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Pramipexole reduces inflammation in the experimental animal models of inflammation

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ABSTRACT
Pramipexole is a dopamine (DA) agonist (D2 subfamily receptors) that widely use in the treatment of Parkinson's diseases. Some epidemiological and genetic studies propose a role of inflammation in the pathophysiology of Parkinson's disease. To our knowledge, there is no study regarding the anti-inflammatory activity of pramipexol. Therefore, the aim of the study was to investigate anti-inflammatory effect of pramipexol. Anti-inflammatory effects of pramipexole were studied in three well-characterized animal models of inflammation, including carrageenan- or formalin-induced paw inflammation in rats, and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema in mice. The animals received pramipexol (0.25, 0.5 and 1 mg/kg, I.P.) 30 min before subplantar injection of carrageenan or formalin. Pramipexol (0.5 and 1 mg/kg) was also injected 30 min before topical application of TPA on the ear mice. Serum malondialdehyde (MDA) levels were evaluated in the carrageenan test. Finally, pathological examination of the inflamed tissues was carried out. Pramipexole significantly inhibited paw inflammation 1, 2, 3 and 4 h after carrageenan challenge compared with the control group (\textit{p} < 0.001). Pramipexol also showed considerable anti-inflammatory activity against formalin-evoked paw edema over a period of 24 h (\textit{p} < 0.001). TPA-induced ear edema was markedly decreased by pramipexol (\textit{p} < 0.001). The pathological evaluation of the paws and ears revealed that pramipexol reduced tissue injury, neutrophil infiltration, and subcutaneous edema. Pramipexol did not alter the increased serum levels of MDA due to carrageenan injection. These data clearly indicate that pramipexol possesses significant anti-inflammatory activity. It seems that its antioxidants do not play an important role in these effects.

INTRODUCTION
It is known that central and peripheral sympathetic nervous system regulate the immune system through modulation of releasing several neurotransmitters, neuropeptides, hormones and cytokines which interact with diverse immune effector cells\textsuperscript{1}. Catecholamines play an important role in this interaction. There are a number of reports available regarding the role of epinephrine (EP), norepinephrine (NE) and central and peripheral dopamine (DA) on the immune system\textsuperscript{2}. The functional importance effect of DA on the immune system is further supported by the presence of its receptors on the immune cells\textsuperscript{3}. Moreover, the presence of specific DA transport system\textsuperscript{4} and endogenous synthesis of DA have been reported about leukocytes\textsuperscript{5}. The function of DA on the immune system becomes more interesting when it is reported that the abnormalities of immune systems well correlate with some of the important neurological diseases such as Parkinson's disease, depression and schizophrenia\textsuperscript{6,7}. In the line of the indicated studies, evidence is growing to suggest that the presence of DA receptors such as D2/D3 DA receptors on the immune cell surface such as natural killer (NK) cell, Th1, monocyte and neutrophils\textsuperscript{8,9}.

Pramipexole is a direct-acting DA agonist that widely uses in the treatment of Parkinson's diseases and neurodegenerative movement disorders. Pramipexole strongly binds to D2 and preferential D3 DA receptors on nerve cell surface and other cells possess DA actions. It has been reported that pramipexole has antioxidant properties\textsuperscript{10,11}. The presence of D2/D3 DA receptors on immune cells suggests the potential effect of pramipexol on the process of inflammation\textsuperscript{12}. Bromocriptine, as agonist DA receptors, exhibited considerable anti-inflammatory effects in some studies\textsuperscript{14,15}. Furthermore, anti-inflammatory activity of pergolide, as another DA agonist, has been shown in animal models of inflammation\textsuperscript{16}. To our knowledge, there is not any report regarding the potential anti-inflammatory properties of pramipexole. Therefore, the aim of this study was to investigate the anti-inflammatory effect of pramipexol in some experimental animal models of acute inflammation.
Materials and methods

Animals

Adult male Wistar rats (200–250 g) and adult male Swiss albino mice (25–35 g) were obtained from Pasteur Institute of Iran (Tehran, Iran). The animals allowed free access to normal diet and water. They were kept in groups of four per standard cage, under a 12:12 h light/dark cycle at 24 ± 2 °C. The experiments were carried out in accordance with local guidelines for the care of laboratory animals of the Yasuj University of Medical Sciences.

