Original Article

A study on the anti-inflammatory effects of new derivatives of 3-hydroxy pyridine-4-one

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Abstract

Background: Derivatives of pyridine-4-one act as iron chelators and possess various pharmacological effects such as antifungal, antimalarial, antiviral, anti-inflammatory, and analgesic effects. The aim of our study was to evaluate the anti-inflammatory effects of the three new derivatives of pyridine-4-one.

Materials and Methods: Carrageenan-induced paw edema in rats and croton oil-induced ear edema in mice were used to evaluate the anti-inflammatry effects of three 3-hydroxy-pyridine-4-one derivatives (compounds A, B, and C). Compound A (10, 20 mg/kg), compound B (200, 400 mg/kg), and compound C (100, 200 mg/kg), vehicle (1 mL/kg), and indomethacin as standard drug (10 mg/kg) were injected intraperitoneally 30 min prior to carrageenan injection and 4 h later, the paw volume was measured using a mercury plethysmograph. The maximum dose of each test compound was used in the croton oil-induced ear edema test.

Results: All compounds showed significant anti-inflammatory activity in both tests. On a molar basis, compound A had the greatest potency, which may be due to the presence of a benzyl group substitution on the pyridine ring.

Conclusions: Because cyclooxygenase and lipoxygenase as key enzymes of the inflammation pathway are heme-dependent, it seems that the anti-inflammatory effect of derivatives of pyridine-4-one may be related to their iron chelating properties. However, more investigations are needed to find out their exact mechanism of actions.

Key Words: Anti-inflammatory, iron chelators, 3-hydroxy pyridine-4-one derivatives

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INTRODUCTION

Rheumatoid arthritis as a common inflammatory joint disease is associated with increased concentrations of synovial fluid iron deposits.[1,2] In addition, iron via a Fenton reaction involves in the production of highly reactive oxygen radicals with ability to initiate and develop inflammatory processes.[3] On the other hand, cyclooxygenase as a key enzyme in inflammation pathway produces prostaglandins, which have important roles in inflammation and pain. Cyclooxygenase contains heme (an iron containing molecule), which acts as a catalytic center for the generation of oxygen radicals.[4,5] Because iron has an important role in the inflammatory process, some investigations have been conducted to evaluate the anti-inflammatory activity of iron chelators.

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Sedgwick *et al*. examined the effect of the iron chelating agent, desferrioxamine, on animal models of acute inflammation induced by carrageenan or calcium pyrophosphate crystals and confirmed that desferrioxamine shows anti-inflammatory effect.[6]

Because desferrioxamine has a very low oral bioavailability, researchers focused on iron chelators with improved oral bioavailability. Hewitt and coworkers synthesized a series of 3-hydroxypyridin-2-one and 3-hydroxypyridin-4-one compounds as iron chelators and examined their anti-inflammatory effects in the carrageenan pleurisy model. They reported that these iron chelators are more potent than desferrioxamine in their iron scavenging abilities, and some of them showed anti-inflammatory effect comparable with indomethacin.[7] Some other derivatives of 3-hydroxy pyridine-4-one have also shown iron chelating activity, anti-inflammatory, and analgesic activities.[8-10] On the basis of these findings, two mechanisms have been suggested for anti-inflammatory potential of iron chelators: (1) inhibition of proinflammatory prostanoid synthesis and (2) inhibition of toxic free radical generation by cyclooxygenase.[10] In a previous study, we showed analgesic activity for some new derivatives of 3-hydroxy pyridine-4-one [Figure 1].[11] Therefore, this study was designed to evaluate their anti-inflammatory activity in animal models.

MATERIALS AND METHODS

Animals

Experiments were performed on male Wistar rats, weighing 200-220 g, and male Swiss mice (18-22 g). All animals were maintained under standard laboratory conditions in the animal house of School of Pharmacy, Isfahan University of Medical Sciences (Isfahan, Iran). These animals were euthanized immediately after each experiment. All experiments were carried out in accordance with local guidelines for the care of laboratory animals of Isfahan University of Medical Sciences (Isfahan, Iran).

