Anti-inflammatory and Anti-allergic Activities of Hydroxylamine and Related Compounds

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The anti-inflammatory activities of several novel oximes and 0-acyl oximes that we synthesized have been reported based on carrageenan-induced rat foot-pad swelling assay and histamine-induced rat vascular permeability assay. A cyclooxygenase (COX)-1 inhibitory effect has also been reported for 4’-piperidinoacetophenone and 4’-morpholinooctetophenone oximes and their 0-acyl derivatives. To further search for more effective non-steroidal anti-inflammatory or anti-allergic drugs, 1-hydroxylamino-1-(4’-piperidinophenyl) ethane (P-HA) and 1-hydroxyamino-1-(4’-morpholinophenyl) ethane (M-HA) were synthesized from the corresponding oximes with sodium cyanoborohydride, and N,O-diacetyl hydroxylamines (P-HA-Ac and M-HA-Ac) were prepared from these hydroxylamines using acetyl chloride. These hydroxylamines and N,O-diacetyl hydroxylamines clearly exhibited inhibitory effects on mouse carrageenan-induced foot-pad swelling induced by oral administration (150, 37.5 mg/kg). An oral dose of P-HA-Ac (150 mg/kg) significantly inhibited the mouse anaphylactic reaction to ovalbumin measured by the abdominal wall (AW) method. Percutaneous administration of P-HA and M-HA significantly inhibited 2,4-dinitrofluorobenzene (DNFB)-induced contact hypersensitivity reaction (type IV) in mice at a dose of 0.5 and 0.1 mg/ear, respectively. All tested hydroxylamines and N,O-diacetyl hydroxylamines clearly inhibited both COX-1 and COX-2 enzyme activities with IC50 values of 1.9—28.7 and 1.6—2.9 mm against COX-1 and COX-2, respectively. Hydroxylamines (P-HA and M-HA) also showed a 5-lipoxygenase inhibitory effect.

Key words hydroxylamine; N,O-diacetyl hydroxylamine; anti-inflammatory activity; anti-allergic activity; cyclooxygenase; 5-lipoxygenase

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesic or antipyretic agents for the clinical treatment of inflammatory diseases such as arthritis, lumbago and rheumatism. These NSAIDs (e.g., ibuprofen, aspirin, and indomethacin) exhibit an inhibitory action on the cyclo- oxygenase (COX) that catalyzes the biosynthesis of prostaglandins and thromboxane from arachidonic acid. There are two isofoms of the membrane-bound COXs, COX-1 expressed constitutively, and COX-2 induced by stimuli of cytokines, mitogens or hormones. The constitutive COX-1 has also been reported to be induced by the stimulus of an angiogenic cytokine vascular endothelial growth factor (VEGF) or by shear stress in vascular endothelial cells. Recently, several COX-2 selective inhibitors such as meloxicam and celecoxib have been developed as NSAIDs because these substances show little gastropathy, a typical side effect of NSAIDs.

We have previously reported the synthesis of a number of novel oximes and their derivatives, with several compounds showing anti-inflammatory activities in carrageenan-induced rat foot-pad swelling assay and histamine-induced rat vascular permeability assay. We also reported the COX-1 inhibitory effect of the 4’-piperidinoacetophenone oxime (P-Ox), 4’-morpholinooctetophenone oxime (M-Ox) and their O-acyt derivatives (P-Ox-Ac or M-Ox-Ac). The metabolic products of arachidonic acid catalyzed by 5-lipoxygenase (LOX) are leukotrienes (LTs) which function as important mediators in various types of inflammation reaction including allergy, asthma, and arthritis. For instance, LTC4 and LTD4, well-known components of the slow-reacting substance of anaphylaxis (SRS-A), induce severe bronchoconstriction and increase vascular permeability, and LTB4 causes leukocyte chemotaxis. Therefore, the development of dual inhibitors of COX and 5-LOX should be promising for the treatment of several inflammatory and allergy symptoms. Many investigations have shown that the enzymes catalyzing the arachidonic acid cascade (COX, 5-LOX, and/or 12-LOX) were inhibited by various hydroxamic acids and their derivatives. Kramer et al. have reported a dual inhibitory action to COX and 5-LOX by hydroxylamine analogs of 2, 6 di-tert-butylphenols.

