Original Article

Evaluation of the central and peripheral effects of doxepin on carrageenan-induced inflammatory paw edema in rat

Mohsen Zabihi[1], Valiollah Hajhashemi[1,*], Mohsen Minaiyan[1], and Ardeshir Talebi[2]

[1]*Department of Pharmacology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.*
[2]*Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.*

Abstract

The anti-inflammatory effects of anti-depressants have been demonstrated recently. Doxepin, a tricyclic antidepressant drug (TCA), has some special properties in comparison with the other members of its family. It has some H_1, H_2, alpha-1 adrenergic and muscarinic receptor blocking effects. It revealed also anti-nociceptive and relatively potent sedative effects. This study was aimed to evaluate its possible anti-inflammatory effect in a well-established animal model. Male Wistar rats weighing 200-250 g were used in carrageenan-induced inflammatory paw edema model. The test and control drugs were injected by intraperitoneal (i.p.) and intracerebral (i.c.v.) routes. The anti-inflammatory activity of doxepin (15, 30 and 60 mg/kg, i.p. and 50 and 100 μg/rat, i.c.v.) and the reference drug, dexamethasone (2 mg/kg, i.p.) were evaluated by determination and comparison of some involved biological markers including the paw volume, cytokine levels (interleukin 6 (IL-6), IL-1β, tumor necrosis factor α (TNFα)), myeloperoxidase (MPO) activity and histopathological parameters. All i.p. doses of doxepin showed significant anti-inflammatory effect. It also significantly reduced MPO activity and cytokine levels and improved histopathologic parameters of carrageenan-injected paw tissues. I.c.v. administration of the drug did not show any significant reduction of carrageenan-induced paw edema. Although the exact mechanism of the anti-inflammatory effect of doxepin is not clear, it seems that reduced leukocyte migration and pro-inflammatory cytokines play important role in its anti-inflammatory effect. Also central sites are not involved in the anti-inflammatory effect of the drug.

Keywords: Anti-inflammatory; Carrageenan; Cytokines; Doxepin

INTRODUCTION

Antidepressant medications are used in many different conditions in addition to depression such as major depressive disorders (MDD), neuropathic pain, panic disorders, attention deficit hyperactivity disorder (ADHD), and anxiety (1,2). Recent investigations suggest that antidepressants produce anti-inflammatory activities (3-6). The anti-inflammatory effects of fluoxetine, amitriptyline, trazodone and clomipramine have been reported on carrageenan-induced paw edema (5,7). Also our previous studies demonstrated the anti-inflammatory activities of amitriptyline, maprotiline, venlafaxine and fluvoxamine (3,6,8,9). Clinically antidepressants have been used in management of various types of inflammatory conditions such as inflammatory bowel syndrome (IBS), and there are important relationship between their anti-inflammatory and analgesic effects (10-13).

Doxepin, a tertiary amine and a tricyclic antidepressant (TCA), has been approved for treatment of MDD, insomnia (14), as a part of the treatment of chronic urticaria (15) and in pain management (16,17). Doxepin was shown to be more effective than venlafaxine in reducing thermal hyperalgesia and mechanical allodynia (18). It inhibits the reuptake of norepinephrine (NE) and serotonin (5HT) and exerts a very weak inhibition of dopamine (DA) reuptake. Doxepin binds strongly to the histamine H_1 and H_2 receptors (19-21). Its selective histamine antagonistic function is responsible for the drug's sleep-promoting properties (22).

*Corresponding author: V. Hajhashemi
Tel: +98-3137927080, Fax: +98-3136680011
Email: vhajhashemi@gmail.com

Doxepin also has some antagonistic effects on 5-HT receptors, alpha1 adrenergic receptors and muscarinic cholinergic receptors (22). On the basis of above-mentioned matters, doxepin is going to be a promising candidate for inflammatory and pain conditions especially in patients with depressive disorders. The anti-inflammatory activity of doxepin has not been examined yet and therefore this study was aimed to assess this effect and its probable mechanisms. Since the central anti-inflammatory effects of some antidepressants was shown in the previous studies (3,8,23), the role of central sites in possible anti-inflammatory activity of the drug was also assessed following its intracerebral (i.c.v.) administration.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200–250 g were purchased from the animal house of the School of Pharmacy, Isfahan University of Medical Sciences. All rats were housed in cages, under a 12:12 h light/dark cycle and had free access to food and water. The study protocol was approved by the Bioethics Committee of Isfahan University of Medical Sciences (Ethical code, 394946) and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Chemicals

Doxepin hydrochloride (Sigma, USA) and carrageenan λ (Fluka Chemical, Switzerland), was dissolved in isotonic saline. Dibasic and monobasic potassium phosphate (Merck, Germany), hexadecyltrimethyl-ammonium bromide (HTAB), o-dianisidine dihydrochloride (o-dianisidine) (Sigma Chemical Co., St.Louis, Mo, USA) were used for evaluation of myeloperoxidase (MPO) activity. TNF-α, IL-6 and IL-1β kits (Boster, China), dibasic and monobasic sodium phosphate and sodium chloride (Merck, Germany) were used for evaluation of the cytokines. Ketamine and xylazine vials (Alfasan, Holand) were used for the rat anesthesia.

