle group. Ibuprofen treatment reverses these reductions (Table 2).

DISCUSSION

The hypothesis of this study was that the inflammation arising from peripheral administration of rotenone is able to induce motor and nonmotor impairments in rats through

a neurodegenerative process. We also speculated that ibuprofen post-treatment in these animals would effectively reverse these deficits.

The present results showed that rotenone induced motor and depressive-like behavior and caused an imbalance in the antioxidant system, resulting in the neurodegeneration of dopaminergic neurons in the SNpc. Ibuprofen was able to reverse these deficits, likely because of its antiinflammatory and antioxidant properties.

The use of ibuprofen in an animal model of PD has been studied previously (Aubin et al. 1998). The authors found that 20 mg/kg ibuprofen was ineffective in inducing neuroprotection against MPTP-induced neurotoxicity. In the present study, we evaluated the effects of several doses of ibuprofen. Our data showed that 5, 15, and 30 mg/kg ibuprofen administered acutely induced antidepressant-like effects in rats (Table 1). Thus, we opted to continue our studies using the 15 mg/kg dose, which was similar to the effective dose used by Aubin et al. (1998).

In the present study, rotenone induced motor impairment. The animals that received a single dose of ibuprofen by gavage 24 h before the test presented an absence of motor impairment (Fig. 1). A recent study that subcutaneously administered rotenone for 5 weeks observed significant motor impairment, and co-treatment with sodium salicylate, an NSAID, was unable to reverse these deficits (Thakur and Nehru 2013).

A reduction of TH immunoreactivity was observed in the SNpc in animals treated with rotenone (Fig. 3, 4), confirming the involvement of the dopaminergic pathway in motor impairment. Other studies also demonstrated the ability of rotenone to promote neurodegeneration in this pathway (Betarbet et al. 2000; He et al. 2003; Höglinger et al. 2003; Sherer et al. 2003; Fleming et al. 2004; Lapointe et al. 2004; Zhu et al. 2004; Huang et al. 2006; Phinney et al. 2006; Luo et al. 2007; Cannon et al. 2009; Madathil et al. 2013). Other recent data showed a significant reduction of DA content in the striatum in animals treated

with rotenone and an increase in the turnover of this monoamine, suggesting the occurrence of a compensatory neurochemical mechanism that could be related to the spontaneous recovery of motor activity observed in the open-field test conducted on day 31 (Morais et al. 2012; Thakur and Nehru 2013).

As shown in Fig. 2, the animals treated with rotenone and subjected to the FST exhibited a significant decrease in total swimming time and an increase in immobility time, suggesting that a more pronounced depressive-like behavior was induced by this toxin. Such behavior was not observed in the animals treated with ibuprofen. These data indicate that ibuprofen is able to induce an antidepressant-like effect in the FST when depressive-like behavior is induced by rotenone. Acute ibuprofen treatment also induced an antidepressant-like effect in the FST in naive animals, but when the vehicle and ibuprofen groups received the same treatment for a long period of time, however, we did not observe this effect.

The present study found an antidepressant-like effect of ibuprofen, but such an effect other NSAIDs, such as rofecoxib (which increases serotonin levels and decreases the maximum number of 5-HT_2 receptors) and acetylsalicylic acid (which might accelerate the onset of action of selective serotonin reuptake inhibitors), has already been characterized (Sandrini et al. 2002; Brunello et al. 2006; Muller et al. 2006).

Our FST data suggest that the increase in norepinephrine and DA levels in the hippocampus is related to the decrease in immobility time (Porsolt et al. 1978; Reneric et al. 2002). The mechanism that underlies the antidepressant-like effect of ibuprofen may be related to COX inhibition and its antioxidant properties. These parameters could lead to a reduction of the synthesis of prostaglandin E_2 (PGE_2), increases in which are related to indoleamine 2,3-dioxygenase (IDO) overexpression (Muller et al. 2011).

Inflammatory mediators, such as cytokines and PGE_2, increase the activity of IDO in patients diagnosed with major depression (Linnoila et al. 1983; Mikova et al. 2001). As a

result of increased IDO activity, a decrease in serotonergic neurotransmission occurs in the CNS because the availability of tryptophan is decreased, which limits the synthesis of serotonin (Muller et al. 2011). *In vitro* studies have demonstrated that antidepressants, such as fluoxetine and amitriptyline, are also able to inhibit PGE_2 synthesis (Yaron et al. 1999), suggesting the involvement of this mediator in depressive behavior.