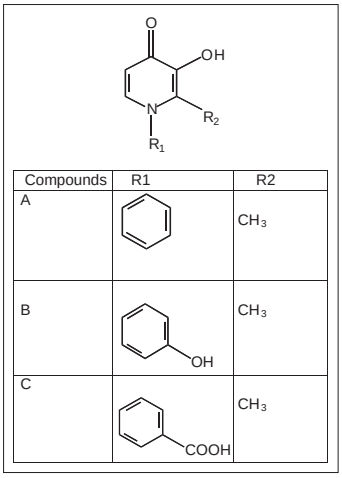

Figure 1: Chemical structures of three derivatives of 4(1H)-pyridinone (compounds A, B, and C), which were used to test their anti-inflammatory activities

Chemicals

Three derivatives of 4 (1*H*)-pyridinone (compounds A, B, and C) as shown in Figure 1, which had been synthesized in Department of Medicinal Chemistry, School of Pharmacy, Isfahan University of Medical Sciences (Isfahan, Iran), were used.[12] Indomethacin (Sigma, USA) was used as a reference anti-inflammatory drug.

Carrageenan-induced paw edema

Compounds A, B, and C were administered intraperitoneally (i.p.) to rats. The control animals received vehicle (10 mL/kg), and the reference group received indomethacin (10 mg/kg). Thirty minutes after carrageenan injection, the rats received a subplantar injection of 100 μL of a 1% (w/v) suspension of carrageenan lambda in the right hind paw.[13] The volume of the paw was measured by a mercury plethysmometer (Ugo Basil, Italy) immediately prior to and 4 h after carrageenan injection. The data were expressed as the volume difference (mL) of carrageenan-treated and control paw.

Croton oil-induced ear edema in mice

The tested compounds were administered i.p. to mice. Compound A (20 mg/kg), compound B (400 mg/kg), and compound C (200 mg/kg) were used. Control animals received vehicle (10 mL/kg), and the reference group received indomethacin (10 mg/kg). Thirty minutes later, 15 μL of croton oil in acetone (100 μg/15 μL) was applied to the inner surface of the right ear of mice. The left ear was considered as control. Six hours after croton oil application, the animals were killed and both ears were cut. Discs of 6-mm diameter were removed from each ear and weighed. The difference in weight between the punches from the right and left ears was considered as ear edema.[14,15]

Statistical analysis

The results were expressed as mean ± SEM. The data obtained in the experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by a Scheffe *post hoc* test. $P < 0.05$ were considered significant.

RESULTS

Three new derivatives of hydroxy pyridinone (compounds A, B, and C) were investigated for their possible anti-inflammatory effect. Indomethacin as a standard anti-inflammatory drug inhibited carrageenan-induced

paw edema by 60% [Figure 2]. The maximum applied dose of compound A (20 mg/kg) produced 67% inhibition in carrageenan-induced paw edema. The anti-inflammatory activity of compound B has been shown in Figure 3. This compound at doses of 200 and 400 mg/kg significantly ($P < 0.001$) inhibited inflammation. The results of the anti-inflammatory activity of compound C are illustrated in Figure 4. Compound C at doses of 100 and 200 mg/kg significantly ($P < 0.001$) reduced carrageenan-induced paw inflammation by 56% and 58%, respectively.

In the croton oil test, indomethacin as a standard anti-inflammatory drug inhibited inflammation by 65%. All of compounds at applied doses significantly inhibited ear edema induced by croton oil [Figure 5]. Compounds A (20 mg/kg), B (400 mg/kg), and C (200 mg/kg) showed anti-inflammatory activity by 37%, 43% and 50%, respectively.

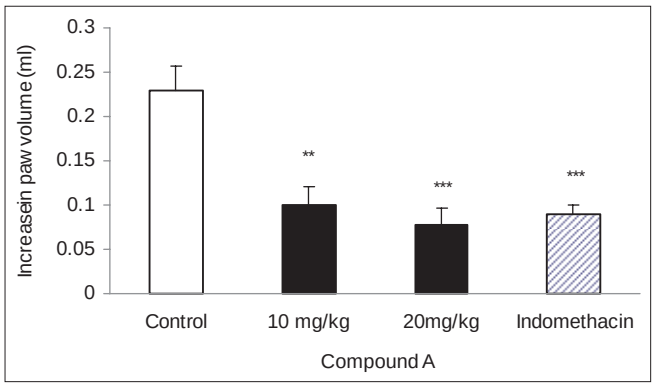

Figure 2: The anti-inflammatory activity of compound A in the carrageenan-induced paw edema test. Vehicle and two doses of compound A were administered 30 min prior to subplantar injection of carrageenan, and the volume of the paw (mL) was measured immediately prior to carrageenan injection and 4 h after injection. Indomethacin (10 mg/kg, i.p.) was used as the reference drug. Data are mean ± SEM of 6 animals in each group. $^{**}P < 0.01$; $^{***}P < 0.001$ significantly different from the control group (ANOVA with Scheffe post hoc test)

