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·Original Article·

Effects of caffeic acid, rofecoxib, and their combination against quinolinic acid-induced behavioral alterations and disruption in glutathione redox status

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Abstract: Objective The neuroprotective roles of cyclooxygenase (COX) and lipooxygenase (LOX) inhibitors have been well documented. Quinolinic acid (QA) is a well-known excitotoxic agent that could induce behavioral, morphological and biochemical alterations similar with symptoms of Huntington's disease (HD), by stimulating NMDA receptors. However, the exact roles of COX and LOX inhibitors in HD have not yet been explained. The present study aims to elucidate the effects of caffeic acid (a specific inhibitor for LOX), rofecoxib (a specific inhibitor for COX-2), and their combination in ameliorating QA-induced neurotoxicity in rats. **Methods** QA was injected into the right striatum of rats to induce neurotoxicity. Caffeic acid and rofecoxib were then orally administered separately. In the combination study, caffeic acid and rofecoxib were administered together. After that, a series of behavioral assessments were conducted to determine the effects of caffeic acid and rofecoxib, respectively, and the co-effect of caffeic acid and rofecoxib, against QA-induced neurotoxicity. **Results** Intrastriatal QA administration (300 nmol) not only induced a significant reduction in body weight and motor incoordination, but also altered the redox status (decreased glutathione and increased oxidized glutathione level) in striatum, as compared to the sham group. Moreover, chronic treatment with caffeic acid (5 mg/kg and 10 mg/kg, respectively, p.o.) or rofecoxib (10 mg/kg, p.o.) could significantly attenuate QA-induced behavioral alterations and restore the redox status in striatum. However, at the dose of 2.5 mg/kg, caffeic acid did not show any significant effects on these parameters in QA-treated rats. Furthermore, the combination of rofecoxib (10 mg/kg) and caffeic acid (5 mg/kg) could significantly protect against QA neurotoxicity. **Conclusion** The *in vivo* study indicates that excitotoxic injury to the brain might affect oxidant/antioxidant equilibrium by eliciting changes in glutathione. Moreover, the LOX and the COX pathways may be both involved in quinolinic-induced neurotoxicity, which provides a promising target for HD treatment.

Keywords: caffeic acid; cyclooxygenase; glutathione; quinolinic acid; rofecoxib

1 Introduction

Glutamate is an important excitatory amino acid, exerting both excitatory and neurotoxic effects. Although excitatory action is essential for normal neuronal activity, neurotoxic effects would appear when excessive excitatory neurotransmitters accumulate in the synaptic cleft. Neurotoxicity then

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causes disturbances in intracellular calcium homeostasis and mitochondrial energy metabolism, and is responsible for cell death in neurodegenerative disorders. For instance, excitotoxicity is partially responsible for the striatal GABAergic spiny neurons death in Huntington's disease (HD), a neurological disorder characterized by involuntary choreiform movements and cognitive impairment[1].

Quinolinic acid (QA) could induce lesions similar as the behavioral, morphological and neurochemical alterations in HD patients, including hyperactivity[2], cognitive impairment[3], depletion of spiny neuron markers[1,4], and reduction in GABAergic and cholinergic neurotransmission[5,6]. Hyperstimulation of NMDA receptors[7] leads to massive Ca^{2+} influxes that would then activate other cellular processes, such as Ca^{2+}-dependent production of phospholipase A2. The phospholipase A2 could cleave membrane phospholipids, leading to the production of arachidonic acid, which is then converted into prostaglandin (PG) G2 by cyclooxygenases (COXs)[8]. Subsequently, PGG2 is reduced to be PGH2 by peroxydase, with the productions of intermediate free radicals that rapidly convert to a reactive hydroxyl radical[9].