The purpose of the present study is to search for more effective anti-inflammatory or anti-allergic drugs such as COX-2 selective and/or 5-LOX inhibitors. Hydroxylamine derivatives, which are substances reduced from anti-inflammatory oximes, are potential candidates for both anti-inflammatory and anti-allergic agents. Thus, anti-inflammatory oximes (P-Ox and M-Ox) were reduced to the corresponding hydroxylamines; (1-hydroxyamino-1-(4’-piperidinophenyl) ethane, P-HA, and 1-hydroxyamino-1-(4’-morpholinophenyl) ethane, M-HA). We also prepared N,O-diacetyl hydroxylamine derivatives, i.e., 1-(N,O-diacetyl hydroxylamo)-1-(4’-piperidinophenyl) ethane (P-HA-Ac) and 1-(N,O-diacetyl hydroxylamo)-1-(4’-morpholinophenyl) ethane (M-HA-Ac), as O-acetyl oximes inhibited the increased vascular permeability induced by histamine in rat skin more strongly than the original oximes.

Anti-inflammatory activities were evaluated by screening with carrageenan-induced mouse foot-pad swelling assay. Anti-allergic activities (type I) were examined by the abdominal wall (AW) method assay with a simple technique for induction and estimation of mouse anaphylactic reaction to ovalbumin (OVA). Anti-allergic activities (type IV) were tested by the 2,4-dinitrofluorobenzene (DNFB)-induced contact hypersensitivity reaction.
tact hypersensitivity reaction.\textsuperscript{21)} Furthermore, in vitro COX and 5-LOX inhibitory tests for the hydroxylamines and related compounds were performed to elucidate the mechanism of their inhibitory effect on inflammation and allergy.

MATERIALS AND METHODS

**Animals** Male ddY mice (SPF, 5 weeks old for type I anti-allergic effect assay and 6 weeks old for anti-inflammation assay), male BALB/c mice (SPF, 7—8 weeks old for type IV anti-allergic effect assay), and male Sprague–Dawley rats (SPF, 120—150 g body weight) were purchased from Japan SLC Co. (Hamamatsu). These animals were maintained under constant temperature (24 ± 2°C) and were given commercial laboratory diet (CE-2, Clea Japan, Tokyo) and tap water ad libitum. This animal study was approved by the Experimental Animal Research Committee of the School of Pharmaceutical Sciences, Mukogawa Women’s University.

**Reagents** The following reagents were used: sodium cyanoborohydride (NaBH₄CN) (Aldrich Chemical, Co., Inc., U.S.A.); acetyl chloride (CH₃COCl), DNFB, sodium carboxymethyl cellulose (CMC·Na), dimethyl sulfoxide (DMSO), ibuprofen, and diphenhydramine hydrochloride (Nacalai Tesque, Inc., Osaka, Japan); hydroxylamine hydrochloride (NH₂OH·HCl), Evans blue, and phenidone (DMSO), ibuprofen, and diphenhydramine hydrochloride (CMC·Na), dimethyl sulfoxide (Japanese Pharmacopoeia grade, Maruishi, Pharmaceutical Co., Ltd., Japan); Freund’s incomplete adjuvant (FIA) (Difco Laboratories, Detroit, MI, U.S.A.); OVA (Sigma Chemical Co., St. Louis, U.S.A.); NS-398 (Cayman Chemical, Co., U.S.A.); acetyl chloride (CH₃COCl), 24.39 (C-4), 24.39 (C-3), 7.24; N, 9.14. Found: C, 62.67; H, 7.38; N, 9.12. IR (film.) cm⁻¹: 2935, 1796, 1517. EI-MS m/z: 304 (M⁺, 19%), 245 (58%), 203 (43%), 188 (100%). \textsuperscript{1}H-NMR (CDCl₃ at 50°C) δ: 1.51 (3H, d, J = 7.3, H-2), 1.58 (2H, m, H-4'), 1.69 (4H, m, H-3',5'), 1.99 (3H, s, NCOCH₃), 2.04 (3H, br s, OCOCH₃), 3.15 (4H, 2H, br, J = 8.8, H-3',5'), 7.20 (2H, d, J = 8.8, H-2',6'). \textsuperscript{13}C-NMR (CDCl₃ at 50°C) δ: 17.11 (C-2', br), 18.12 (OCOCH₃), 20.95 (NCOCH₃), 24.39 (C-4'), 25.85 (C-3',5'), 50.34 (C-2',6'), 56.12 (C-1', br), 115.87 (C-3',5'), 128.37 (C-2', 6', br), 129.36 (C-1', br), 151.79 (C-4'), 168.46 (OCO), 170.36 (NCO, br). 1-(N,O-Diacetylhydroxylamino)-1-(4'-piperidinophenyl)ethane, M-HA-Ac: mp 86—88°C, from diethylether–petroleum ether, 59%. Anal. Caled for C₁₇H₂₄N₂O₃: C, 67.08; H, 7.95; N, 9.20.

