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RESEARCH PAPER

Activity Profile of Glycolamide Ester Prodrugs of Ibuprofen

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ABSTRACT

Glycolamide esters of ibuprofen (I), namely, unsubstituted (II), N,N dimethyl (III), and N,N diethyl (IV), were synthesized and studied for different physicochemical, pharmacological, and toxicological properties. They were comparable with I in respect of anti-inflammatory and analgesic activity but did not exhibit reduction in the ulcerogenicity on oral administration. However, all three exhibited significantly better topical activity in carrageenan-induced rat paw edema assay. In the same assay, they provided significant protection against inflammation when applied at a site remote to the inflammation site.

INTRODUCTION

The gastric ulcerogenicity of acidic nonsteroidal anti-inflammatory drugs (NSAIDs) remains the most troubling side effect that often forces alteration in therapy. Inhibition of prostaglandin biosynthesis is implicated in both pharmacological and gastrotoxicity of NSAIDs. Endogenous prostaglandins have a cytoprotective action on gastric mucosa (1) and NSAIDs by imparting their biosynthesis that causes gastric ulceration (2–4). In a review article, concluded McCormack and Brune (5) that NSAIDs reach particularly high concentrations in those compartments in which they cause effects and side effects. Specifically, the accumulation of NSAIDs within gastric mucosal cells a priori is a principal factor associated with the intervention of intracellular biochemical events and resulting gastric mucosal damage.

The gastrointestinal lesions produced by the acidic NSAI agents are generally believed to be caused by two mechanisms: (1) a direct contact mechanism on the gastrointestinal mucosa, and (2) a generalized systemic action appearing after absorption that can be demonstrated

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after intravenous dosing. The relative importance of these mechanisms may vary from drug to drug (6–10). Cioli et al. (9) concluded that gastric ulcerogenicity of ibuprofen is to a large extent connected to local type factors.

Temporary masking of the carboxylic function can reduce or abolish the gastrointestinal toxicity owing to direct contact mechanism (11,12). Ester and amide prodrugs are synthesized with this very aim because they neither possess a free carboxylic group nor inhibit the prostaglandin biosynthesis, toxicological, and pharmacological profile of some esters and amide of ibuprofen that have been reported (13–18).

Glycolamide ester prodrugs were suggested as novel prodrug form for NSAIDs because they cleave remarkably fast in human plasma and are highly stable in aqueous medium (11,14). This was considered a major advantage because most of the prodrugs fail owing to slow hydrolysis of the parent drug.

Three glycolamide esters—unsubstituted (II), *N*,*N* dimethyl (III), and *N*,*N* diethyl (IV)—of ibuprofen were investigated as possible prodrugs for improved oral and topical delivery of ibuprofen. Physicochemical properties, namely aqueous solubility, octanol-water partition coefficient, hydrolysis kinetics in aqueous buffer, and human plasma, were studied. Their performance in established animal models was also studied.

MATERIALS AND METHODS

The following equipment was used: IR spectrophotometer (Perkin Elmer); NMR spectrometer (Perkin Elmer E-32); elemental analyzer (Heraeus); HPLC-Waters 510 (Millipore), equipped with Waters automatic gradient controller, Water 490E programmable Multiwave length detector, and Waters 745B data module; HPLC column (u Bondapak C18, 3.9 × 300-mm reverse phase); and UV spectrophotometer (CE 594, CECIL Double Beam Spectrophotometer).

Chemicals

Ibuprofen was a gift from M/s Lark Laboratories (New Delhi, India). 2-Chloroacetamide (Fluka Chemie AG, Switzerland), 2-chlor-*N*,*N* diethylacetamide, and 2-chlor-*N*,*N* dimethylacetamide (E. Merck, Germany) were of synthesis grade. All other chemicals were of analytical grade.

Synthesis and Characterization of Glycolamide Ester Prodrugs

The general formula of glycolamide ester prodrugs was $(CH_3)_2CH\text{-}CH_2\text{-}C_6H_4\text{-}CH(CH_3)\text{-}COOR$.