Chemicals

Pramipexole powder (SND 919; 2 Amino 4,5,6,7 tetrahydro-6-propyl-Amino benzothiazole dihydro-chloride) was donated by the Sobhan Darou (Rasht, Iran). Trichloroacetic acid (TCA), thiobarbituric acid (TBA), diethyl ether and formalin obtained from Merck (Darmstadt, Germany). Carrageenan (lambda) was purchased from Sigma-Aldrich (St. Louis, MO). Indomethacin and 12-O tetra decanoyl phorbol-13-acetate (TPA) were purchased from Sigma-Aldrich (St. Louis, MO).

Carrageenan-induced paw edema in rats

Rats were randomly divided into five groups of six animals; including a control group (vehicle group); treatment groups (0.25, 0.5 and 1 mg/kg) and indomethacin group (10 mg/kg), as the standard drug. The method of inducing inflammation was similar to that described in our previous works. At first, the animals were pretreated with pramipexole (0.25, 0.5 and 1 mg/kg, I.P.) or indomethacin (10 mg/kg, I.P.) 30 min before subplantar injection of carrageenan. Then, the rats received 100 µl of a 1% (w/v) suspension of λ-carrageenan in the right hind paw. The paw thickness was recorded from the ventral to the dorsal surfaces using a digital caliper (Mitutoyo, Sakado, Japan) immediately before carrageenan injection and again at 1, 2, 3 and 4 h after that. The data were expressed as the variation in the paw thickness (mm) and were compared to pre-injection values. Then, under ether anesthesia, blood samples were collected from the heart of the animal and serum was separated for malondialdehyde (MDA) assessment. Finally, the animals were euthanized by ether and the right paws removed and fixed in 10% formaldehyde solution for histological examination.

TPA-induced mouse ear edema

Mice were randomly assigned to four groups, including a control group (vehicle group); pramipexole groups (0.5 and 1 mg/kg, I.P.) and indomethacin group (10 mg/kg, I.P., as the standard drug). Pramipexole and indomethacin were given 30 min before topical application of TPA on the mouse’s ear. Ear edema was induced by topical application of 20 µl of TPA (2.5 mg/ear) dissolved in acetone to both surfaces of the right ear of each mouse. The animals were euthanized 4 h after the TPA application and one ear punch (6 mm diameter) were taken from each ear of a mouse. Edema was expressed as the difference between to the weight (mg) of punched ears. Three ear samples were fixed in formalin 10% for histological examination.

Determination of MDA

The measurement of serum MDA concentration was performed according to the method previously described. In brief, MDA reacted with thiobarbituric acid in the acidic high temperature and produced a red-complex TBARS. The absorbance of TBARS was read at 532 NM. Serum MDA concentration was expressed as nmol/ml.

Histological examination

Three samples of the ears or paws of the animals were taken and fixed in 10% formaldehyde solution for one week. Then, the fixed biopsies were embedded in paraffin and cut into 3–4 µm slices. The slices were mounted on the glass slides and stained with hematoxylin and eosin for pathological analysis.

Statistical analysis

The data are expressed as the means ± SD. The differences between the control and treatment groups were tested by ANOVA followed by the Tukey post-hoc test, using SPSS 13.0 software (SPSS Inc., Chicago, IL). The probability of $p < .05$ was considered to show considerable differences for all comparisons made.