The involvement of oxidation in the cytotoxicity of excitatory amino acids has been well documented[10]. The term of oxidative stress describes the imbalance between prooxidant and antioxidant which is beyond the capacities of cellular antioxidants[11]. Among the antioxidants, the reduced form of tripeptide glutathione (GSH) is the most abundant sulfur-containing molecule in brain tissue[12-14]. Lipid peroxides and hydrogen peroxide (H_2O_2) are produced during enzymatic scavenging of superoxide radicals, and could be reduced by GSH peroxidase that converts GSH into oxidized species (GSSG). Regeneration of GSH and the concomitant oxidation of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) are catalyzed by GSH reductase[13], an enzyme responsible for maintaining the reduced form of brain glutathione almost exclusively (> 99%).

Being a natural phenolic compound, caffeic acid (3, 4-dihydroxycinnamic acid) has been reported to exert neuroprotective effects against brain ischemia due to its potent antioxidative, anti-inflammatory and free radical scavenging characteristics[15, 16]. Rofecoxib [4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone] is a selective inhibitor for COX-2 and has been proved to be efficacious in treating inflammation, post-operative pain, rheumatoid arthritis, and other musculo-skeletal and joint disorder-like conditions[17]. Since the antioxidative effects of rofecoxib and caffeic acid have been reported previously[8, 9], the present study aims to evaluate the effects of caffeic acid and rofecoxib, respectively, and the co-effect of them in ameliorating QA-induced behavioral alterations and in restoring the glutathione redox status.

2 Materials and methods

2.1 Animals Male Wistar rats (weighing 220-250 g) were raised in Central Animal House of Panjab University and housed under standard laboratory conditions, with free access to food and water under a 12-h light/dark cycle. Animals were acclimatized to laboratory conditions before the test. Tests were performed between 9:00 and 17:00. All the experiments were approved by Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the Care and Use of Experimental Animals.

2.2 Drugs and treatment schedule QA (Sigma-Aldrich, St Louis, Mo USA) was dissolved in normal saline and injected unilaterally in the striatum (75 mmol/L, 4 μL). Caffeic acid (Himedia, Mumbai) and rofecoxib (Panacea Biotec. Ltd, New Delhi, India) were prepared in 0.25% *(m/V)* sodium carboxymethyl-cellulose (CMC) and given a daily oral administration at the same time in the morning for 21 d in a dose of 5 mL/kg. In the combination study, caffeic acid and rofecoxib were administered together.

A total number of 11 groups were included in the present study: group-1 naïve (without surgery), group-2 sham (intrastriatal normal saline injection), group 3 receiving single intrastriatal injection of QA 300 nmol, group 4 receiving only caffeic acid treatment (5 mg/kg, p.o.), group 5 receiving only caffeic acid treatment (10 mg/kg, p.o.), group 6-8 receiving caffeic acid administration at graded doses of 2.5 mg/kg, 5 mg/kg and 10 mg/kg, respectively, in QA-treated (300 nmol) animals, group 9 receiving only rofecoxib treatment (10 mg/kg, p.o.), group 10 receiving rofecoxib treatment (10 mg/kg, p.o.) in QA-treated (300 nmol) animals, and

group 11 receiving a combination of caffeic acid (5 mg/kg, p.o.) and rofecoxib (10 mg/kg, p.o.) treatment in QA-treated (300 nmol) animals. Each group consisted of 10 animals. The caffeic acid and rofecoxib treatment started on the next day of animal recovery from anesthesia after intrastriatal injection of QA, and the first day of the treatment was designated as day 1.

2.3 Intrastriatal administration of QA Animals were anesthetized with thiopental sodium (45 mg/kg, i.p.). The skull was exposed by making an incision on the scalp, and a l-2 mm diameter hole was made in the skull using a small hand drill at a position anterior+1.7 mm, lateral±2.7 mm, and ventral–4.8 mm from bregma and dura as described by Paxinos and Watson[18]. QA injection into the right striatum was made through a 28-gauge stainless steel needle attached to a Hamilton syringe. QA was injected in a volume of 4 μL for 2 min, and the injection needle was kept still for another 1.5 min to allow diffusion of the QA solution.