**Preparation of Hydroxylamine Compounds** Hydroxylamines (P-HA and M-HA) were prepared from the P-Ox or M-Ox reported previously\textsuperscript{5} according to a method using NaBH₄CN described by Borch et al. with some modifications.\textsuperscript{22} The detailed preparation method and the MS, IR, and NMR spectrometric and elemental analysis data of their compounds have been reported elsewhere.\textsuperscript{5,17}

Preparation of N,O-Diacetyl Hydroxylamine Compounds (P-HA-Ac or M-HA-Ac) A solution of CH₃COCl (2.5 eq) in anhydrous CHCl₃ was added dropwise to a mixture of the hydroxylamine (P-HA or M-HA) (1 eq), triethylamine (2.5 eq), and anhydrous CHCl₃ under ice-cooling. The solution was washed with 10% NaHCO₃ and water, then dried over anhydrous MgSO₄, and evaporated in vacuo. The products were purified by column chromatography on silica gel and their structures confirmed by elemental analysis, IR, EI-MS and \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectrometry.

1-(N,O-Diacetylhydroxylamino)-1-(4'-piperidinophenyl)ethane, P-HA-Ac: mp 55—56°C (from petroleum ether, 22%). Anal. Caled for C₂₃H₂₆N₂O₇: C, 67.08; H, 7.95; N, 9.20. Found: C, 67.01; H, 8.08; N, 9.15. IR (film.) cm⁻¹: 2935, 1796, 1517. EI-MS m/z: 304 (M⁺, 19%), 245 (58%), 203 (43%), 188 (100%). \textsuperscript{1}H-NMR (CDCl₃ at 50°C) δ: 1.51 (3H, d, J = 7.3, H-2), 1.58 (2H, m, H-4'), 1.69 (4H, m, H-3',5'), 1.99 (3H, s, NCOCH₃), 2.04 (3H, br s, OCOCH₃), 3.15 (4H, m, H-2',6'), 5.65 (3H, brq, J = 6.6), 6.85 (2H, d, J = 8.8, H-3',5'), 7.20 (2H, d, J = 8.8, H-2',6'). \textsuperscript{13}C-NMR (CDCl₃ at 50°C) δ: 17.11 (C-2', br), 18.12 (OCOCH₃), 20.95 (NCOCH₃), 24.39 (C-4'), 25.85 (C-3',5'), 50.34 (C-2',6'), 56.12 (C-1', br), 115.87 (C-3',5'), 128.37 (C-2', 6', br), 129.36 (C-1', br), 151.79 (C-4'), 168.46 (OCO), 170.36 (NCO, br). 1-(N,O-Diacetylhydroxylamino)-1-(4'-morpholinophenyl)ethane, M-HA-Ac: mp 86—88°C, from diethylether–petroleum ether, 59%. Anal. Caled for C₁₇H₂₄N₂O₃: C, 67.08; H, 7.95; N, 9.20. Found: C, 67.01; H, 8.08; N, 9.15. IR (film.) cm⁻¹: 2935, 1796, 1517. EI-MS m/z: 306 (M⁺, 26%), 247 (72%), 205 (54%), 190 (100%). \textsuperscript{1}H-NMR (CDCl₃ at 50°C) δ: 1.51 (3H, d, J = 7.0, H-2), 1.99 (3H, s, NCOCH₃), 2.06 (3H, brs, OCOCH₃), 3.14 (4H, m, H-3',5'), 3.84 (4H, m, H-2',6'), 5.67 (3H, brq, J = 6.6), 6.84 (2H, d, J = 8.8, H-3',5'), 7.27 (2H, d, J = 8.8, H-2',6'). \textsuperscript{13}C-NMR (CDCl₃ at 50°C) δ: 17.05 (C-2', br), 18.12 (OCOCH₃), 20.93 (NCOCH₃), 49.21 (C-3',5'), 66.92 (C-2',6'), 55.98 (C-1', br), 115.29 (C-3',5'), 128.49 (C-2',6', br), 130.57 (C-1', br), 150.93 (C-4'), 168.42 (OCO), 170.41 (NCO, br).