The substitutions present in different prodrugs were compound I, H; compound II, $-CH_2CONH_2$; compound III, $-CH_2CON(CH_3)_2$; and compound IV, $-CH_2CON(C_2H_5)_2$.

The compounds were synthesized according to the method of Nielsen and Bundgaard (20). The synthesized compounds were characterised by TLC, IR, and NMR spectroscopy, and elemental analysis.

Determination of Aqueous Solubility and Partition Coefficient

The solubility of ibuprofen and synthesized esters was determined in water at 25°C by adding an excess of compound to water and vigorously shaking for 4 hr at 27°C on a mechanical shaker to initially exceed solubility at 25°C. The mixture was then stored at 25°C for 2 hr to attain equilibrium at this temperature. After filtration/centrifugation, the aqueous layer was analyzed using the UV spectrophotometric method. The details of parameters during the UV spectrophotometric determination are given (see Table 5).

Partition coefficient was determined in octanol-water system using the shake-flask method. Accurately weighed compound was dissolved in 1-octanol, and it was shaken with a water layer ar 25 ± 2°C for 1 hr in a 50-ml conical flask with an ungreased glass stopper. Both layers were separated by centrifugation and aqueous layer withdrawn with the use of Pasteur pipette. The amount of compound in aqueous layer was quantified UV spectrophotometrically and partition coefficient determined from the formula, $P = C_oV_w/C_wV_o$. C_o and C_w are solute concentrations in octanol and water phase, respectively, after equilibration. V_w and V_o are the volumes of water and octanol, respectively.

Hydrolysis Kinetics in Human Plasma

Human plasma diluted to 50% with 20 mM phosphate buffer (pH 7.4) was used because the hydrolysis in 100% plasma was too fast to allow manual sampling for quantification. The reaction was initiated by adding the stock solution of the ester (II, III, and IV) in methanol (1.5 mg/ml) to 5 ml of plasma, preheated to 37°C. The final concentration of the compounds for reaction initiation were 2.8, 1.7, and 1.8×10^{-5} M for II, III, and IV, respectively. The samples were kept at 37°C, and after appropriate intervals

(5, 10, 20, and 30 min) samples of 500 μl were withdrawn and mixed with 2000 μl of methanol to deproteinize the plasma. Samples were centrifuged at 5000 rpm for 5 min and passed through a 2.0-micron filter. The filtrate (100 μl) was analyzed using HPLC for the unhydrolyzed ester.

In the HPLC method, a reverse-phase C18 column was eluted at ambient temperature with methanol : 0.05% phosphoric acid (7:3) at a flow rate of 2 ml min^{-1}, and detection was carried out at 220 nm. Quantitation of the compounds was carried out by measurement of the peak area in relation to those of standards chromatographed under similar conditions.

Stability in Aqueous Solution

Buffer solution (20 mM; pH 7.4) containing 7.6, 6.8, and 6.2×10^5 of II, III, and IV, respectively, was kept at 37°C and at appropriate intervals samples were analyzed for the remaining ester using the HPLC method as described above.

Hydrolysis Studies in Simulated Gastric Fluid

The compounds were analyzed for hydrolysis in the presence of simulated gastric fluid (USP XXII, pH 2.0) by TLC studies on silica-coated plates. A solvent system of chloroform : acetone (4:1) was used for resolving the components. Compounds II, III, and IV were dissolved in methanol (2.5 mg/ml). Of this stock solution 1 ml was diluted to 5 ml with simulated gastric fluid, and digestion was monitored at 37°C for the next 2 hr by drawing samples at regular intervals of 30 min. Each sample was extracted with chloroform (3×2 ml) and subjected to TLC along with ibuprofen to detect the hydrolysis of glycolamide ester prodrugs of ibuprofen. Detection was performed in a chamber of iodine vapors.