With regard to the motor and nonmotor impairment observed in the present study, oxidative stress may be associated with these observations. Rotenone inhibits the activity of NADH-ubiquinone reductase (Schuler and Casida 2001), leading to the formation of reactive species, such as superoxide anions ($O_2^{\bullet-}$). The formation of these reactive species is especially important in the SNpc because of high levels of DA metabolism, the high prevalence of glial cells, and particularly the low levels of antioxidant defenses in this region (Kim et al. 2000; Thakur and Nehru 2013). To confirm our hypothesis, we assessed LOOH levels, GSH content, SOD activity, and CAT activity in the striatum and hippocampus in rotenone-treated animals.

Lipid peroxidation occurs as consequence of an imbalance between the generation of reactive species and the amount of endogenous antioxidants, such as GSH, resulting in the oxidation of these lipids and subsequently their degradation. Polyunsaturated fatty acids found widely in the central nervous system are the most susceptible to this process (Sanders and Greenamyre 2013). In the present study, we found no difference in lipid peroxidation in the hippocampus between groups, but we observed a decrease in GSH content in the hippocampus in animals treated with rotenone. Ibuprofen effectively reversed this depletion. Similarly, rotenone did not appear to induce lipid peroxidation or GSH depletion in the striatum, whereas ibuprofen was able to increase the basal content of GSH in this region.

The lack of an increase in lipid peroxidation observed in the present study could be attributable to the long time interval (i.e., 24 days) between the last rotenone administration

and the assessment of this parameter. Thus, a different approach may be necessary to evaluate this parameter in future studies.

The mechanism responsible for the increase in GSH content may be related to (*i*) a direct antioxidant effect, in which ibuprofen could act as a scavenger of reactive species to preserve GSH content, or (*ii*) an indirect antioxidant effect, in which ibuprofen might induce the synthesis of GSH and reduce its degradation through mechanisms that are not yet understood. Thus, further studies are needed to confirm these possibilities.

Superoxide dismutase and CAT are important parts of the enzymatic antioxidant defense system. Superoxide dismutase is the first line of defense against reactive species and responsible for catalyzing $O_2^{\bullet -}$, which is highly reactive and toxic, to H_2O_2 (hydrogen peroxide), which is less reactive and toxic (Winterbourn 1993). Although this enzyme is a potent antioxidant defense, its physiological role appears to be ambiguous because this can lead to an increase in oxidative stress through excessive H_2O_2 formation, especially if CAT activity is impaired (Michel et al. 2012). In models of neurodegeneration, an increase in CAT levels has been shown to be beneficial, whereas a decrease in CAT activity leads to the increased susceptibility of neurons to oxidative injury (Baker et al. 1998; Klivenyi et al. 2000; Clausen et al. 2012).

In the present study, SOD activity was decreased in the hippocampus and striatum in animals treated with rotenone and ibuprofen (Table 2). The activity of CAT was reduced in both the hippocampus and striatum in animals treated with rotenone. Interestingly, ibuprofen treatment effectively reversed this impairment (Table 2). Madathil et al. (2013) recently demonstrated that rotenone infusion in rats does not alter SOD activity. However, when these animals were treated with sodium salicylate, an NSAID with potent antioxidant properties (Esposito et al. 2007), SOD activity was significantly increased.

The decrease in SOD activity observed in the animals treated with rotenone and ibuprofen may be related to an increase in GSH content in these animals, which could be responsible for neutralizing and reducing the formation of $O_2^{-\bullet}$, a substrate for SOD. Supporting this possibility, we did not observe an increase in basal CAT activity in these animals.

The production of reactive species in the central nervous system comes from two main sources: (*i*) the mitochondrial respiratory chain and (*ii*) microglial activation (Thakur and Nehru 2013). The neuroprotection provided by ibuprofen against rotenone-induced oxidative stress may derive from both sources, including its antiinflammatory effects and its effect on increasing GSH content.

In summary, the present data suggest that rotenone is able to induce motor and depressive-like effects, accompanied by an imbalance in the antioxidant system that leads to the neurodegeneration of dopaminergic neurons in the SNpc. Posttreatment with ibuprofen was able to reverse these deficits, possibly because of its antioxidant and antiinflammatory properties.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflicts of interest.

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Table 1. Ibuprofen dose-response curve. Naive male Wistar rats received acute ibuprofen by gavage (24, 8, and 1 h before the behavioral tests). After the acute treatment, the animals were subjected to the open-field test and forced swim test.