**Inhibitory Effect on Carrageenan-Induced Foot-Pad Swelling in Mice** All test compounds and ibuprofen were prepared by suspending them in 0.5% CMC-Na solution, immediately before the start of the experiments. Mice were starved overnight before the experiment, and then administered orally with the CMC-Na suspension of the test compounds (150 mg/15 ml/kg or 37.5 mg/15 ml/kg). Control experiments were performed with the 0.5% CMC-Na suspension of distilled water alone (15 ml/kg). After 30 min, 0.02 ml of a 1% α-carrageenan suspension in sterile saline was injected into the right hind paw (foot-pad) of mice and the left foot-pad of mice was injected with sterile saline alone (0.02 ml). The thickness of the foot-pads on both sides was measured with a Peacock Dial Thickness Gauge (Ozaki, Japan), before and at 1, 2, 3, 4, 5, 6, and 7 h after carrageenan injection, and the differences in the thickness were calculated. The degree of foot-pad swelling was expressed as follows:

\[
\text{increase in foot-pad thickness (mm)} = (Rt - Ro) - (Lt - Lo)
\]

where Rt: right foot-pad thickness after carrageenan injection, Ro: right foot-pad thickness before carrageenan injection, Lt: left foot-pad thickness after saline injection, Lo: left foot-pad thickness before saline injection.
Swelling in Rats  Inhibitory assay of P-HA on carrageenan-induced foot-pad swelling in rats was carried out using the same conditions as previously described. Differences between the experiments using rat and mouse were as follows. Swelling reactions of rats were produced in the right foot-pad by subplantar injection of 0.1 ml of a 1% λ-carrageenan suspension in sterile saline. The dose of P-HA tested was 75 mg/kg or 30 mg/kg of body weight, respectively. From the rat data, we expressed the % swelling as well as the data from a previous report.

Inhibitory Effect on Anaphylactic Reaction (AW Method)  The experiments were carried out according to a method reported previously. Briefly, mice were sensitized intraperitoneally with a 1:1 emulsion of OVA (2 mg/ml of saline) and FIA. CMC·Na suspension (0.5%) of the test compounds (150 mg/15 ml/kg) were administered orally to mice which were starved overnight before the challenge, 14 d after the sensitization of OVA. Control mice were administered 0.5% CMC·Na suspension of distilled water alone (15 ml/kg). In the case of diphenhydramine hydrochloride, mice of the experimental group were orally administered the drug dissolved in distilled water and control mice were given water only. Thirty minutes after the oral administration, 0.1 ml of 1% Evans blue dye was injected intravenously, and the animals were anesthetized with diethyl ether. The skin of the abdomen was detached, and 5 min after injection of the dye, OVA solution (5 µg/0.05 ml/site, 0.1 mg/ml of saline) was injected in the exposed abdominal wall. The mouse was killed by cervical dislocation 7 min after the challenge, and after removal of the abdominal wall, the diameter of the blue area of the abdominal wall was measured. This anti-allergic activity (type I) was expressed as the percentage inhibition compared with the control group.

Inhibitory Effect on DNFB-Induced CHR  The dorsal skin of the mice was shaved on the day before the experiment, and the mice were sensitized with 0.1 ml of 0.5% DNFB in acetone–olive oil (4:1) applied to the dorsal skin on days –1 and 0. Five days after the second sensitization, mice were dosed percutaneously with ethanol–acetone–olive oil (5:4:1) solution of the test compounds (0.5 or 0.1 mg/0.02 ml/ear) or phenidone (1 or 0.5 mg/0.02 ml/ear) on both right and left ears. As controls, both ears of the mice were painted with the vehicle used for the dissolution of the test compounds (0.02 ml/ear). Immediately after the above percutaneous administration, the mice were challenged on the right ear with 0.5% DNFB in acetone–olive oil (4:1) (0.02 ml/ear) and the left ear was painted with the vehicle, acetone–olive oil (4:1) alone (0.02 ml/ear).

