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Synergistical neuroprotection of rofecoxib and statins against malonic acid induced Huntington's disease like symptoms and related cognitive dysfunction in rats

Anil Kumar*, Neha Sharma, Jitendriya Mishra, Harikesh Kalonia

Pharmacology Division, University Institute of Pharmaceutical Sciences, UGC-Centre of Advanced Study (UGC-CAS), Panjab University, Chandigarh 160014, India

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ABSTRACT

Malonic acid (MA) is a reversible inhibitor of succinate dehydrogenase (SDH) which induces mitochondrial dysfunction followed by secondary excitotoxicity and apoptosis due to generation of reactive oxygen species. Therapeutic potential of rofecoxib and statins have been well documented in several experimental models of neurodegenerative disorders, however, its exact mechanism of action is not known properly. Therefore, the present study is an attempt to investigate the effect of rofecoxib along with the statins against MA induced behavioural and biochemical alterations in rats. Single intrastriatal MA (6 μmol) significantly caused motor incordination, memory dysfunction and alteration in the antioxidant enzyme levels, mitochondrial enzyme complex (I, II, IV) activities, mitochondrial redox ratio and pro-inflammatory cytokine [tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6)] levels in the striatum as compared to the naive group. Fourteen days treatment with rofecoxib, atorvastatin, simvastatin significantly attenuated these behavioural, biochemical, and cellular alterations as compared to control (MA treated group). However, the treatment of rofecoxib along with atorvastatin or simvastatin significantly attenuated these behavioural, biochemical, and cellular alterations as compared to their individual effects. The results of the present study demonstrated that rofecoxib modulates the protective effects of statins against MA-induced neurobehavioral and related biochemical and cellular alterations in rats. This further provides evidence toward the involvement of neuroinflammatory cascade in the pathogenesis of Huntington's disease.

1. Introduction

Huntington disease is an inherited neurodegenerative disorder, mainly caused due to the expansion of a polymorphic CAG trinucleotide repeat encoding polyglutamine tract within htt gene (Bates, 2003). Huntington disease is primarily characterized by loss of medium spiny neurons in basal ganglia, which involves progressively worsening chorea followed by cognitive and psychiatric disturbances (Kent, 2004). Several studies suggest that neuro-inflammation, apoptosis, oxidative stress and mitochondrial dysfunction are widely involved in the pathophysiology of Huntington disease (Walker and Raymond, 2004; Kumar et al., 2007; Estrada Sanchez et al., 2008; Tasset et al., 2009).

Malonic acid (MA) is a reversible inhibitor of succinate dehydrogenase (SDH) which induces mitochondrial dysfunction followed by secondary excitotoxicity and apoptosis due to generation of reactive oxygen species (Dedeoglu et al., 2002). Intrastriatal MA induces a similar pattern of cell damage as seen in Huntington disease patients by a mechanism that involves interference with the activity of an oxidative phosphorylation enzyme complex (Perez-De La Cruz et al., 2009). Malonate increases the conversion of salicylate to 2,3- and 2,5-dihydroxybenzoic acid, an index of hydroxyl radical generation suggesting an involvement of oxidative damage mechanisms in malonate toxicity (Klivenyi et al., 2000; Schulz et al., 1996). Further, elevated 3-nitrotyrosine concentrations are reported after intrastriatal malonate injection, and malonate lesions were attenuated by free radical spin traps and nitric oxide synthase (NOS) inhibitors. Hence there is substantial evidence that NO-mediated oxidative damage is involved in cell death processes following energetic disruption (Matthews et al., 1998). Hence, the compounds that can attenuate the energy impairment and oxidative stress caused by MA may demonstrate therapeutic benefit in the management of Huntington disease like symptoms.

Statins, 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors, imparts neuroprotective effects in various neurodegenerative diseases including Huntington disease, but the

* Corresponding author. Tel.: +91 172 2534106; fax: +91 172 2543101.
E-mail address: kumaruips@yahoo.com (A. Kumar).

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underlying mechanism is poorly understood (Patassini et al., 2008). Various preclinical and retrospective studies demonstrated the potential of stains in attenuation of oxidative stress as well as the process of neuro-inflammation in various neurodegenerative conditions. (Franke et al., 2007; Ginter and Simko, 2009; Gaur and Kumar, 2011; Kumar et al., 2012a). However, some studied in the recent past have demonstrated about the involvement of altered cholesterol homeostasis in the increased excitotoxicity in the brain (del Toro et al., 2010; Simons and Ehehalt, 2002; Distl et al., 2003; Valenza and Cattaneo, 2006).

Cyclooxygenases (COX), the key enzymes in arachidonic acid pathway, exist in two isoforms i.e. COX-1 and COX-2 (Botting, 2000). Recently, a third variant of COX has been described as COX-3 (more appropriately renamed COX-1b), is a splice variant of COX-1 (Chandrasekharan et al., 2002). COX-1 is constitutive and participates in normal physiology, whereas COX-2 is induced in pathological states and exerts effect in homeostasis (Boyd et al., 2007; Saldana et al., 2008; Huntjens et al., 2009). Various COX inhibitors have already been reported to possess neuroprotective potential against colchicines, 3-nitropropionic acid, quinolinic acid and kainic acid induced neurotoxicity (Kumar et al., 2006, 2012b; Kalonia et al., 2009, 2010a). Further, increased COX-2 expression has also been demonstrated in Huntington's disease like conditions (Breder et al., 1995).