Surgical procedure

The animals were anesthetized by i.p. injection of ketamine (40 mg/kg) and xylazine (7 mg/kg). Then, the animals were placed in a stereotaxic frame (Stoelting, USA) (stereotaxis), and an i.c.v. cannula was implanted with stereotaxic coordinates: AP, −0.8 mm; L, 1.4 mm; and V, 3.3 mm, according to Paxinos and Watson rat atlas (24). The implanted cannula was then fixed by dental cement. A needle (no. 22) with a 2 mm length as a cannula and a needle (no. 30) inside the first one were used for the drug injection. The rats with i.c.v. cannulas were euthanized at the end of the experiments, and their brains were examined to confirm the correct implantation of the cannula. To reduce stress, rats were handled daily for five days before the experiments.

Carrageenan-induced rat paw edema

One hundred microliters of a 1% (w/v) suspension of carrageenan was injected into the subplantar space of the right hind paw of rats (25). The paw volume was measured by a plethysmometer (Ugo Basil, Italy) just before subplantar injection of carrageenan and after 4 h, in this context the volume difference was used as an index of inflammation.

Experimental design

The doses of doxepin were chosen based on a pilot study. Finally three doses, 15, 30 and 60 mg/kg as i.p. (n = 6) and two doses 50 and 100 μg/rat as i.c.v. (10 μL, n = 6) were chosen for doxepin groups. Animals were treated 30 min prior to subplantar injection of carrageenan. Control groups received only vehicle. A group of animals were pretreated with dexamethasone (2 mg/kg, i.p.; n = 6) and used as the positive control. Finally, animals were euthanized, and the carrageenan injected paws were collected for the evaluation of biochemical parameters. Doxepin and vehicle in i.c.v. groups were injected slowly for 1 min through the cannula in a volume of 10 μL.

Histopathologic examination

A 2-mm sample of carrageenan-injected paws were removed 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 light microscopy analysis. Then, the fixed biopsies evaluated

by a pathologist in a blinded way. A histopathologic index for each sample was assigned for comparison to each other.

Histopathologic index (*unit*)
= (*Fraction of epiderm hydropic degeneration*
× *length of epiderm hydropic degeneration*)
+ *derm inflammation index*

Myeloperoxidase assay

The MPO activity in paw tissue was measured based on the modified method of Bradley (26). The biopsies of inflamed paws were removed and weighed and then each sample was finely chopped in 1 mL of 50 mM potassium phosphate buffer containing 0.5% HTAB. The chopped tissue was transferred to a homogenizing tube and the container was rinsed with 2 × 1 mL HTAB in buffer solution. If needed more buffer was added to obtain a concentration equivalent to 5 mL per 0.1 g of paw tissue and homogenized (15,000 rpm) for 4 × 45 s at 1 min intervals. After homogenizing, the samples were sonicated in an ice bath for 10 s, then subjected to a sequence of freezing and thawing 3 times, and sonicated again for 10 s.

The suspensions were centrifuged at 15,000 rpm for 15 min in 4 °C and then the supernatant separated for examination. 0.1 mL of the supernatant was added to 2.9 mL of 50 mM potassium phosphate (K_3PO_4) buffer (pH = 6.0) containing o-dianisidine (0.167 mg/mL) and 0.005% hydrogen peroxide. The absorbance of the reaction mixture was determined at 450 nm spectrophotometrically. The activity of MPO was determined and reported in units (U) per gram weight of wet tissue.

Determination of the TNF-α, IL-6 and IL-1β levels in the rat paw

TNF-α, IL-6 and IL-1β levels in the paw tissues were evaluated by enzyme-linked ELISA. Four hour after carrageenan challenge, the collected samples were homogenized in 0.01 M phosphate buffered saline (PBS) (pH = 7.2-7.6) containing 8.5 g NaCl, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 mL distilled water. 1 mL PBS per 50 mg tissue was used to prepare the supernatants. The supernatants were stored at -70 °C and then the cytokines levels were measured according to kit brochures provided by the manufacturer (Boster, China).