Ear thickness was measured with a Peacock Dial Thickness Gauge before and 24 h after the DNFB challenge, and the swelling percentage (%) was calculated. The degree of ear swelling was expressed as follows:

\[
\text{swelling percentage} \% = \frac{(E_t - E_o)}{E_o} \times 100
\]

where \(E_o\) and \(E_t\) represent the ear thickness before and after challenge.

Inhibitory Assays of COX-1, COX-2 and 5-LOX  Screening for COX-1 and COX-2 inhibitors was performed using a COX (ovine) inhibitor screening assay kit according to the manufacturer’s instructions.

Preliminary screening assay for the 5-LOX inhibitor was performed using a LOX inhibitor screening assay kit for 15-LOX, with modifications. Briefly, immediately before a test, an assay buffer solution of 3-fold dilution of 5-LOX (potato) purified with an ultrafiltration membrane (30 KDA cut off) was used instead of the 15-LOX packed as the standard enzyme.

Test compounds dissolved in DMSO were added to each assay buffer, and the final solvent used for the preparation of the samples was used as a positive control. The IC\(_{50}\) value, the concentration necessary to obtain 50% inhibition of the responses, was determined from the logarithmic regression line of a dose-response curve.

Statistical Analysis  The data from the inhibitory tests of carrageenan-induced swelling and DNFB-induced CHR were analyzed using Williams’ multiple comparison test. The data from the inhibitory test of the anaphylactic reaction were examined using analysis of variance followed by Fisher’s Protected Least Significant Difference (PLSD) test. Each value represents the mean±S.E. Asterisks indicate significant differences from the control group (*, \(p<0.01\) and *, \(p<0.05\)).

RESULTS AND DISCUSSION  The structures and abbreviations of the compounds used in this study are shown in Table 1. These hydroxylamines and diacetyl hydroxylamines were used as racemic compounds. In the present study, the assay of the inhibitory effect on carrageenan-induced foot-pad swelling using the mouse model was conducted to evaluate the in vivo anti-inflammatory effects because the total doses of the necessary test compounds in the mouse model were smaller than those in the rat model, although it has been reported that the susceptibility to the anti-inflammatory test by NSAIDs in carrageenan-induced foot-pad swelling using the mouse model is lower than that of rats. To confirm whether the foot-pad swelling response in mice is induced by carrageenan and the swelling can be inhibited by NSAIDs, we conducted the following experiments. The level of the foot-pad swelling response of the mice to the λ-carrageenan (Sigma Chem. Co.), which was used previously for rats, was low, however, we obtained satisfactory results by using another λ-carrageenan purchased from Zushikagaku Laboratory Inc. At 5 to 6 h after its injection, the increase in thickness of mouse foot-pad reached maximal level. This carrageenan-induced foot-pad swelling in mice could be inhibited by ibuprofen used as a reference drug, and maximum inhibition was observed 5 h after carrageenan injection (25 mg/kg, per os; percentage inhibition of foot-pad swelling compared with the vehicle-treated control group at 5 h, 28.81±6.59, \(p<0.05\)).

The time course of the foot-pad swelling response in mice of P-HA (150 and 37.5 mg/kg, per os) is presented in Fig. 1a. The inhibitory effects of carrageenan-induced foot-pad swelling in rats of P-HA (75 mg/kg and 30 mg/kg, per os) are also shown in Fig. 1b to confirm whether the method using mouse in this study could give results consistent with the previous method using rat. The results show that P-HA displays concentration-dependent inhibition activity on the carrageenan-induced foot-pad swelling in both mice and rats. The swelling reaction in mice was markedly inhibited by P-HA at 4 to 6 h after carrageenan injection and the inhibitory activity in rats began from immediately after carrageenan in-
Injection. Thus, although the inhibitory patterns differ in mouse and rat, the inhibitory effect of the swelling reaction between the two animals showed the same tendency. Figure 2 shows the anti-inflammatory activities (% of inhibition) of the hydroxylamines and \(\text{N, O-diacetyl hydroxylamines} \) 5 h after carrageenan injection. All tested compounds clearly inhibited carrageenan-induced foot-pad swelling in mice. Interestingly, P-HA derived from P-OX potently inhibited the carrageenan-induced foot-pad swelling reaction although the original P-OX had no inhibitory effect on the reaction. The