2.4 Behavioral assessment

2.4.1 Body weight Body weight was measured on day 1 and day 21 of the study.

2.4.2 Assessment of gross behavioral activity (locomotor activity) The locomotor activity was assessed by using actophotometer (IMCORP, Ambala, India) on day 1, 7, 14 and 21. Animals were placed individually in the activity chamber for 3 min as a habituation period before the 5-min motor activity task. Total activity (horizontal and vertical) was expressed as counts per 5 min as described by Kumar[19].

2.4.3 Rotarod activity Motor coordination and balance was evaluated by rotarod test on day 1, 7, 14 and 21 after QA injection. Before actual recording on rotarod apparatus (IMCORP, Ambala, India), the animals were given a prior training session for acclimatization. During the rotarod test, rats were placed on the rotating rod (7 cm in diameter) at a speed of 25 rpm, and the duration that an animal stayed on the rod within 90 s was recorded. Each rat performed 3 separate trials with 5-min intervals as described by Kulkarni[20].

2.5 Biochemical assessment

2.5.1 Dissection and homogenization On day 21, animals were sacrificed by decapitation immediately after the behavioral assessment. The striatum was isolated from the brain, weighed and homogenized (10%, *m/V*) in 0.1 mol/L phosphate buffer (pH 7.4). The homogenate was centrifugated at 10 000 g for 15 min at 4 ºC and supernatant was collected for biochemical assessment.

2.5.2 Measurement of reduced glutathione (GSH) Glutathione content in striatum was estimated according to the method described by Ellman[21]. In this method, 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) is reduced by –SH groups and 1 mole of 2-nitro-5-mercaptobenzoic acid is produced by per mole of SH. The released nitro mercaptobenzoic acid anion exhibits an intense yellow color, the density of which could be measured at 412 nm to indicate the contents of –SH groups.

2.5.3 Detection of total glutathione level Total glutathione level in striatum region of brain was measured according to the previous report by Zahler and Cleland[22]. The method is based on the reduction with dithioerythritol and the determination of the resulting monothiols with DTNB in the presence of arsenite.

2.5.4 Detection of oxidized glutathione (GSSG) level The level of GSSG was quantified by subtracting the value of GSH from the content of total glutathione. Redox ratio of GSH to GSSG (GSH/GSSG) was also calculated.

2.5.5 Protein estimation Protein estimation was performed as reported by Biuret[23]. Bovine serum albumin was used as the standard agent.

2.6 Statistical analysis A total number of 10 animals were employed in each drug treatment group. Data were analyzed using one-way analysis of variance (ANOVA) (for body weight and biochemical parameters) and two-way ANOVA (for locomotor activity and rotarod performance) followed by Tukey's test. Data were expressed as mean±SEM. $P < 0.05$ was considered as significantly different.

3 Results

3.1 Effects of caffeic acid and rofecoxib on body weight in QA-treated rats As shown in Table 1, intrastriatal QA injection (300 nmol) significantly decreased the body weight as compared to sham group on day 21, which was significantly attenuated by caffeic acid at the doses of 5 mg/kg and 10 mg/kg (p.o.), and rofecoxib (10 mg/kg, p.o.), respectively. Furthermore, co-treatment of rofecoxib (10 mg/kg, p.o.) and

caffeic acid (5 mg/kg, p.o.) significantly potentiated the protective effects in QA-treated rats, as compared to their individual effect in QA-treated group. However, at a lower dose of 2.5 mg/kg, caffeic acid did not show any significant attenuation in QA-induced body weight loss.