Ulcerogenic Studies

The method of Wax et al. (21) was used with slight modifications. Male Wistar rats (average group weight, 146 to 180 g; $n = 6$) were fasted for 24 hr in all-wire mesh cages to prevent caprophagy before the experiment. Animals were divided into groups of six and orally given 250 mg per kg of body weight of ibuprofen or molecular equivalent quantity of the ester as suspension/emulsion in 0.5% acacia. The control group was administered only 0.5% acacia solution. The volume given was 6.7 ml per kg of body weight. Allowing for hydrolysis of the prodrugs to parent drug and maximum ulcerogenic effect to take place, 8 hr later the animals were sacrificed under ether anesthesia and isolated stomach was opened along its greater curvature. Gastric contents were gently washed off with normal saline and hemorrhagic lesions, produced in the glandular region, were measured along their longest diameter, with the help of a magnifying lens. The numbers of ulcers and their severity on a millimeter scale were noted and area lesion index was calculated (19). The number of petechiae was also counted. An area of hemorrhage 2 mm or greater was taken as another indication of positive ulcerogenic response, giving an ulcer percent, which was the percentage of rats with ulcers per rats treated (13). Rats treated topically with I, II, III, and IV were also sacrificed and studied for gastric damage using a similar procedure.

Activity in Carrageenan-Induced Rat-Paw Edema on Oral Administration

The method of Winter et al. (22) was used with minor modifications. Male Wistar rats (average group weight, 152 to 178 g; $n = 6$) were fasted for 18 hr before experiments. Ibuprofen (30 mg per kg of body weight; 0.145 mmol per kg of body weight) or molecular equivalent quantity of the ester was orally given to rats in a volume of 1 ml per 150 g of body weight. One hour later, 0.1 ml of 1% carrageenan solution in sterile saline was injected subplantarly into the rat hind paw. The volume displaced by the paw just before the injection and after every hour was recorded using a mercury displacement plethysmometer. Statistical parameters such as mean percent protection, SEM, and significance (Student's t test) were calculated.

Activity in Carrageenan-Induced Rat-Paw Edema on Topical Application

The procedure was essentially similar to that described above except that the drug was applied topically on the hind paw of rats. An excess of compound (50 mg) was uniformly spread on the surface of the paw to allow for zero-order flux conditions. Compounds were applied, as neat samples, to lightly anesthesized rats and covered with aluminium foil and occluded with surgical tape. Excess unabsorbed compounds were wiped off after a treatment time of 2 hr, and 0.1 ml of 1% carrageenan was administered subplantarly. Recordings were made every hour after the injection of the phlogistic agent using plethysmometry.

Before the main experimentation, topical activity of ibuprofen was studied in two different physical forms: in semimolten state and as supersaturated solution in light liquid paraffin.

Activity in Carrageenan-Induced Rat-Paw Edema on Application at a Site Remote from Site of Inflammation

The procedure was essentially similar as that described above, but the drug was applied on the shaved backs of rats in a 2.6-cm diameter. Application time of 2 hr was allowed, and hourly recordings of the hind paw swelling were taken.

RESULTS AND DISCUSSION

Characterization of the Synthesized Prodrugs

The recordings of IR and NMR spectroscopy and elemental analyses follow and findings are in agreement with the expected values: compound II-IR (Neat), max. $\surd$−3380 (N-H stretch), 1760 (C = O ester), 1650 (C = O primary amide) and 635 cm^{-1} (broad N-H out of plane bend). NMR, δ $CDCl_3$ 4.30-4.46 (1H, d, -COO-CH(H)-, J = 15.76 Hz), 4.60-4.76 (1H, d, -COO-CH(H)-, J = 15.75 Hz). Elemental analysis, calculated for $C_{15}H_{21}O_3N$. C-68.42, H-8.04, O-18.23, N-5.32; found C-68.29, H-7.69, O-18.82, N-5.20%.