<table>
<thead>
<tr>
<th>Hydroxylamines and diacetyl hydroxylamines</th>
<th>R</th>
<th>R_1</th>
<th>Abbreviation</th>
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<tr>
<td>(-\text{H}) P-HA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(-\text{COCH}_3) P-HA-Ac</td>
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<tr>
<td>(-\text{H}) M-HA</td>
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<td>(-\text{COCH}_3) M-HA-Ac</td>
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<th>Oximes and acetyl oximes</th>
<th>R</th>
<th>R_1</th>
<th>Abbreviation</th>
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<td>(-\text{H}) M-Ox</td>
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<td>(-\text{COCH}_3) M-Ox-Ac</td>
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Fig. 2. Anti-inflammatory Activities of the Hydroxylamines and \(N,O\)-Diacyl Hydroxylamines on Carrageenan-Induced Foot-Pad Swelling in Mice

0.5% CMC Na suspensions of the test compounds (150 mg/kg, or 37.5 mg/kg) were given orally 0.5 h before the injection of 1% \(\lambda\)-carrageenan. Control group mice were administered 0.5% CMC Na suspension of distilled water alone (15 ml/kg). Each column and bar of the percentage of inhibition at 5 h after \(\lambda\)-carrageenan injection represent the mean\(\pm\)S.E. for 5—6 mice. Statistical analyses were as in Fig. 1. Asterisks indicate significant differences from control group (**, \(p<0.01\); *, \(p<0.05\)). See text for further details.

Fig. 3. Type I Anti-allergic Activities of Hydroxylamine and Oxime-Related Compounds

Mice were sensitized intraperitoneally (50 \(\mu\)g/50 \(\mu\)l/mouse) with ovalbumin (OVA) in Freund’s incomplete adjuvant on day 0. On day 14, 0.5% CMC Na suspension of distilled water alone (15 ml/kg). Each column and bar of the percentage inhibition (%) represent the mean\(\pm\)S.E. for 5—6 mice. Statistical analyses were as in Fig. 1. Asterisks indicate significant differences from control group (**, \(p<0.01\); *, \(p<0.05\)). See text for further details.

Fig. 4. Type IV Anti-allergic Activities of Hydroxylamine and Oxime-Related Compounds

(a) Hydroxylamines and \(N,O\)-diacyl hydroxylamines. (b) Oximes and acetyl oximes. Mice were sensitized with 0.1 ml of 0.5% DNFB in acetone–olive oil (4 : 1) painted onto the shaved dorsal skin on days −1 and 0. Five days later, the mice were challenged percutaneously with ethanol–acetone–olive oil (5 : 4 : 1) solution of the test compounds (0.5 or 0.1 mg/ear) on both right and left ears (0.02 ml/ear). As a control, both ears were painted with the vehicle (ethanol : acetone : olive oil = 5 : 4 : 1). Immediately after the above percutaneous administration of the test compounds, mice were challenged on the right ear with 0.5% DNFB in acetone–olive oil (4 : 1) (0.02 ml/ear) and the left ear was painted acetone–olive oil (4 : 1) alone (0.02 ml/ear). Ear thickness was measured before and 24 h after the challenge. Each column and bar of the swelling percentage inhibition of right ear represent the mean\(\pm\)S.E. for 6—7 mice. No swelling re-action of the left ear by the test compounds was observed under this condition (data not shown). Statistical analyses were as given in Fig. 1. Asterisks indicate significant differences from control group (**, \(p<0.01\)). See text for further details.