Statistical analysis

The data are expressed as Mean ± S.E.M. The differences between the control and treatment groups were tested by ANOVA statistical method, followed by the Tukey Post Hoc test, using SPSS 21 Software. The *P* value < 0.05 was considered to show significant differences for all comparisons made.

RESULTS

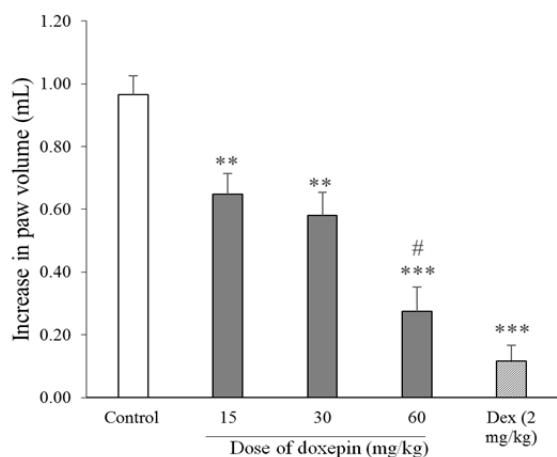### Effects of i.p. injection of doxepin on carrageenan-induced rat paw edema

As shown in Fig. 1, i.p. injection of doxepin (15, 30 and 60 mg/kg) significantly reduced paw edema 4 h after carrageenan application. 60 mg/kg doxepin decreased 72% paw volume in comparison with control group. Dexamethasone (2 mg/kg, i.p.) also significantly inhibited the paw edema after carrageenan test. The effects between doxepin (60 mg/kg) and doxepin (15 or 30 mg/kg) was also significant (*P* < 0.01). There wasn't any difference between dexamethasone (2 mg/kg) and doxepin (60 mg/kg) and between doxepin (15 mg/kg) and doxepin (30 mg/kg).

### Effects of i.c.v. administration of doxepin on carrageenan-induced rat paw edema

As it is shown in Fig. 2, i.c.v. injection of both doses of doxepin (50 μg/rat and 100 μg/rat) did not produce any significant anti-inflammatory activity four hour after injection of carrageenan in comparison with vehicle-treated control group.

Histological examination

As shown in Figs. 3 and 4, cellular infiltration and edema were noticeable in the paw biopsies of control animals. All applied doses of doxepin significantly (*P* < 0.05 for doses of 15 and 30 mg/kg and *P* < 0.01 for dose of 60 mg/kg) reduced tissue damage, polymorphonuclear (PMN) infiltration, and swelling.

Fig. 1. Effects of doxepin on carrageenan induced rat paw edema. Doxepin (15, 30 and 60 mg/kg) and dexamethasone (2 mg/kg) were injected i.p. 30 min before carrageenan and paw volume measured at 4 h after carrageenan injection. Results are presented as mean ± S.E.M (n = 6). **$P < 0.01$, ***$P < 0.001$ in comparison with the control group, #$P < 0.01$ in comparison with doxepin (15 and 30 mg/kg, i.p.), one-way ANOVA followed by Tukey test. Dex, dexamethasone.
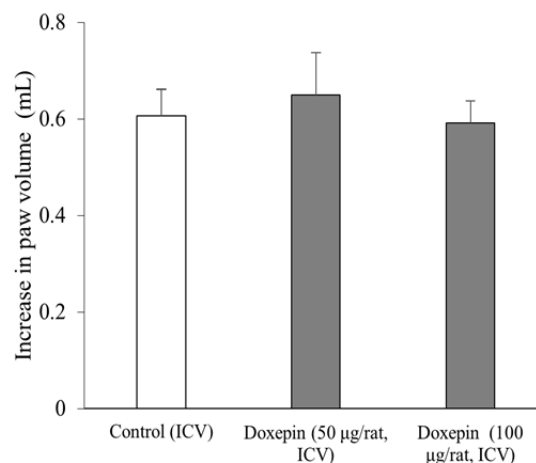
Fig. 2. Effects of i.c.v. treatment with doxepin on carrageenan induced rat paw edema. Doxepin (50 and 100 µg/rat) were injected 30 min before carrageenan, and paw volume measured at 4 h after carrageenan injection. Each value are presented as mean ± S.E.M (n = 6).
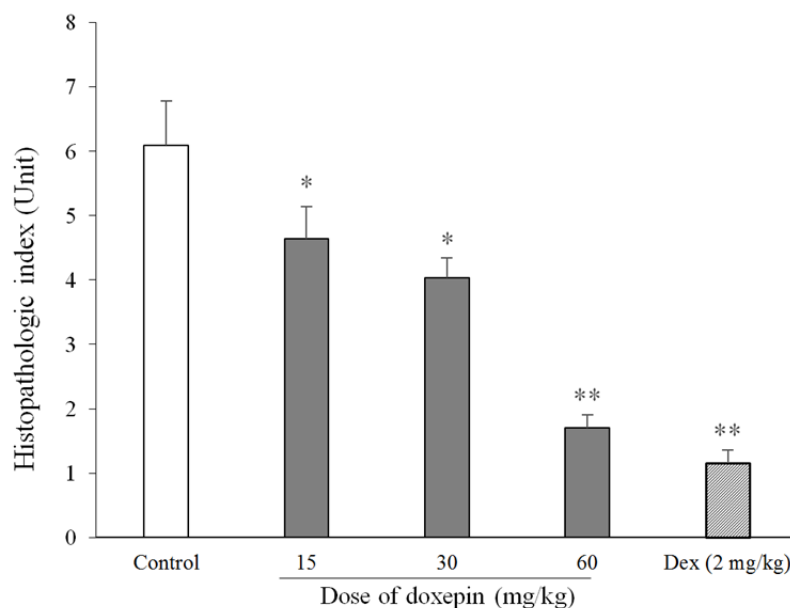
Fig. 3. Effects of doxepin on histopathologic index of paw tissue in carrageenan-induced rat paw edema. Doxepin (15, 30 and 60 mg/kg) and dexamethasone (2 mg/kg) were injected i.p. 30 min before carrageenan. Each value represents as mean ± S.E.M (n = 6). *$P < 0.05$, **$P < 0.01$ in comparison with the control group, one-way ANOVA followed by Tukey test. Dex, dexamethasone.