Compound III-IR, max $\surd$1760 (C = O ester), 1660 (C = O tertiary amide), 1132 cm^{-1}. NMR, δ $CDCl_3$ 2.87 (3H, s, -N-$CH_3(CH_3)$, 2.93 (3H, s, -N- $CH_3(CH_3)$, 4.48-4.62 (1H, d, -COO-CH(H)-, J = 14.28 Hz), 4.71-4.86 (1H, d, -COO-CH(H)-, J = 14.28 Hz),] geminal coupling. Elemental analysis, calculated for $C_{17}H_{25}O_3N$. C-70.07, H-8.65, O-16.47, N-4.81; found C-70.08, H-8.32, O-16.66, N-4.94%.

Compound IV-IR, max $\surd$1756 (C = O ester), 1668 (C = O tertiary amide). NMR, δ $CDCl_3$ 3.08-3.27 (2H, m, -N-$CH_2(CH_3)CH_2(CH_3)$, 3.27-3.48 (2H, m, -N-$CH_2(CH_3)CH_2(CH_3)$, [4.47-4.61 (1H, d, -COO-CH(H)-, J = 14.28 Hz), 4.73-4.88 (1H, d, -COO-CH(H)-, J = 13.92 Hz)] geminal coupling. Elemental analysis, calculated for $C_{19}H_{29}O_3N$. C-71.44, H-8.52, O-15.02, N-4.38; found C-71.60, H-8.83, O-14.98, N-4.59%.

Physicochemical Properties of the Prodrugs

The glycolamide esters of ibuprofen are more soluble in water than is ibuprofen (I) and have a higher log *p* value. The aqueous solubility of the prodrugs decreases as the bulkiness of the substituent group increases. The trend of aqueous solubility is II > III > IV. Glycolamide ester prodrugs are rapidly hydrolyzed in human plasma. Compound IV has the fastest hydrolysis rate in human plasma, followed by compounds III and II. The esters follow first-order rate kinetics as indicated by the linear plot of log % unhydrolyzed ester versus time. The slope of the linear plot gave the value of rate constant K, and half-life was calculated from the equation $t_{1/2} = 0.693/K$. The distributed glycolamide esters are 8 to 10 times more stable in the aqueous buffer and they cleave 5 to 7 times more quickly in human plasma compared with the unsubstituted ester.

Table 1.

Physicochemical Properties of Ibuprofen (I) and Its Glycolamide Ester Prodrugs

Compound	Aqueous Solubility (mcg/ml)	log *p*	Plasma Half-Life	Aqueous Buffer Half-Life
I	71.2	2.22	—	—
II	189.1	2.7	75.82	405
III	133.3	2.65	15.07	4950
IV	122.1	2.47	9.94	3150

Table 1 shows the values of aqueous solubility, partition coefficient, and hydrolysis half-lives of the prodrugs in aqueous buffer and human plasma. The hydrolysis profile of prodrugs in human plasma is shown in Table 2.

Hydrolysis of Prodrugs in Simulated Gastric Fluid

The TLC studies show partial hydrolysis of the esters to parent drug from the 30-min reading onward (Table 3).

Table 2.

Hydrolysis Kinetics of Ibuprofen (I) and Its Glycolamide Ester Prodrugs in 50% Human Plasma

Sampling Time (Min)	% Ester Remaining		
	II	III	IV
0	100	100	100
5	79.8[a]	58.6	17.6
10	78.6	26.6	13.6
20	70.9	12.4	3.09
30	48.4	—	—
R[b]	0.91	0.98	0.93
K[c]	0.0091	0.046	0.69

[a] Each reading is a mean of two observations.
[b] Coefficient of correlation.
[c] First order rate constant (min^{-1}).
Both these quantities have been calculated from the slopes of the linear plots of log % remaining ester versus time in minutes.

Table 3.

Hydrolysis Studies of Prodrugs in Simulated Gastric Fluid

	Time in Minutes[a]			
Compound	30	60	90	120
II	+	+	+	+
III	+	+	+	+
IV	+	+	+	+

[a] Recordings were made 30, 60, 90, and 120 min after incubation of the prodrug sample.
+ Indicates positive detection of ibuprofen spot on TLC plate.