With the above background, the present study has been undertaken to evaluate the neuroprotective effect of COX-inhibitors, HMG-CoA reductase inhibitors and their combination against MA induced Huntington disease like symptoms and cognitive dysfunction in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g), bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were housed under standard laboratory conditions and maintained on natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. The experimental procedures have been carried out between 09.00 h and 17.00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy (INSA) Guidelines for the use and care of experimental animals.

2.2. Drugs and treatment schedule

MA (6 μmol/4 μl) (Sigma, St. Louis, MO, USA), rofecoxib (10, 20 mg/kg, p.o.), atorvastatin (10, 20 mg/kg, p.o.) and simvastatin (15, 30 mg/kg, p.o.) (Ranbaxy Research Laboratories, Gurgaon, India) were used in present study. Rofecoxib (Rof), atorvastatin (Atr) and simvastatin (Sim) were suspended in 0.25% w/v sodium carboxy methyl cellulose (Na-CMC) and were administered in a constant volume of 0.5 ml/100 g body weight of rats.

Eleven groups were used in the present study, comprising of 12 animals in each group ($n=12$). Group I: Naive (without treatment); Group II: Sham (surgery performed, vehicle administered); Group III: Control (MA, 6 μmol/4 μl; single Intrastriatal injection); Group IV and V: MA (6 μmol/4 μl)+Rof (10 and 20 mg/kg) respectively; Group VI and VII: MA (6 μmol/4 μl)+Atr (10 and 20 mg/kg) respectively; Group VIII and IX: MA (6 μmol/4 μl)+Sim (15 and 30 mg/kg) respectively; Group X: MA (6 μmol/4 μl)+Rof (10 mg/kg) +Atr (10 mg/kg); Group XI: MA (6 μmol/4 μl)+Rof (10 mg/kg)+Sim (15 mg/kg) (Table 1).

Several previous studies in our laboratory reported that, rofecoxib (20 mg/kg, p.o.), atorvastatin (20 mg/kg, p.o.) and simvastatin (30 mg/kg, p.o.) per se groups did not exhibit any significant difference in the behavioral, biochemical and mitochondrial parameters as compared to the naive animals (Kalonia et al., 2010a, 2011a). Therefore, the per se groups for rofecoxib, atorvastatin and simvastatin at the above mentioned doses have been excluded from the present study protocol in order to minimize the use of experimental animals as per CPCSEA guidelines. Hence the protocol has been designed with an assumption that rofecoxib (20 mg/kg, p.o.), atorvastatin (20 mg/kg, p.o.) and simvastatin (30 mg/kg, p.o.) do not possess any per se effect in healthy rats.

2.3. Experimental protocol

MA (6 μmol/4 μl) was administrated as a single unilateral injection into the right striatum with a 28-gauge Hamilton syringe. Rofecoxib, atorvastatin and simvastatin were given per oral (p.o.) for a period of 14 days following surgery. Behavioral parameters (locomotor activity, rotarod and body weight) were assessed in weekly intervals (day 1, 7 and 14) whereas balance beam walk and Morris water maze performance test has been recorded on day 14 only. After 2 weeks of drug treatment following MA administration, animals were sacrificed by cervical dislocation, striatum were quickly removed, perfused with ice-cold normal saline, weighed and used for several biochemical and mitochondrial analysis. All behavioral parameters were assessed in a dimly illuminated and quiet environment (Fig. 1).

2.4. Intrastriatal administration of MA

Animals were anesthetized by thiopental sodium (45 mg/kg, i.p.) and 6 μmol of MA (4 μl) was injected into the right striatum with a 28-gauge Hamilton syringe over a period of 2 min, and the needle was left in the place for another 2 min to prevent back diffusion of the injected drug solution (Kalonia et al., 2010b). Injections were made via an l–2 mm diameter hole made in the skull using a small hand drill at anterior +1.7 mm; lateral ±2.7 mm; ventral −4.8 mm from bregma and dura as described in Paxinos and Watson (2007).

Table 1
Grouping of the experimental animals.

Group no.	Group name	Treatment
Group I	Naive	Without treatment
Group II	Sham	Surgery performed, vehicle administered
Group III	Control	MA (6 μmol/4 μl) (Single, intrastriatal injection)
Group IV and V	MA (6)+Rof (10 and 20)	MA (6 μmol/4 μl, intrastriatal)+Rofecoxib (10 and 20 mg/kg) respectively
Group VI and VII	MA (6)+Atr (10 and 20)	MA (6 μmol/4 μl, intrastriatal)+Atorvastatin (10 and 20 mg/kg) respectively
Group VIII and IX	MA (6)+Sim (15 and 30)	MA (6 μmol/4 μl, intrastriatal)+Simvastatin (15 and 30 mg/kg) respectively
Group X	MA (6)+Rof (10)+Atr (10)	MA (6 μmol/4 μl, intrastriatal)+Rofecoxib (10 mg/kg)+Atorvastatin (10 mg/kg)
Group XI	MA (6)+Rof (10)+Sim (15)	MA (6 μmol/4 μl, intrastriatal)+Rofecoxib (10 mg/kg)+Simvastatin (15 mg/kg)