Total histopathologic index was decreased by 72% in doxepin (60 mg/kg) group in comparison with control group.

The microscopic appearance of paw tissues in doxepin 60 mg/kg group was similar to the dexamethasone (2 mg/kg) group as the reference drug.

Myeloperoxidase activity

Subplantar injection of carrageenan enhanced the activity of MPO in comparison with the control group. The reduction of MPO activity was significant with all examined doxepin IP doses (62% for 60 mg/kg group) and also dexamethasone (2 mg/kg. i.p.).

There was not any significant differences between dexamethasone and each of doxepin doses (Fig. 5).

Effects of doxepin on TNF-α levels in carrageenan-injected paws

TNF-α concentration remarkably raised in the carrageenan-treated paws in comparison with the control group. All of the groups with i.p. injected doxepin produced a considerable reduction in the levels of TNF-α (73% reduction for 60 mg/kg doxepin in comparison with the control group, $P < 0.01$). There were no differences between the three doses of doxepin in decreasing TNF-α level. The effect of doxepin on TNF-α was more pronounced than dexamethasone ($P < 0.05$) (Fig. 6).

### Effects of doxepin on IL-6 levels in the carrageenan-injected paws

Doxepin in all of the examined i.p. doses decreased IL-6 concentration without any meaningful differences between the doses. It decreased this biologic parameter for about 80% more than control group (Fig. 7).

Effects of doxepin on IL-1β levels in the carrageenan-injected paws

Same as the other examined cytokines, IL-1β concentration significantly raised in the control group in comparison with doxepin groups (about 70%) and there wasn't any significant difference between doxepin and dexamethasone (Fig. 8).
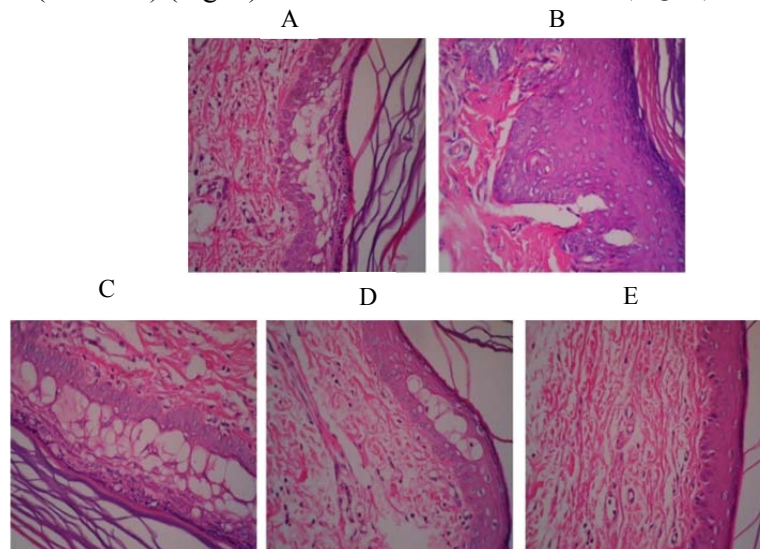