This raises the possibility that prodrug might hydrolyze before absorption from the gastrointestinal tract.

Ulcerogenic Studies

All the prodrugs show greater incidence of ulceration compared with that of the parent drug when administered orally in equimolar concentration (Table 4). This behavior can be attributed to the preabsorption hydrolysis of the prodrug on oral administration as discussed above. This defeats the aim of prodrug design targeted at reduction of gastroulcerogenicity associated with oral administration of ibuprofen. The hydrolysis products are 2-hydroxyacetamide, 2-hydroxy *N*,*N* dimethylacetamide, and 2-hydroxy *N*,*N* diethylacetamide. The latter is a skin-penetration enhancer capable of altering the integrity of biological membranes. This might be contributing a synergestic effect on the overall ulcerogenicity of the compounds. These results also indicate that a direct contact mechanism plays a dominant role in overall ulcerogenicity of ibuprofen. However, animals administered I, II, III, and IV topically did not show any signs of ulcerogenicity. Topical delivery abolishes the direct contact of the drug with gastric mucosa and consequent accumulation of acidic NSAID within the gastric mucosal cells, which are the principal factors responsible for ulcerogenicity (Table 5).

Table 4.

Gastroulcerogenicity Caused by Ibuprofen and Its Glycolamide Ester Prodrugs

Group	Ulcer%	Severity[a]	No. of Ulcer/Stomach	p[b]	ALI[c]
Control	0	0.2	0	1.0	0.2
I					
1.21	100	2.7	7.0 (1.2)	14.2	9.7
0.91	40	1.0	1.6 (0.9)	1.4	2.6
0.60	40	0.2	0.2 (0.2)	1.4	0.4
II					
1.21	100	5.8	8.0 (0.6)	4.6	13.8
0.91	100	4.0	10.4 (1.4)	7.8	14.4
0.60	20	0.6	1.0 (1.0)	2.0	1.6
III					
1.21	100	3.6	8.1 (1.5)	7.6	11.7
0.91	80	3.4	4.0 (1.4)	7.8	7.4
0.60	80	2.0	3.2 (0.5)	1.8	5.2
IV					
1.21	100	4.6	7.6 (3.0)	7.2	12.2
0.91	100	1.4	5.8 (1.7)	1.2	7.2
0.60	40	0.8	1.0 (0.4)	1.8	1.8

Dose in mmol/kg body weight.
[a] Severity measured in approximate millimeter gradings, i.e., 1+ = 1 mm, 2+ = 2 mm, etc.
[b] No. of Petechiae.
[c] Area Lesion Index (see Ref. 19).

Effect on Carrageenan-Induced Edema on Oral Administration

The effect of ibuprofen and the synthesized prodrugs on the carrageenan-induced edema in rat hind paw is given in Figure 1. All the prodrugs retain the pharmacological activity profile of the parent drug (I) owing to rapid hydrolysis kinetics in plasma.

Effect on Carrageenan Induced Edema on Topical Application

Ibuprofen exhibited an identical activity profile when applied as neat semimolten sample or as supersaturated solution in light liquid paraffin. This indicates that dissolution or thermodynamic activity is not the limiting factor in its topical penetration. This afforded direct comparison among neat samples of all four compounds (I–IV). The pharmacological activity profile in this particular

Table 5.

Details of UV Spectrophotometric Quantification of Ibuprofen (I) and Its Glycolamide Ester Prodrugs

Compound	Solvent System	Abs. Maxima (nm)	Range of Beer's Lambert Law mcg/ml
I	Acetonitrile-water 1:1	220	0–35
II	Propan-2-ol-water 1:1	220	0–38
III	-do-	224	0–36
IV	-do-	224	0–17