Results

Effect of pramipexole on carrageenan-induced paw edema in rats

Injection of carrageenan into the hind paw of rats led to a time-dependent increase in paw thickness (Figure 1). Pramipexole at doses of 0.5 and 1 mg/kg significantly inhibited carrageenan-induced paw edema at the end of 1, 2, 3 and 4 h ($p < .01$). The maximal anti-inflammatory effect of pramipexole was detected at 1 mg/kg and 2 h after carrageenan challenge. The anti-inflammatory effect of pramipexole at dose of 1 mg/kg was comparable to that found with indomethacin. The dose of 0.25 mg/kg pramipexole also significantly reduced the paw thickness at the end of 3 and 4 h.
As expected, indomethacin (10 mg/kg) considerably decreased paw inflammation (p < .001). Pramipexol (0.5 mg/kg, I.P.) significantly inhibited the development of paw edema at 1, 2, 3, 4 and 24 h after injection of formalin, compared to control group (p < .001). Indomethacin also elicited a significant anti-edematogenic effect on this model (p < .001).

**Effect of pramipexole on formalin-induced paw edema**

As presented in Figure 2, subplantar injection of formalin (2%, 100 μl) induced a progressive edema, immediately began after formalin injection and lasted for 24 h. Pramipexol (0.5 mg/kg, I.P.) significantly inhibited the development of paw edema at 1, 2, 3, 4 and 24 h after injection of formalin, compared to control group (p < .001). Indomethacin (10 mg/kg, I.P.) also noticeably attenuated the formalin-induced paw edema (p < .01 and p < .001).

**Effect of pramipexole on TPA-induced mouse ear edema**

As shown in Figure 3, ear weight noticeably increased at 4 h following topical application of TPA (p < .001). Pretreatment with pramipexole (0.5 and 1 mg/kg, I.P.) showed significant inhibitory effect on TPA-induced weight increase in mouse ear (p < .001). Indomethacin also elicited a significant anti-edematogenic effect on this model (p < .001).

**Effect of pramipexole on serum MDA concentration**

As illustrated in Figure 4, the serum concentration of MDA considerably increased after subplantar injection of carrageenan compared to vehicle-treated group (p < .001). Pramipexol at 0.5 and 1 mg/kg dose and indomethacin (10 mg/kg) were not able to restore the elevated serum levels of MDA due to injection of carrageenan.

**Histological examination**

Pathological assessment of the paw tissues revealed that subplantar injection of carrageenan elicited some injuries such as hyperplasia, edema, congestion of vessels and infiltration of inflammatory cells such as lymphocytes and neutrophils into the site of inflammation. Pretreatment with pramipexole...
(0.5 mg/kg) and indomethacin (10 mg/kg) greatly reduced the indicated changes (Figure 5).

Ear biopsies of TPA-treated group showed (Figure 6) that TPA increased the epidermis thickness, infiltration of polymorphonuclear (PMN) leukocytes into the inflamed ear. Furthermore, the ear edema in this group was noticeable. Pramipexole (0.5 mg/kg) and indomethacin (10 mg/kg,) were able to inhibit these inflammatory lesions due to TPA.

**Discussion**

Our current investigation, to our knowledge, verifies for the first time the *in vivo* anti-inflammatory effect of pramipexole in different animal models of inflammation, including carrageenan- and formalin-evoked paw edema in rat and TPA-induced ear edema in mice.

In accordance with Figure 1 pramipexole significantly prevented the development of paw edema due to carrageenan challenge. Carrageenan-induced inflammation is a valid model for screening anti-inflammatory activity of different experimental substances. The carrageenan-induced paw edema is a time dependent and biphasic event with participation of various mediators. The early phase (0–1 h) is attributed to changes in vascular permeability and release of histamine, bradykinin, serotonin and cyclooxygenase (COX) products. The later phase is owing to infiltration of leukocytes and releasing of prostaglandins (PG), leukotrienes, platelet-activating factor (PAF), pro-inflammatory cytokines, and free radicals of oxygen-derivatives. Pramipexole exhibited a potent anti-inflammatory effect against two phases of carrageenan-induced edema; hence, it is reasonable to assume that pramipexole, in some way, interfered with the synthesis or secretion of pro-inflammatory mediators involved in the two phases of carrageenan test. Pathological evaluation of the inflamed paw also supported the anti-inflammatory effect of pramipexol. The drug reduced paw injury, edema and infiltration of PMN to the inflammatory...
tissue, following injection of carrageenan. These results were in good agreement with the previous work about anti-inflammatory of pergolide, as a DA D2 receptor agonist. Alison and coworkers reported that pergolide elicited significant reduction in development of paw edema induced by carrageenan in rats. They proposed that the anti-inflammatory effect of pergolide relate to the sympathetic nervous system not to induction of corticosterone secretion.