Fig. 4. Histopathologic appearance of paw epidermis and dermal tissue in carrageenan induced rat paw edema after 4 h (400 fold), (A) control; (B) dexamethasone 2 mg/kg i.p.; (C) doxepin 15 mg/kg i.p.; (D) doxepin 30 mg/kg i.p.; (E) doxepin 60 mg/kg i.p. It shows severe epidermal and dermal damage in the control group and less damage in the treatment groups.
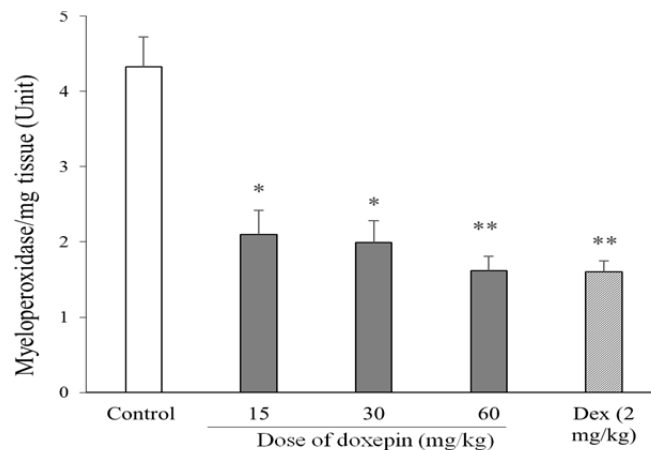
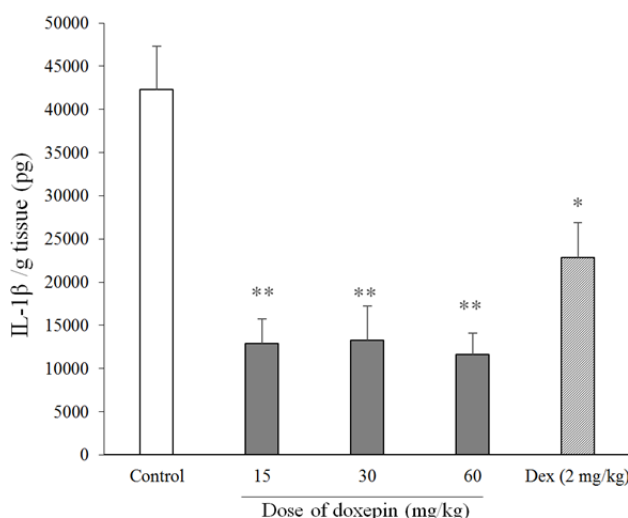**Fig. 5.** Effects of doxepin and dexamethasone on myeloperoxidase (MPO) activity in carrageenan injected paws. Rats were treated with doxepin or dexamethasone at the indicated doses 30 min before carrageenan challenge. MPO activity was evaluated at 4 h after injection of carrageenan. Results are presented as mean ± S.E.M (n = 6). *P < 0.05, **P < 0.01 in comparison with the control group, one-way ANOVA followed by Tukey test.
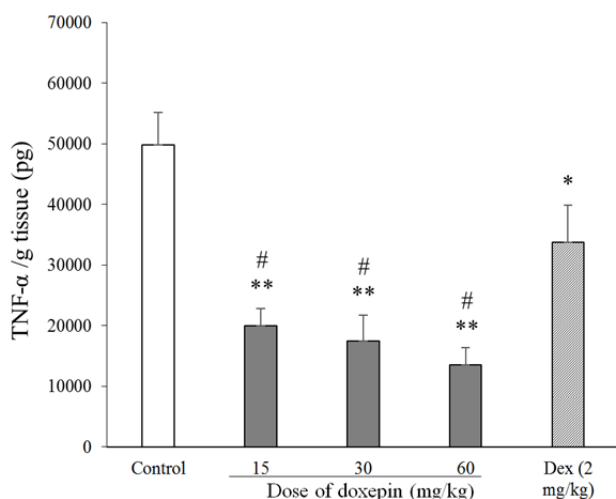
Fig. 6. Effect of doxepin and dexamethasone on TNF-α levels in carrageenan-injected paws. Rats were given indicated doses of doxepin or dexamethasone 30 min before carrageenan challenge. Paw tissues were dissected 4 h after carrageenan, and TNF-α level were measured. Results are presented as mean ± S.E.M (n = 6). *$P < 0.05$, **$P < 0.01$ in comparison with the control group, #$P < 0.05$ in comparison with the dexamethasone group one-way ANOVA followed by Tukey test. Dex, dexamethasone.
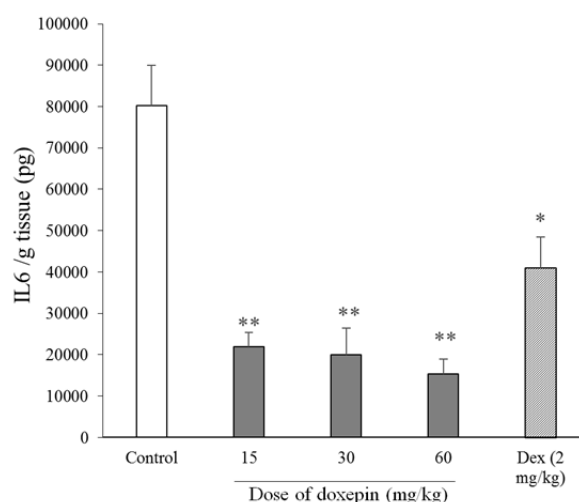
Fig. 7. Effect of doxepin and dexamethasone on IL-6 levels in carrageenan-injected paws. Rats were given indicated doses of doxepin or dexamethasone 30 min before carrageenan challenge. Paw tissues were dissected 4 h after carrageenan, and IL-6 level was measured. Results are presented as mean ± S.E.M (n = 6). *$P < 0.05$, **$P < 0.01$ in comparison with the control group, one-way ANOVA followed by Tukey test. Dex, dexamethasone.