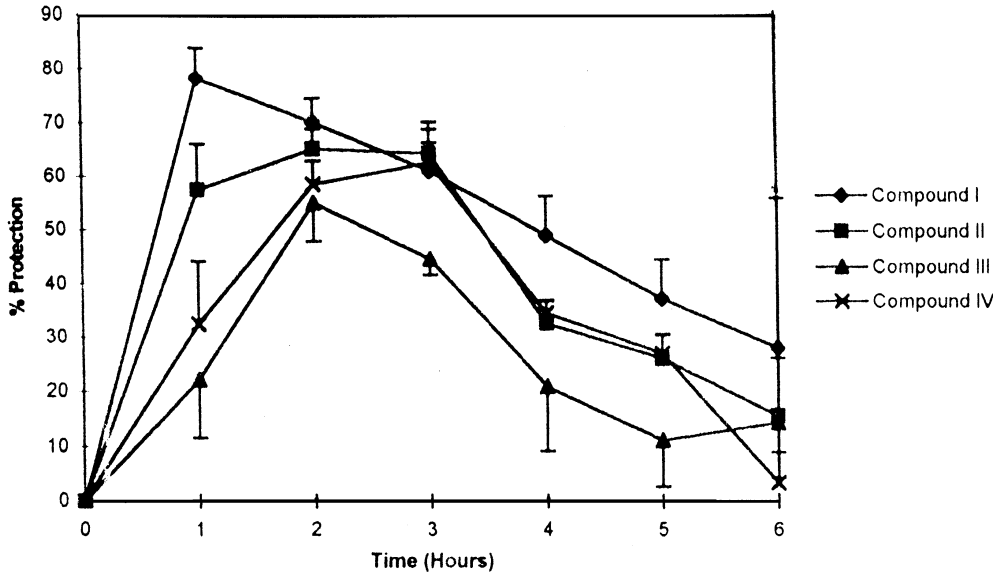

Figure 1. Anti-inflammatory activity of ibuprofen (I) and its glycolamide ester prodrugs (II–IV) in carrageenan-induced rat paw edema (dose level 0.145 m mol/kg body weight) on oral administration.

assay on topical application is shown in Figure 2. The synthesized prodrugs II, III, and IV show a significantly better activity in the experiment during the period of 3 to 6 hr. The response produced by a prodrug can be a function of permeation kinetics, rate of prodrug hydrolysis in the body tissues, and extent of absorption into systemic circulation. These esters, aided by a favorable shift in lipophilicity, are able to penetrate the skin easily. This, followed by hydrolysis of prodrug to the parent drug, might help in maintaining pharmacologically significant concentrations in the tissues. Formation of a "depot" of the drug in the tissues and the structural closeness