Table 1. Effects of caffeic acid and rofecoxib on QA-induced reduction in the body weight on day 21

Treatment	Change in body weight (%)
Naïve	0.645
Sham	0.648
QA 300	-9.127[a]
QA 300 + CA (2.5)	-7.928
QA 300 + CA (5.0)	-5.938[b,c]
QA 300 + CA (10)	-3.664[b,c,d]
QA 300 + Rof (10)	-5.884[b]
QA 300 + CA (5.0) + Rof (10)	-2.474[b,d,e]

[a]$P < 0.05$ *vs* sham, [b]$P < 0.05$ *vs* QA 300, [c]$P < 0.05$ *vs* QA 300 + CA (2.5), [d]$P < 0.05$ *vs* QA 300 + CA (5.0), [e]$P < 0.05$ *vs* QA 300 + Rof (10).

3.2 Effects of caffeic acid and rofecoxib on locomotor activity in QA-treated rats As shown in Fig. 1, there were no significant changes in the locomotor activity between sham group and naïve group on day 21. Intrastriatal QA injection (300 nmol) significantly improved the locomotor activity on day 7 as compared to sham group, while it significantly decreased the locomotor activity on day 14 and 21. Besides, both caffeic acid (5 mg/kg and 10 mg/kg, p.o.) and rofecoxib (10 mg/kg, p.o.) could significantly improved the locomotor activity in QA-treated animals on day 14 and 21. Moreover, co-treatment of rofecoxib (10 mg/kg, p.o.) and caffeic acid (5 mg/kg, p.o.) exerted a significantly stronger protective effect in locomotor activity in QA-treated group, as compared to their individual effects. Also, at the lower dose of 2.5 mg/kg, caffeic acid did not show any significant improvement in the locomotor activity as compared to QA-treated group.

3.3 Effects of caffeic acid and rofecoxib on rotarod activity in QA-treated rats As shown in Fig. 2, intrastriatal injection of QA (300 nmol) significantly shortened the rat duration on the rod, compared to that in sham group. Both caffeic acid (5 mg/kg