In the formalin test similar to that found in carrageenan test, pramipexol showed a considerable inhibitory effect on the development of paw inflammation lasting until 24 h. The rat paw edema induced by formalin has been described to biphasic events. In the initial phase (also called neurogenic phase) bradykinin, and substance p are released while histamine, serotonin and PG are involved in the second phase. Therefore, this finding confirmed the earlier finding about the anti-inflammatory of pramipexole on the carrageenan test. Moreover, the results of formalin test showed that the anti-inflammatory effect of pramipexole lasting for 24 h that comparable with indomethacin as a reference anti-inflammatory drug.

TPA-induced ear edema is a model of skin inflammation for assessment of local and systemic anti-inflammatory compounds. TPA develops local inflammation through leukocyte infiltration and generation of free radicals due to activation of protein kinase C (PKC). This enzyme induces a number of active enzymatic pathways such as phospholipase A2 (PLA2) and mitogen activated protein kinas (MAPK). These events result in the secretion of platelet activation factor (PAF) and arachidonic acid. This cascade of event stimulates vasodilation, vascular permeability and release of serotonin and histamine or commences production of PG and leukotrienes through COX and 5-lipoxygenase (5-LOX) enzyme, respectively. Pramipexole showed a considerable inhibitory effect against TPA-induced phlogistic response. Histological analysis of the inflamed ears confirmed that pramipexole was able to reduce edema and PMN cell migration.

![Figure 6](image)

**Figure 6.** Pathological evaluation of ear tissues after topical application of 12-O-tetradecanoylphorbol-13-acetate. (A) Normal ear. (B) Control: Topical application of TPA (2.5 mg/ear) induced inflammatory lesion with edema and epidermal hyperplasia. (C) Indomethacin (10 mg/kg, I.P.) reduced the indicated changes by TPA. (D) Pretreatment with pramipexole (0.5, I.P.) reduced edema and ear hyperplasia. Sections were stained with hematoxyline and eosin, magnification ×20.

E: edema; I: infiltration of neutrophil
into the site of injury. According to the mechanisms of TPA-induced inflammation, it is possible that the drug interfered with the PKC or MAPK pathways.

As mentioned earlier, reactive oxygen species (ROS), such as superoxide, hydroxyl and peroxynitrite radicals are involved in the carrageenan or TPA-induced inflammation. In addition, the antioxidant activity of pramipexole has been established in some studies. MDA, as an indicator of the rate of lipid peroxidation, increased due to attack of free radicals to lipids in cell membranes. Lipid oxidation not only gives out as an indicator of cellular damage but also has been known to be the inducer of inflammatory development. The lack of a significant effect of pramipexole on the increased serum levels of MDA because of carrageenan injection ruled out the impotence of antioxidant properties of pramipexole in the anti-inflammatory response. However, our results do not allow us to elucidate the mechanisms of anti-inflammatory effect of pramipexole. Peripheral and central actions of the drug are plausible.

In conclusion, pramipexole exerted potent anti-inflammatory properties in three well-known experimental models of inflammation. This was supported by pathological analysis of the injured tissues. Additional investigations are needed to elucidate the exact mechanism of anti-inflammatory properties of pramipexole.

Disclosure statement

The authors declare that they have no conflict of interest.

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