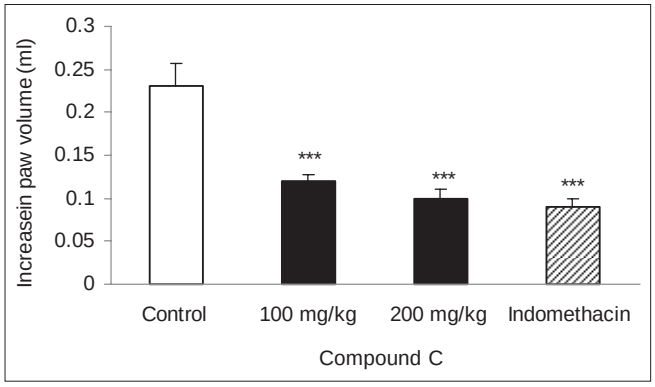

Figure 4: The anti-inflammatory activity of compound C in the carrageenan-induced paw edema test. Vehicle and two different doses of compound B were administered 30 min prior to subplantar injection of carrageenan and the volume of the paw (mL) was measured immediately prior and 4 h after carrageenan injection. Indomethacin (10 mg/kg, i.p.) was used as the reference drug. Data are mean ± SEM of 6 animals in each group. $^{***}P < 0.001$ significantly different from the control group (ANOVA with the Scheffe post hoc test)

DISCUSSION

In this study, anti-inflammatory effects of three new derivatives of 3-hydroxy-pyridine-4-one were evaluated. As discussed in the "Results" section, all compounds show significant anti-inflammatory effects in both the carrageenan-induced paw edema test and croton-induced ear edema.

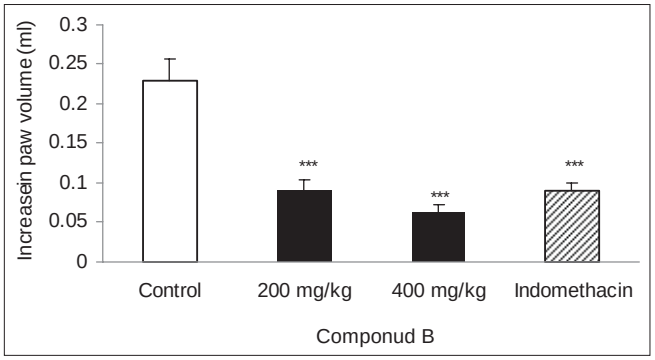

Figure 3: The anti-inflammatory activity of compound B in the carrageenan-induced paw edema test. Vehicle and two different doses of compound B were administered 30 min prior to subplantar injection of carrageenan and the volume of the paw (mL) was measured immediately prior to carrageenan injection and 4 h afterward. Indomethacin (10 mg/kg, i.p.) was used as the reference drug. Data are mean ± SEM of 6 animals in each group. $^{***}P < 0.001$ significantly different from the control group (ANOVA with the Scheffe post hoc test)