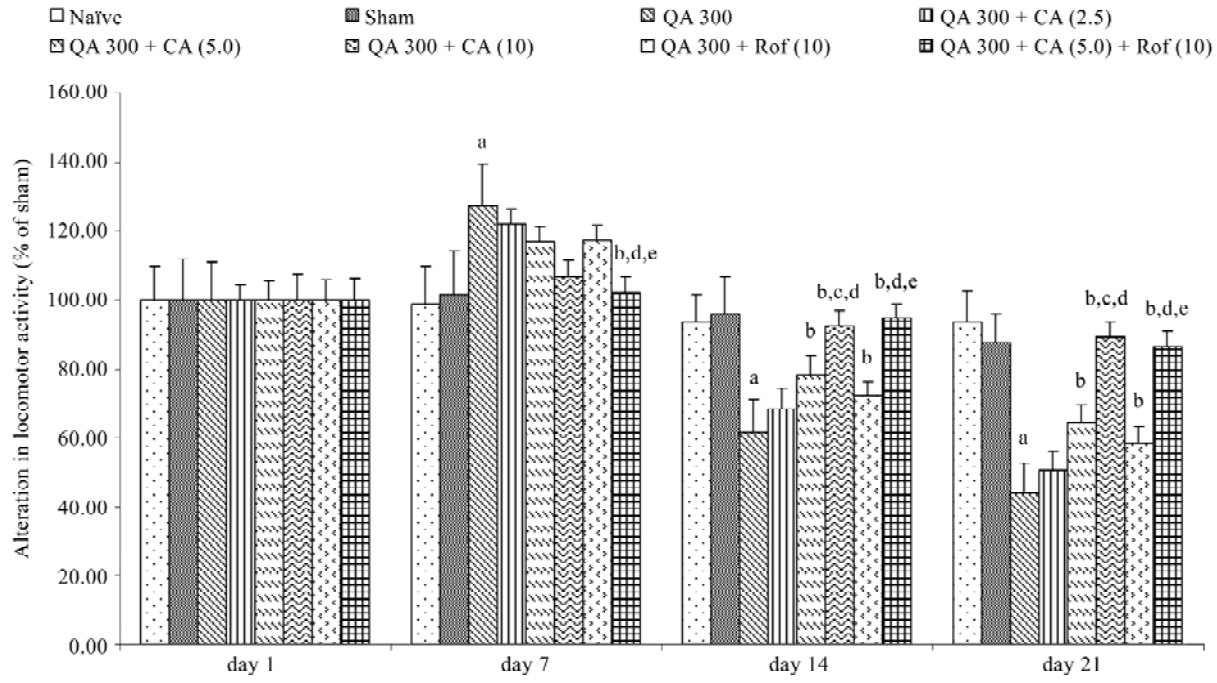

Fig. 1 Effects of caffeic acid and rofecoxib on QA-induced alterations in the locomotor activity. Values were expressed as mean±SEM (% of sham). [a]$P < 0.05$ *vs* sham, [b]$P < 0.05$ *vs* QA 300, [c]$P < 0.05$ *vs* QA 300 + CA (2.5), [d]$P < 0.05$ *vs* QA 300 + CA (5.0), [e]$P < 0.05$ *vs* QA 300 + Rof (10).

and 10 mg/kg, p.o.) and rofecoxib (10 mg/kg, p.o.) could significantly improve the rotarod activity (delayed fall off time) in QA-treated rats. Further, combination of rofecoxib (10 mg/kg, p.o.) and caffeic acid (5 mg/kg, p.o.) significantly potentiated the protective effects (delayed fall off time) as compared to their individual effect in QA-treated rats. However, lower dose of caffeic acid (2.5 mg/kg, p.o.) did not show any significant improvement in rotarod performance