	Open-field test		Forced swim test		
	Locomotion	Rearing	Swimming	Climbing	Immobility
Saline	101.9 ± 6.5	30.7 ± 2.8	184.9 ± 10.7	70.5 ± 11.1	44.6 ± 1.2
Ibuprofen 5 mg/kg	94.8 ± 7.2	27.4 ± 2.0	195 ± 8.1	72.1 ± 8.7	32.9 ± 1.2*
Ibuprofen 15 mg/kg	95.2 ± 6.6	27.1 ± 3.7	206.9 ± 8.8	59.0 ± 7.7	34.0 ± 1.6*
Ibuprofen 30 mg/kg	93.1 ± 11.1	23.2 ± 2.8	213.3 ± 8.6	54.9 ± 8.6	31.7 ± 1.3*
Ibuprofen 50 mg/kg	104.4 ± 8.4	23.6 ± 3.3	199.0 ± 12.6	62.0 ± 12.3	38.9 ± 2.0

The results are expressed as mean ± SEM (n = 8-14). *$p < 0.05$, compared with saline group (ANOVA followed by Tukey's test).

Table 2. Effect of ibuprofen (15 mg/kg. p.o.) on LOOH content (mmol/mg of tissue), GSH content (µg/g of tissue), SOD activity (U/g of tissue), and CAT activity (mmol•min^{-1}/mg of protein) in the hippocampus and striatum. The animals were treated with rotenone (2.5 mg/kg, i.p.) for 10 consecutive days and post-treated with ibuprofen once daily for 22 days.

	Vehicle + saline	Vehicle + ibuprofen	Rotenone + saline	Rotenone + ibuprofen
LOOH				
Hippocampus	68.55±6.55	68.39±3.74	55.86±2.45	60.51±2.44
Striatum	53.31±2.31	59.08±2.05	48.79±1.62	48.68±2.35§
GSH				
Hippocampus	1395±95.92	1423±80.48	717.4±99.33*	1176±137.20$^{\#}$
Striatum	387.20±55.30	771.80±63.22*	406.70±40.50	623.80±49.35$^{\#}$
SOD				
Hippocampus	19.30±1.40	21.19±1.03	18.52±0.78	17.63±0.70§
Striatum	19.13±0.69	20.71±1.00	17.24±1.38	15.53±1.21§
CAT				
Hippocampus	268.40±38.81	203.50±19.90	95.47±9.15*	169.80±15.75$^{\#}$
Striatum	180.40±18.42	136.30±12.09*	78.07±3.79*	116.00±4.43$^{\#}$

The results are expressed as mean ± SEM (n = 4-13). $^{*}p < 0.05$, compared with vehicle+saline group; $^{\S}p < 0.05$, compared with vehicle+ibuprofen group; $^{\#}p < 0.05$, compared with rotenone+saline group (ANOVA followed by Tukey's test).

FIGURE LEGENDS

Fig. 1. Effect of ibuprofen (15 mg/kg, p.o.) on locomotor activity in male Wistar rats pretreated with rotenone (2.5 mg/kg, i.p.). The animals were treated with rotenone for 10 consecutive days and posttreated with ibuprofen once daily for 22 days. The results are expressed as mean ± SEM (n = 16-19). *$p < 0.05$, compared with vehicle+saline group; $^{\#}p < 0.05$, compared with rotenone+saline group (ANOVA followed by Tukey's test).

Fig. 2. Antidepressive effect of ibuprofen (15 mg/kg, p.o.) in the modified forced swim test in male Wistar rats pretreated with rotenone (2.5 mg/kg, i.p.). The animals were treated with rotenone for 10 consecutive days and posttreated with ibuprofen once daily for 22 days. The results are expressed as mean ± SEM (n = 11-13). *$p < 0.05$, compared with vehicle+saline group; $^{\#}p < 0.05$, compared with rotenone+saline group (ANOVA followed by Tukey's test).

Fig. 3. Quantification of optical density of TH immunoreactivity in the SNpc. The animals were treated with rotenone for 10 consecutive days and posttreated with ibuprofen once daily for 22 days. The results are expressed as a percentage of the control group (mean ± SEM; n = 3). *$p < 0.05$, compared with vehicle+saline group; $^{\#}p < 0.05$, compared with rotenone+saline group (ANOVA followed by Tukey's test).