Fig. 8. Effect of doxepin and dexamethasone on IL-1β levels in carrageenan-injected paws. Rats were given indicated doses of doxepin or dexamethasone 30 min before carrageenan challenge. Paw tissues were dissected 4 h after carrageenan, and IL-1β level was measured. Results are presented as mean ± S.E.M (n = 6). *$P < 0.05$, **$P < 0.01$ in comparison with the control group, one-way ANOVA followed by Tukey test. Dex, dexamethasone.

DISCUSSION

Results of the present study clearly showed that doxepin has anti-inflammatory effect in carrageenan-induced paw edema. This animal model is a well-characterized model which resembles to human inflammation (25).

Following carrageenan injection edema, hyperalgesia and redness occurs which is due to the action of pro-inflammatory agents released in the paw tissue. Mediators such as histamine, prostaglandins, bradykinin, tachykinins, cytokines and substance P are involved in this process. Another feature of the

inflammatory process is infiltration of polymorphonuclear cells into the tissue. Increase in paw volume as an index of inflammation reaches a peak of 4 h following carrageenan injection and is modulated by some inhibitory molecules of the inflammatory cascade (27,28).

This study showed that i.p. injection of doxepin inhibits development of paw edema 4 h following carrageenan injection. However i.c.v. injection didn't show any effect on paw edema and it means that central sites do not play any role in its anti-inflammatory effect. Our previous investigations indicated that the anti-inflammatory activity of drugs such as maprotiline and amitriptyline are mediated to somewhat by central sites (3,8,23) but for doxepin these sites are not involved.

Some studies have shown that inhibition of H_1 and H_2 receptors and histamine release (29,30), serotonin (31) and muscarinic receptors (32) can suppress the inflammation. Doxepin as mentioned in introduction section has antagonistic effects on these receptors and they may contribute in its anti-inflammatory effects.

It has also been reported that a part of doxepin may be metabolized to desmethyldoxepin (nordoxepin) in the liver (33-35), and this active metabolite might share the parent drug in producing anti-inflammatory activity.

In any case evaluation of the anti-inflammatory activity of desmethyldoxepin in future studies may determine the definite role of this active metabolite in suppression of inflammation.

In this research based on pathological assessment of paw biopsies, doxepin reduced the tissue destruction induced by carrageenan injection. Doxepin significantly diminished the MPO activity and cytokines levels in the carrageenan-injected paw tissue. MPO activity reduction by doxepin explains that this drug inhibits neutrophil migration into the inflamed tissue.

Also consistent with previous studies which showed that some antidepressant medications such as amitriptyline, maprotiline, venlafaxine and fluvoxamine reduce the release of pro-inflammatory cytokines such as IL-2, IL-1β,

IL-12 and IFN-γ (3,4,8,23,36,37), in the current study i.p. injection of doxepin (15, 30 and 60 mg/kg) significantly reduced TNF-α, IL-6 and IL-1β levels in inflamed paw tissue.