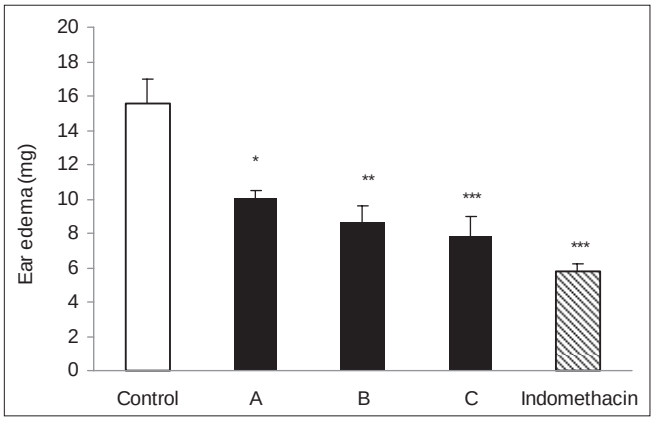

Figure 5: The anti-inflammatory activity of compounds A, B, and C in the croton oil test. All compounds and vehicle were administered i.p. The reference group received indomethacin (10 mg/kg). Thirty minutes later, 15 μL of croton oil solution applied on the inner surface of the right ear of each mouse. The left ear was considered as control. After 6 h, discs of 6-mm diameter were removed from each ear and weighed. The differences between weights of the left and right ear were considered as edema. Data are mean ± SEM of 6 animals in each group. $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$ significantly different from the control group (ANOVA with the Scheffe post hoc test)

Carrageenan-induced paw edema is a well-known model of acute inflammation that consists of a biphasic inflammatory response and a number of mediators participate in this inflammatory response.[13,16] Following the injection of carrageenan into the rat paw, several mediators are sequentially released, including histamine, serotonin, and bradykinin in the initial phase (0-1 h), followed by an increase in the production of prostaglandins (PGs) through the activation of cyclooxygenase-2 (COX-2) and the release of nitric oxide (NO) in the second phase (1-6 h).[17-19] It is well-known that reactive oxygen species, NO and PGE_2 are considered as inflammatory factors and play important roles in the tissue damage by inflammation.[20,21] It was found that the injection of carrageenan into the rat paw induced the liberation of bradykinin, which later induced the biosynthesis of PGs and other autacoids that are responsible for the formation of the inflammatory exudates.[22,23] PGs play an important role in the inflammatory response, and it is currently recognized that a variety of organizations stimulated by physical, chemical, and biological factors lead to the synthesis and release of a variety of PGs.[24,25] Therefore, as inflammation is a peripheral process, it is suggested that new derivatives of hydroxyl pyridinone exerted peripheral effects. The anti-inflammatory effect of new derivatives of hydroxyl pyridinone may be due to a decrease in the production of PGs, NO, bradykinin, or other inflammatory mediators.

In our study, the anti-inflammatory effects of new hydroxyl pyridinone derivatives were also evaluated by ear edema induced by croton oil in mice. Ear edema induced by croton oil has been widely accepted as a useful pharmacological model for the investigation of new anti-inflammatory drugs.[26] Croton oil contains 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and other phorbol esters as main irritant agents.[27] Application of croton oil can induce significant inflammatory response as characterized by edema, neutrophil infiltration, prostaglandin production, and increases in vascular permeability.[28] It is reported that cyclooxygenase inhibitors and also 5-lipoxygenase inhibitors are highly effective against inflammation caused by TPA.[29] In our previous study, we tested the analgesic effects of these derivatives of hydroxyl pyridinone. All compounds showed analgesic effects in the acetic acid-induced writhing test and formalin test. The results of this study along with the results of the previous work clearly indicate the beneficial effects of these compounds in alleviating pain of inflammatory origin.

In our previous work, the analgesic effect of the tested compounds was more significant in the late phase of the formalin test. In addition, it has been reported that the late phase depends on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord. The tested compounds indicate an action related to the inflammatory process. The results of this study are in agreement with the previous studies,[3–5] and indicate that 3-hydroxy-pyridine-4-one have analgesic and anti-inflammatory effects. Cyclooxygenase and lipoxygenase are important enzymes for inflammation and pain responses, and these enzymes depend on iron. Since compounds with 3-hydroxy-pyridine-4-one structure have iron chelating activity, it seems that the analgesic and anti-inflammatory effects of hydroxyl pyridinone derivatives might be due to their iron chelating activity.[10,30] In addition, free radicals are involved in the inflammatory process and iron chelators have antioxidant activity.[31] Therefore, perhaps their anti-inflammatory effects are to some extent related to their antioxidant properties. In conclusion, based on the previous and present study, new derivatives of 3-hydroxy-pyridine-4-one have analgesic and anti-inflammatory effects.

CONCLUSIONS

All of three compounds which had been designed and synthesized as iron chelators showed anti-inflammatory activity in the carrageenan-induced paw edema and croton oil-induced ear edema test. Further studies are necessary to understand the underlying and implicated mechanisms of observed pharmacologic effects.

ACKNOWLEDGMENTS

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