Table 2. IC\(_{50}\) Values of Hydroxylamines and \(N,O\)-Diacyl Hydroxylamines for COX-1 and COX-2 Activities

<table>
<thead>
<tr>
<th>Compound(^a)</th>
<th>IC(_{50}) ((\mu)M)(^b)</th>
<th>Selectivity COX-1/COX-2</th>
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<tbody>
<tr>
<td>P-HA</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>P-HA-Ac</td>
<td>27.8</td>
<td>2.1</td>
</tr>
<tr>
<td>M-HA</td>
<td>11.5</td>
<td>2.9</td>
</tr>
<tr>
<td>M-HA-Ac</td>
<td>18.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>NS-398</td>
<td>&gt;200</td>
<td>0.11</td>
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\(^{a}\) Ibuprofen and NS-398 were used as the reference compounds. \(^{b}\) IC\(_{50}\) values are the means of duplicate determinations.

anti-inflammatory activity of P-OX was estimated by the inhibitory effect on increased vascular permeability induced by histamine.\(^6\)

The AW method is one of the simplest techniques for inducing and detecting anaphylactic reaction in in vivo models.\(^1,9,20\) As shown in Fig. 3, oral administration of P-HA-Ac significantly decreased the diameter of the blue spot compared with the control groups. Also, the anti-allergic effect of 150 mg/kg body weight of P-HA-Ac was nearly the same as that of the 5 mg/kg body weight of diphenhydramine hydrochloride which has been widely used against allergic disorders as a histamine \(H_1\)-receptor antagonist.

P-HA and M-HA showed significant inhibitory effects on DNFB-induced CHR (type IV) in mice after percutaneous administration at a dose of 0.5 and 0.1 mg/ear, respectively (Fig. 4). These hydroxylamines showed higher inhibitory activity than the COX/5-LOX inhibitor phenidone, which was used as a reference agent, because phenidone showed a significant inhibitory effect at the dose of 1 mg/ear, but not at a dose of 0.5 mg/ear (1 mg/ear; percentage inhibition of ear swelling compared with the vehicle-applied control group at 24 h, 49.1 ± 14.7, \(p<0.01\)).

COX-1 and COX-2 enzyme inhibitory activities of the hydroxylamines and \(N,O\)-diacyl hydroxylamines and reference drug and agent are shown in Table 2. All tested compounds clearly inhibited both COX-1 and COX-2 activity and
the COX-inhibitory activities of P-HA were almost as strong as that of ibuprofen. Furthermore, acetylation of these hydroxylamines was shown to be slightly effective for increasing the COX-2 selectivity. That is, hydroxylamine compounds derived from oximes which have COX-1 inhibitory action but little inhibitory action of COX-2, suppressed not only COX-1 but also COX-2. However, no selective inhibitory action against COX-2 was observed. Hydroxylamines (P-HA: IC$_{50}$ 16.6 µM and M-HA: IC$_{50}$ 198 µM) showed a 5-LOX inhibitory effect, while N$_2$O-diacylated hydroxylamines (P-HA-Ac and M-HA-Ac) had no effect (IC$_{50}$ >200 µM). The reference agent phenidone inhibited 5-LOX with an IC$_{50}$ of 2.74 µM.

On the other hand, no clear inhibitory effects of oxime (P-Ox and M-Ox) and acetyl oxime (P-Ox-Ac and M-Ox-Ac) were noted on both type I and IV allergy and 5-LOX activity.

The present findings indicate that all tested hydroxylamines (P-HA and M-HA) and N$_2$O-diacylated hydroxylamines (P-HA-Ac and M-HA-Ac) can inhibit carrageenan-induced foot-pad swelling in mice, and suggest that the anti-inflammatory action of these compounds is primarily due to inhibition of both COX-1 and COX-2 activities. It is remarkable that COX-2 inhibitory action appeared on conversion to hydroxylamines or N$_2$O-diacylated hydroxylamines from the corresponding oxime. Furthermore, P-HA and M-HA showed dual inhibitory actions of COXs and 5-LOX. P-HA-Ac suppressed the type I allergy reaction although more weakly than diphenhydramine hydrochloride. P-HA and M-HA significantly inhibited the type IV allergy reaction more strongly than phenidone did, but these compounds showed a lower 5-LOX inhibitory effect than phenidone. The anti-inflammatory action (type IV) of percutaneously applied P-HA and M-HA may be involved in the 5-LOX inhibitory effect of these compounds, at least in part, or their action may partly be due to their anti-inflammatory effects. The detailed mechanism of the anti-allergic effect remains unclear.

In conclusion, the present study suggests that hydroxylamine derivatives are promising candidates for anti-inflammatory and anti-allergic agents.

REFERENCES