These cytokines have a well-established role in inflammatory response. They are produced by various cells including macrophages, lymphoid cells, endothelial cells and mast cells. It has been reported that these cytokines induce the production of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and prostaglandin E2 (PGE2) synthase, thereby increasing the levels of their products e.g. PGE2 and nitric oxide which develop vascular permeability and edema (38). It seems that doxepin exerts its anti-inflammatory activity by lowering pro-inflammatory cytokines and their contributing products.

Generally, inflammation is a very complex cascade of biochemical events with a wide number of contributors and it is difficult to find out an exact mechanism of action for a drug such as doxepin which affects several receptors and transporter systems and only further studies will help to have a better understanding of the involved mechanisms.

CONCLUSIONS

This study clearly shows anti-inflammatory effect of doxepin based on measurement of paw swelling, biochemical and pathological findings. This activity may be explained by the direct or indirect effects of the drug on immune system. Inhibition of both neutrophil migration and the resulting reduction of pro-inflammatory cytokines levels might be the key contributors of anti-inflammatory effects. Since the drug has considerable sedative and antidepressant effect, it can be a promising drug for various inflammatory diseases associated with depression or insomnia.

ACKNOWLEDGMENTS

REFERENCES

1. Thase ME. Overview of antidepressant therapy. Manag Care. 2001;10(8 Suppl):6-9. Discussion 18-22.
2. Geddes JR, Carney SM, Davies C, Furukawa TA, Kupfer DJ, Frank E, *et al*. Relapse prevention with antidepressant drug treatment in depressive disorders: a systematic review. The Lancet. 2003;361(9358):653-661.
3. Hajhashemi V, Minaiyan M, Banafshe HR, Mesdaghinia A, Abed A. The anti-inflammatory effects of venlafaxine in the rat model of carrageenan-induced paw edema. Iran J Basic Med Sci. 2015;18(7):654-8.
4. Janssen DG, Caniato RN, Verster JC, Baune BT. A psychoneuroimmunological review on cytokines involved in antidepressant treatment response. Hum Psychopharmacol. 2010;25(3):201-215.
5. Kostadinov I, Delev D, Petrova A, Stanimirova I, Draganova K, Kostadinova I, *et al*. Study on anti-inflammatory and immunomodulatory effects of clomipramine in carrageenan-and lipopolysaccharide-induced rat models of inflammation. Biotechno Biotechnol Equip. 2014;28(3):552-558.
6. Sadeghi H, Hajhashemi V, Minaiyan M, Movahedian A, Talebi A. Further studies on anti-inflammatory activity of maprotiline in carrageenan-induced paw edema in rat. Int Immunopharmacol. 2013;15(3):505-510.
7. Abdel-Salam OM, Nofal SM, El-Shenawy SM. Evaluation of the anti-inflammatory and anti-nociceptive effects of different antidepressants in the rat. Pharmacol Res. 2003;48(2):157-165.
8. Hajhashemi V, Sadeghi H, Minaiyan M, Movahedian A, Talebi A. The role of central mechanisms in the anti-inflammatory effect of amitriptyline on carrageenaninduced paw edema in rats. Clinics (Sao Paulo). 2010;65:1183-1187.
9. Sadeghi H, Hajhashemi V, Minaiyan M, Movahedian A, Talebi A. A study on the mechanisms involving the anti-inflammatory effect of amitriptyline in carrageenan-induced paw edema in rats. Eur J Pharmacol. 2011;667(1-3):396-401.
10. Ford AC, Talley NJ, Schoenfeld PS, Quigley EM, Moayyedi P. Efficacy of antidepressants and psychological therapies in irritable bowel syndrome: systematic review and meta-analysis. Gut. 2009;58(3):367-378.
11. Ruepert L, Quartero AO, de Wit NJ, van der Heijden GJ, Rubin G, Muris JW. Bulking agents, antispasmodics and antidepressants for the treatment of irritable bowel syndrome. Cochrane Database Syst Rev.. 2011;10(8):CD003460.
12. Lyttle JR, Urquhart DM, Cicuttini FF, Wluka AE. Antidepressants for osteoarthritis. The Cochrane Library. 2016;893:393-397.
13. Saravanan V, Krishnaraju V. Comparative effect of antidepressants (duloxetine) and nsaid (dexibuprofen) in a new rat model of chronic pain induced depression associated with monosodium iodo acetate (mia) induced osteoarthritis in rats. Br J Pharm Res. 2014;4(1):113-124.
14. Wichniak A, Wierzbicka A, Jernajczyk W. Sleep and antidepressant treatment. Curr Pharm Des. 2012;18(36):5802-5817.
15. Negro-Alvarez JM, Carreno-Rojo A, Funes-Vera E, Garcia-Canovas A, Abellan-Aleman AF, Rubio del Barrio R. Pharmacologic therapy for urticaria. Allergol Immunopathol. 1997;25(1):36-51.
16. Godfrey RG. A guide to the understanding and use of tricyclic antidepressants in the overall management of fibromyalgia and other chronic pain syndromes. Arch Intern Med. 1996;156(10): 1047-1052.
17. Sansone RA, Sansone LA. Pain, pain, go away: antidepressants and pain management. Psychiatry (Edgmont). 2008;5(12):16-19.
18. Wrzosek A, Obara I, Wordliczek J, Przewlocka B. Efficacy of tramadol in combination with doxepin or venlafaxine in inhibition of nociceptive process in the rat model of neuropathic pain: an isobolographic analysis. J Physiol Pharmacol. 2009;60(4):71-78.
19. Shibuya K, Funaki Y, Hiraoka K, Yoshikawa T, Naganuma F, Miyake M, *et al*. [(11)C]Doxepin binding to histamine H_1 receptors in living human brain: reproducibility during attentive waking and circadian rhythm. Front Syst Neurosci. 2012;6:45.
20. Shimamura T, Shiroishi M, Weyand S, Tsujimoto H, Winter G, Katritch V. Structure of the human histamine H_1 receptor complex with doxepin. Nature. 2011;475(7354):65-70.
21. Sawynok J, Esser MJ, Reid AR. Antidepressants as analgesics: an overview of central and peripheral mechanisms of action. J Psychiatry Neurosci. 2001;26:21-29.
22. Singh H, Becker PM. Novel therapeutic usage of low-dose doxepin hydrochloride. Expert opin investig drugs. 2007;16(8):1295-1305.
23. Hajhashemi V, Sadeghi H, Minaiyan M, Movahedian A, Talebi A. Central and peripheral anti-inflammatory effects of maprotiline on carrageenan-induced paw edema in rats. Inflamm Res. 2010;59(12):1053-1059.
24. Budantsev A, Kisliuk OS, Shul'govskii VV, Rykunov DS, Iarkov AV. [The brain in stereotaxic coordinates (a textbook for colleges)]. Zh Vyssh Nerv Deiat Im I P Pavlova. . 1993;43(5):1045-1051.
25. Winter CA, RisLey EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544-547.
26. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol. 1982;78(3):206-209.
27. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol. 1971;104(1):15-29.
28. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol.. 2003;225:115-121.