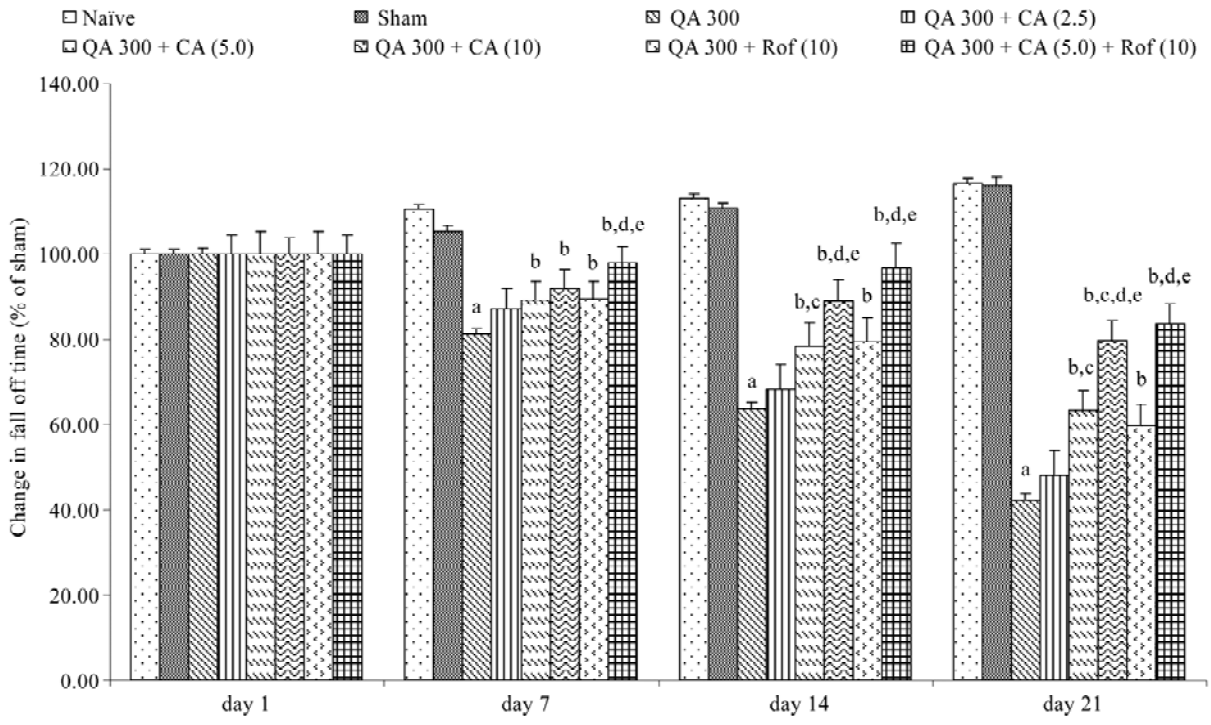

Fig. 2 Effect of caffeic acid and rofecoxib on QA-induced alteration of the rotarod activity. Values were expressed as mean±SEM (% of sham). [a]*P* < 0.05 *vs* sham, [b]*P* < 0.05 *vs* QA 300. [c]*P* < 0.05 *vs* QA 300 + CA (2.5), [d]*P* < 0.05 *vs* QA 300 + CA (5.0), [e]*P* < 0.05 *vs* QA 300 + Rof (10).

Table 2. Effects of QA, caffeic acid and rofecoxib on the levels of total glutathione, reduced glutathione, oxidized glutathione and redox ratio in brain striatum on day 21

Treatment	Total glutathione (% of sham)	Reduced glutathione (% of sham)	Oxidized glutathione (% of sham)	Redox ratio (% of sham)
Naive	101±1.82	102.54±3.33	97.50±2.34	104.00±2.31
Sham	100±1.92	100±15.00	100±1.56	100±1.51
QA 300	98.03±2.02 [NS]	38.50±1.98[a]	154.76±1.79[a]	24.87±1.72[a]
QA 300 + CA (2.5)	98.52±1.89[NS]	44.81±2.72[NS]	145.68±2.03[NS]	36.22±2.03[NS]
QA 300 + CA (5.0)	98.60±2.56 [NS]	56.31±3.54 [b,c]	132.72±1.22 [b,c]	56.22±2.05 [b,c]
QA 300 + CA (10)	99.08±1.76 [NS]	79.77±2.98 [b,d]	122.30±1.62 [b,d]	72.95±1.59 [b,d]
QA 300 + Rof (10)	98.42±1.89	52.81±2.12[b]	140.68±2.03[b]	49.22±2.03[b]
QA 300 + CA (5.0) + Rof (10)	96.30±2.56 [NS]	93.01±3.34 [b,d,e]	107.72±1.92 [b,d,e]	93.22±2.05 [b,d,e]

Values were expressed as mean±SEM (% of sham). [a]*P* < 0.05 *vs* sham, [b]*P* < 0.05 *vs* QA 300, [c]*P* < 0.05 *vs* QA 300 + CA (2.5), [d]*P* < 0.05 *vs* QA 300 + CA (5.0), [e]*P* < 0.05 *vs* QA 300 + Rof (10). [NS] No significant.

as compared to QA-treated group.

3.4 Effect ofs caffeic acid and rofecoxib on glutathione redox status in QA-treated rats Intrastriatal injection of QA (300 nmol) significantly reduced the levels of glutathione (GSH) and redox ratio (GSH/GSSG), and increased the oxidized glutathione level in rat brain striatum, as compared to sham group. However, there was no significant difference in the total glutathione level between QA and sham group. Besides, caffeic acid (5 mg/kg and 10 mg/kg, p.o.) and rofecoxib (10 mg/kg, p.o.) could significantly restore the levels of GSH and GSH/GSSG, and attenuate the rise in oxidized glutathione level in QA-treated rats. Furthermore, co-treatment of rofecoxib (10 mg/kg, p.o.) and caffeic acid (5 mg/kg, p.o.) potentiated their protective effect which was significant as compared to their individual effect in QA treated animals (Table 2). However, at a lower dose of 2.5 mg/kg, caffeic acid did not induce any significant change in the levels of GSH, GSH/GSSG or oxidized glutathione, as compared to the QA-treated group. Moreover, neither caffeic acid (5 mg/kg and 10 mg/kg, p.o.) nor rofecoxib (10 mg/kg, p.o.) produced any significant effect on the behavior as well as the glutathione redox status, as compared to QA-treated group (data not shown).