Fig. 4. Histological analysis of the SNpc in animals treated with rotenone for 10 consecutive days and posttreated with ibuprofen once daily for 22 days. Representative photomicrographs of TH-immunoreactive neurons in the SNpc are shown. (a) Vehicle+saline group. (b) Vehicle+ibuprofen group. (c) Rotenone+saline group. (d) Rotenone+ibuprofen group.

Fig. 1.

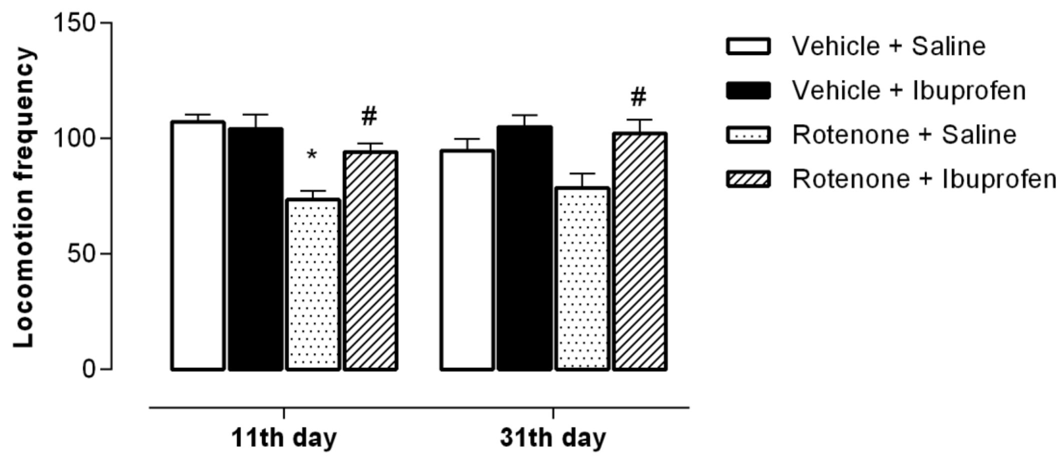


Fig. 2.

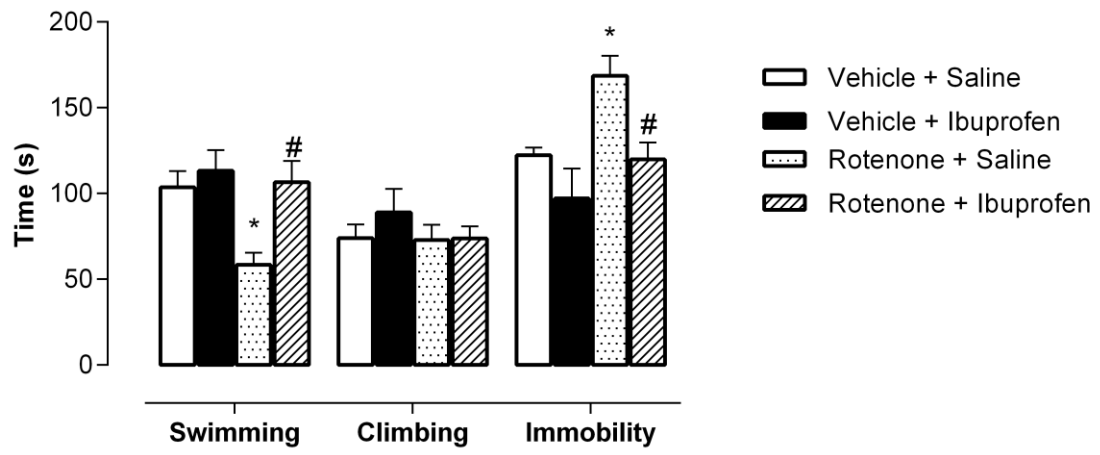


Fig. 3.