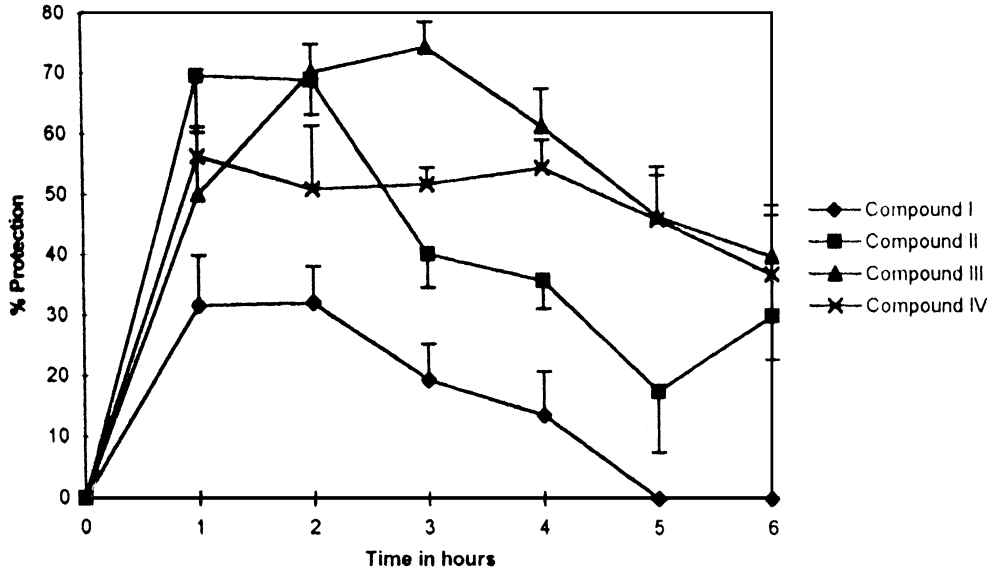

Figure 2. Anti-inflammatory activity of ibuprofen (I) and its glycolamide ester prodrugs (II–IV) in carrageenan-induced rat paw edema on topical application.

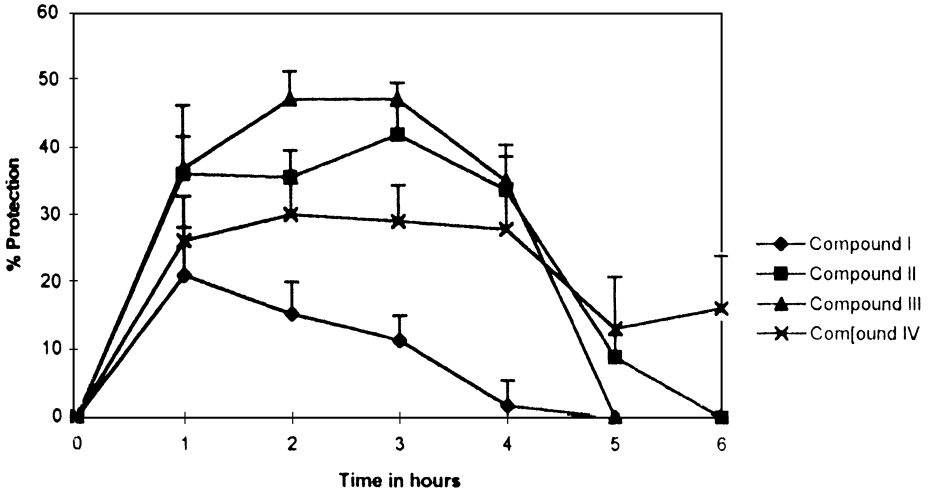

Figure 3. Anti-inflammatory activity of ibuprofen (I) and its glycolamide ester prodrugs (II–IV) in carrageenan-induced rat paw edema when applied transdermally.

of prodrug hydrolysis products 2-hydroxyacetamide, 2-hydroxy *N*,*N* dimethylacetamide and 2-hydroxy *N*,*N* diethylacetamide that structurally resemble *N*,*N* dimethylacetamide, a known skin-penetration enhancer, might also contribute a "self-enhancing" effect on topical penetration of the prodrugs. However, these hypotheses need to be explored individually to pinpoint the role of each one in overall activity profile.

Effect on Carrageenan-Induced Edema when Applied at a Site Remote from Site of Inflammation

Figure 3 depicts the percentage of protection provided against carrageenan-induced edema when the compounds (I–IV) were applied at a site remote to the site of inflammation. Glycolamide ester prodrugs (II–IV) provide better protection compared with I. Activity in this experiment gives a measure of delivery of drug to the systemic circulation on dermal application. Because the site of application is remote to the site of inflammation, the only way drug can elicit pharmacological response is via systemic circulation. Factors similar to those discussed in "Ulcerogenic Studies" above may be responsible for the better performance of prodrugs II–IV in this study.

CONCLUSIONS

The three glycolamide ester prodrugs of ibuprofen studied cleave rapidly inside the biological system and on oral administration elicit a pharmacological-toxicological profile quite similar to that of the parent drug. The prodrugs offer no advantage in terms of reduced gastroulcerogenicity and exploitable improvement in therapeutic ratio. However, in topical and transdermal delivery, the combined effect of favorable shift in lipophilicity, rapid hydrolysis kinetics, and self-enhancing effect exerted by hydrolysis products results in a better activity profile characterized by prompt onset and extended duration of activity.

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