4 Discussion

The present study demonstrates that QA-induced behavioral alterations and striatal damage are associated with impairment of glutathione system. QA is an excitotoxic agent, which has been implicated in the pathogenesis of several neuroinflammatory and neurodegenerative diseases including HD[24, 25]. It has been now well elucidated that increased expression level of COX-2 occurs mainly through activation of NMDA receptors[26], although few studies have reported the involvement of LOX pathway in these neurodegenerative disorders[27,28]. LOX has also been shown to mediate kainic acid-induced excitotoxicity[29] and apoptosis[30].

In the present study, intrastriatal injection of QA could induce significant alterations in behavioral parameters, such as reduction in body weight, impairment in locomotor activity (initial hyperactivity followed by hypoactivity) and motor coordination (grip strength), suggesting the behavioral alterations and HD-like symptoms. These findings are consistent with earlier reports[2,31,32]. The decrease in body weight might be a consequence of impaired energy balance and oxidative damage (impaired redox ratio level) due to excitotoxicity. The initial hyperactivity may be due to the initial increased levels of the excitatory neurotransmitters[33,34], and the following hypoactivity may be due to striatal neuronal degeneration and energy impairment[32]. Evidence suggests that QA induces the degeneration of striatal neurons that express dopamine receptors, therefore causing attenuation of dopamine signaling and disturbances in motor symptoms, such as altered locomotor, impaired motor in coordination and rotational behavior[35]. Consistent with earlier reports, our study suggests that intrastriatal QA injection induces motor incoordination, which may be due to the degeneration of striatal neurons. Antioxidative and protective effects of COX and LOX inhibitors have been well documented in various neurodegenerative conditions[19, 29]. In the present study, both caffeic acid and rofecoxib significantly attenuated the behavioral alterations in QA-treated rats. Furthermore, co-treatment of caffeic acid and rofecoxib exerted a significantly stronger effect in improving the behavioral alterations, as compared to their individual effect. These results indicate that these drugs could protect against QA-induced behavioral alterations possibly through antioxidative mechanisms and their free radical scavenging capabilities. Also, the neuroprotective- and antioxidative-like actions of the selective inhibitors for LOX and COX-2 have been well documented[19,29].

According to previous reports, calcium overload is the major cause for excitotoxic neuronal death, which in turn activates some enzymes such as proteases, endonucleases and phospholipases that contribute to the degradation of different cell components and neuronal death[36]. Intracellular concentration of Ca^{2+} is regulated by energy-dependent systems (cytoplasmic and endoplasmic reticulum Ca^{2+}-ATPases)[37]. Massive influx of Ca^{2+} occurs during excitotoxicity, leading to Ca^{2+} overload in mitochondrial and disruption in ATP production[37, 38]. Another key event involved in excitotoxic death is the generation of free radicals as a result of mitochondrial dysfunction[39]. Studies have shown that ATP acts as a co-substrate of the enzymes involved in GSH synthesis[40,41].

Therefore, we propose that less production of ATP could hinder the synthesis of GSH by altering γ -glutamylcysteine synthetase and glutathione synthetase (enzymes involved in GSH synthesis).