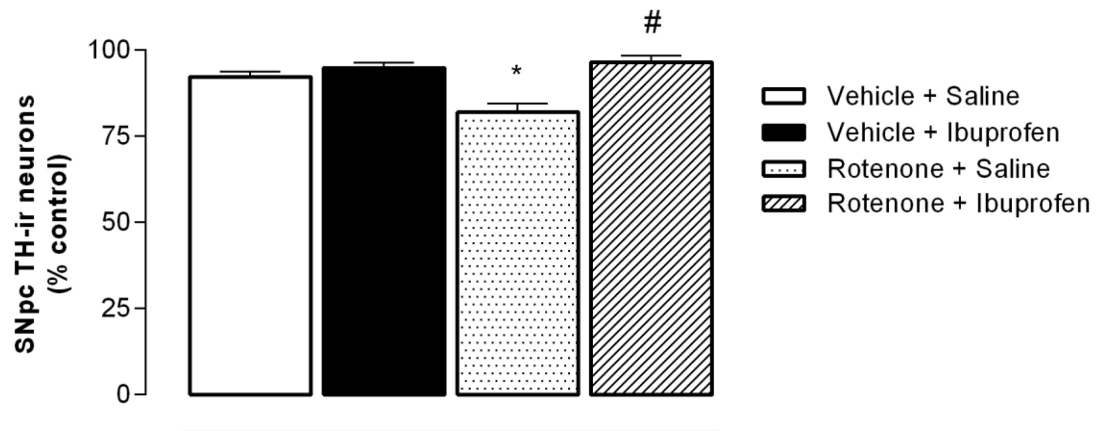
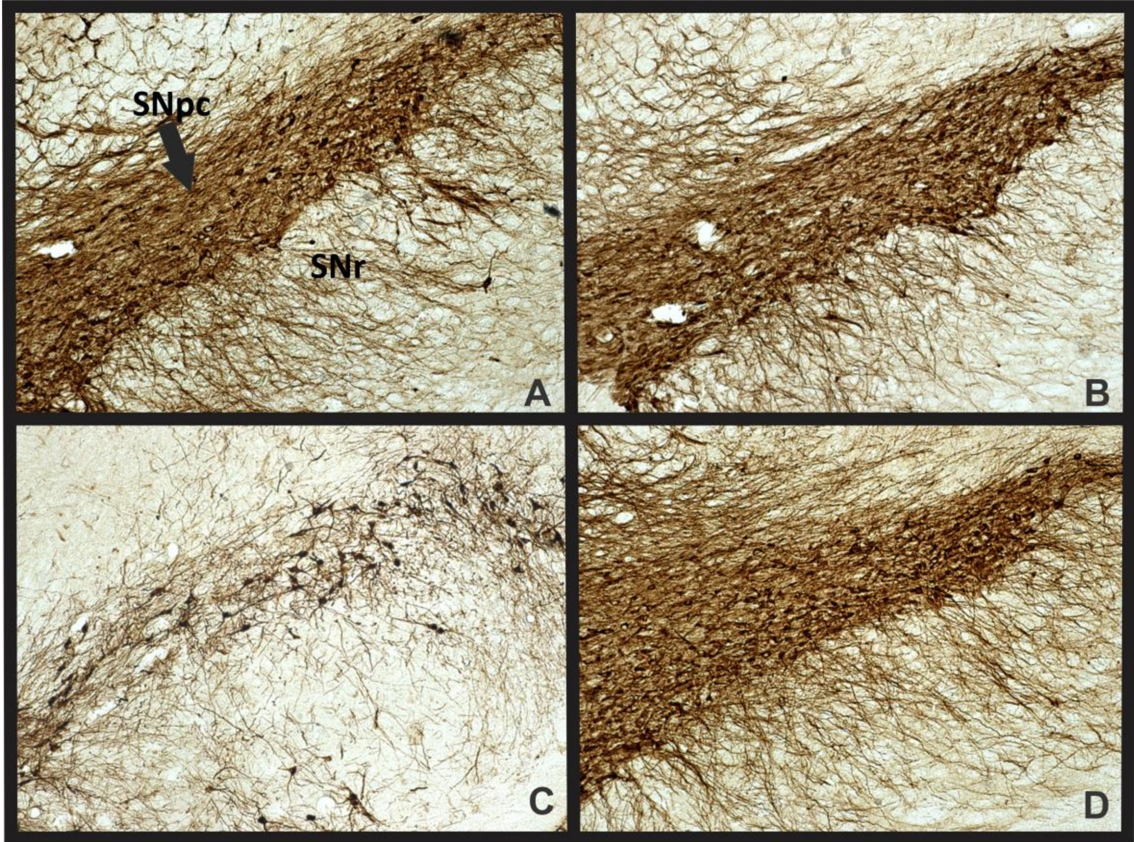


Fig. 4.



4. CONCLUSÕES

Nossos resultados sugerem que a administração de rotenona induz prejuízo motor e comportamento tipo-depressivo em ratos, acompanhado de um desequilíbrio no sistema redox, resultando em neurodegeneração dos neurônios dopaminérgicos da SNpc.

Da mesma forma, demonstramos que o pós-tratamento com ibuprofeno é capaz de reverter estes prejuízos, possivelmente por mecanismos dependentes de seu efeito antioxidante e inibitório sobre a enzima COX.

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