29. Al-Haboubi HA, Zeitlin IJ. Re-appraisal of the role of histamine in carrageenan-induced paw oedema. Eur J Pharmacol. 1983;88(2-3):169-176.

30. Je I-G, Kim H-H, Park P-H, Kwon TK, Seo S-Y, Shin T-Y, *et al*. SG-HQ2 inhibits mast cell-mediated allergic inflammation through suppression of histamine release and pro-inflammatory cytokines. Exp Biol Med (Maywood). 2015;240(5):631-638.

31. Shajib MS, Khan WI. The role of serotonin and its receptors in activation of immune responses and inflammation. Acta Physiol (Oxf). 2015;213(3): 561-574.

32. Verbout NG, Jacoby DB. Muscarinic receptor agonists and antagonists: effects on inflammation and immunity. Handb Exp Pharmacol. 2012;(208):403-27.

33. Frahnert C, Rao ML, Grasmäder K. Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. J Chromatogr B Analyt Technol Biomed Life Sci. . 2003;794(1):35-47.

34. Gronewold A, Dettling A, Haffner HT, Skopp G. Doxepin and nordoxepin concentrations in body fluids and tissues in doxepin associated deaths. Forensic Sci Int. 2009;190(1-3):74-79.

35. Shu YZ, Hubbard JW, Cooper JK, McKay G, Korchinski ED, Kumar R, *et al*. The identification of urinary metabolites of doxepin in patients. Drug Metab Dispos. 1990;18(5):735-741.

36. Castanon N, Leonard BE, Neveu PJ, Yirmiya R. Effects of antidepressants on cytokine production and actions. Brain Behav Immun. 2002;16(5): 569-574.

37. Hajhashemi V, Sadeghi H, Minaiyan M, Movahedian A, Talebi A. Effect of fluvoxamine on carrageenan-induced paw edema in rats evaluation of the action sites. Iran J Pharm Res. 2011;10(3):611-618.

38. Gądek-Michalska A, Tadeusz J, Rachwalska P, Bugajski J. Cytokines, prostaglandins and nitric oxide in the regulation of stress-response systems. Pharmacol Rep. 2013;65(6):1655-1662.