Studies have also reported that excitotoxic processes enhance COX-2 activity, through both increased substrate production and elevated COX-2 expression, thereby exacerbating neuronal damage by free radical- and prostanoid-mediated mechanisms[9, 42, 43]. The broad spectrum of toxic effects produced by Ca^{2+} in biological systems include oxidative damage through reduction in cellular antioxidant defense, protein oxidation/nitration, and inhibition of mitochondrial respiration[44-46]. Glutathione constitutes more than 90% of the intracellular non-protein thiol pool. It regulates endogenous protective mechanism and glutathione redox cycle against various xenobiotic compounds[47-49]. GSH is known to react readily with a wide variety of free radical species, leading to the formation of superoxide anions[50]. The product of GSH oxidation is GSSG. Furthermore, GSH is regenerated from GSSG within the cells which is catalyzed by the flavoenzyme glutathione reductase. Therefore, decrease in the level or activity of any component of this cycle ultimately causes imbalance of redox status (GSH/GSSG) in cells, leading to oxidative stress. In the present study, intrastriatal injection of QA caused depletion of GSH, which in turn led to a relatively higher level of GSSG, and the later increased formation of GSSG would in turn induce decreased formation of GSH, ultimately leading to disturbance in glutathione homeostasis in the brain.

Moreover, intrastriatal QA injection significantly altered the redox status. QA treatment caused a significant decrease in reduced glutathione level and redox ratio, and a significant increase in oxidized glutathione level, while it had no significant effect on total glutathione level. The present findings are consistent with the vulnerability of GSH-related enzymes to oxidative damage, due to the presence of sulfhydryl groups at GSH active sites[51, 52]. It is particularly worth mentioning that cysteine uptake inhibition, nitric oxide synthase stimulation, and GSH depletion are common results of high levels of glutamatergic agonists such as QA in the glutathione metabolism[53-56]. These findings further explain Ca^{2+} overload and free radical generation. Further, caffeic acid and rofecoxib treatment could significantly revert these glutathione alterations in the QA-treated animals, restoring the reduction in glutathione level and redox ratio, and attenuating the increase of oxidized glutathione level. Moreover, combination of rofecoxib and caffeic acid strengthened the protective effect, which is also consistent with previous findings that COX, LOX and dual inhibitors could exert neuroprotective effect at different neurodegenerative conditions[19, 29, 57].

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咖啡酸、罗非考昔及两者连用对喹啉酸诱导的大鼠行为变化及谷胱甘肽氧化还原紊乱的改善与修复作用

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摘要：目的 环氧和酶(COX)抑制剂和脂肪氧化酶(LOX)抑制剂已被证实具有神经保护作用，但对其具体机制目前研究甚少。喹啉酸具有兴奋毒性作用，通过激活 NMDA 受体，引起类似于亨廷顿舞蹈症(Huntington's Disease，HD)的症状，包括行为、形态以及生化水平上的各种异常。本研究旨在探讨咖啡酸(LOX特异性抑制剂)和罗非考昔(COX特异性抑制剂)各自以及两者连用对喹啉酸引起的大鼠神经毒性的改善和修复作用。**方法** 在大鼠右侧纹状体内注射喹啉酸，诱导神经毒性。随后每天给大鼠口服咖啡酸或罗非考昔，或两者同时服用。用一系列行为学及生化检测方法检测咖啡酸和罗非考昔，以及两者连用对喹啉酸诱导的大鼠行为变化及谷胱甘肽氧化还原紊乱的改善和修复作用。**结果** 在纹状体注射喹啉酸不仅能降低大鼠体重，引起运动失调，而且能破坏纹状体内氧化还原间的平衡，表现为谷胱甘肽水平降低，以及氧化谷胱甘肽水平升高。长期服用咖啡酸或罗非考昔，以及两者连用都能显著减轻喹啉酸引起的行为变化，修复氧化还原水平的平衡。而当剂量为 2.5 mg/kg 时，咖啡酸未表现出任何保护作用。**结论** 本实验结果表明，大脑的兴奋性中毒有可能通过改变谷胱甘肽的水平影响氧化与抗氧化间的平衡。环氧合酶和脂肪氧化酶通路都可能参与了喹啉酸诱导的神经毒性过程。这些结果为治疗 HD 提供了研究靶点。

关键词： 咖啡酸；环氧合酶；谷胱甘肽；喹啉酸；罗非考昔