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QUANTITATION OF ACTIVITY OF ALKYL ESTER PRODRUGS OF IBUPROFEN

A.K. Bansal * ^, R. Dubey * AND R.K. Khar ~

* College of Pharmacy, Pushp Vihar, Sector 3, New Delhi 110 017. India.

~ Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062. India.

ABSTRACT

A quantitative relationship has been derived for the physicochemical properties and pharmacological activity of alkyl ester prodrugs of ibuprofen. A comprehensive study consisting of aqueous solubility, octanol-water partition coefficient, hydrolysis kinetics in aqueous buffer (pH 7.4) & human plasma, ulcerogenic studies, anti-inflammatory and analgesic activity was carried on alkyl ester prodrugs of ibuprofen. n-Propyl and n-butyl esters offered significant improvement in oral delivery of ibuprofen in terms of reduced gastroulcerogenicity and maintenance of pharmacological activity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the management of conditions characterised by pain and inflammation. Ibuprofen, a propionic acid derivative, is one of the most commonly prescribed NSAID. It, like other NSAIDs , causes gastrointestinal side-effects such as gastric ulceration and haemorrhage (1,2,3). The search for safer NSAIDs still continues and despite the availability of over 100 drugs for the treatment of arthritic conditions, aspirin, the oldest of all NSAIDs, is still regarded as drug of first choice (4,5).

NSAIDs produce gastrotoxicity with two different mechanisms : a direct contact mechanism on the mucosa and a generalised systemic action which appears after absorption(6). The local contact effect, which is unrelated

^ for correspondence

to the pharmacological activity , varies from drug to drug. This effect has been reported to be predominant in case of ibuprofen (6) and hence its gastrointestinal tolerance can be improved by reducing the factors responsible for local erosive effects.

Temporary masking of the free carboxylic acid group of NSAIDs can improve their gastrointestinal tolerability. The ester and amide prodrugs of NSAIDs are designed to achieve this very objective. It has been observed that out of many prodrugs evaluated only alkyl esterification achieves anything approaching total abolition of gastric injury by drugs, without adversely affecting their anti-inflammatory activity (7,8).

This study examines the suitability of alkyl esters of ibuprofen as potential prodrugs for its oral delivery. A quantitative relationship was derived between physicochemical properties and pharmacological activity.

MATERIALS AND METHODS

Ibuprofen was a gift sample from M/s Lark Laboratories, New Delhi. Chemicals used were of either laboratory or analytical grade.

U.V. - Vis double beam spectrophotometer - CECIL CE 594, Cecil Instruments, Cambridge, U.K.
Infra-red spectrophotometer - Perkin Elmer 1800
N.M.R. spectrometer - Perkin-Elmer R-32
Elemental analyser - Heraeus
HPLC - Waters 510, Millipore, USA equipped with Waters Automated Gradient Controller, Waters 490 E Programmable Multi wavelength detector and Waters 745 B, Data module.

Synthesis of Ester Prodrugs

$(CH_3)_2$-CH-CH_2-C_6H_4-CH(CH_3)-COOR

R = H in case of ibuprofen (I) and R was replaced with suitable alkyl group to get the following esters - methyl(II), ethyl (III), n-propyl (IV), iso-propyl (V), n-butyl (VI), iso-butyl (VII), sec-butyl (VIII), tert-butyl (IX), n-pentyl (X), hexyl (XI), heptyl (XII), octyl (XIII), lauryl (XIV), cetyl (XV) and octadecyl (XVI) ester of ibuprofen.

The esters were synthesised by converting I to its acid chloride followed by its treatment with appropriate alcohol.

Determination of Aqueous Solubility of the Compounds- Aqueous solubility of all the compounds I-XVI were determined, by shaking an excess (nearly 200 mg) of the compound with 40 ml of water, sealed in a glass stoppered conical flask, for 4 h. The temperature during shaking was kept at 27° C$\pm$ 2° to initially exceed solubility at 25°. The mixture was then stored at $25^{\circ} \pm 2^{\circ}$ for 2 h to attain equilibrium at this

temperature. The concentration of the compound in aqueous layer was quantified U.V. spectrophotometrically.

Determination of Partition Coefficient- Partition coefficient in octanol-water system was determined using "Shake-flask" method.

Hydrolysis Kinetics in Aqueous Buffer (pH 7.4)- Hydrolysis kinetics of the esters II-XVI, at $37^{o} \pm 2^{o}C$, were studied in 20 mM phosphate buffer using a High Pressure Liquid Chromatography method.

The mobile phase was methanol and 0.05% phosphoric acid. The C 18 u Bondapak column (3.9 X 300 m.m.) was eluted at a flow rate of 2.0 ml/min. Phenylbutazone was used as the internal standard and quantitation was done using the regression polynomials derived for the linear plot of concentration versus ratio of compound and internal standard peak area ratio. The reaction was initiated by incubating 1.3 to 1.0 X 10^{-5} M of the ester.

Hydrolysis Kinetics in Human Plasma- Hydrolysis kinetics in human plasma were studied using a HPLC method, as described in the previous section. Quantitation was done by comparing with standard chromatographed under similar conditions. Reactions were initiated by incubating 2.0 to 8.0 X 10^{-5} concentrations of the ester.

Hydrolysis Studies in Simulated Gastric Fluid- Some of the compounds (II,III,V,VII and IX) were studied for hydrolysis to parent drug (I), in the presence of simulated gastric fluid. Studies for a 2 h period, were carried using thin layer chromatography. Resolving was done using a solvent system of chlorform : water (4:1).

Study of the Ulcerogenic Property of the Synthesised Prodrugs Method of Wax et al (9) was used with slight modifications. Wistar male rats (140 - 210 g) were fasted for 24 h prior to dose administration but water was allowed ad libitum. The control group received 0.5 % acacia solution at 6.7 ml/kg body weight of the animal. Other groups received either I or the esters (II-XVI) par orally, at a dose level of 1.213 m mol/kg suspended/emulsified in 0.5 % acacia. After 8 h, isolated stomach was studied for haemorrhagic lesions and quantitation was done by measuring the lesions along their longest diameter, with the help of ruler and magnifying lens. Five petechiae (pin-point ulcers) were taken equivalent to 1 mm of the ulcer length. Ulcer index and percentage of rats showing ulceration were calculated (6).

Activity in Carrageenan Induced Rat Hind Paw Oedema - The method followed was essentially as described by Winter et al (10). Ibuprofen (I) or esters (II-XVI) suspended/emulsified in 0.5% acacia were administered p.o. at a dose level of 0.145 m mol/kg. Swelling was recorded using a mercury - displacement plethysmometer.

TABLE - 1

Compound	% yield	Aq.sol.* mcg/ml	log P	Half-life in h Aq.buffer	Plasma	S.G.F.** 30	60	90	120 min.
I	---	71.28	2.22	---	---				
II	76.4	55.02	2.97	227.9	11.25	-	-	-	-
III	73.0	43.82	3.02	98.7	10.5	-	-	-	-
IV	67.2	46.96	2.88	72.4	7.8				
V	66.0	67.75	2.30	40.5	11.3	-	-	-	-
VI	77.0	36.33	3.13	42.9	4.9				
VII	72.0	43.37	2.68	63.5	17.3	-	-	-	-
VIII	66.1	13.45	3.06	79.7	14.7				
IX	70.0	89.94	2.37	34.0	14.5	-	-	-	-
X	78.2	30.75	3.30	74.6	12.5				
XI	80.0	24.74	3.55	157.1	49.1				
XII	67.1	21.10	3.75	151.3	45.3				
XIII	68.0	20.21	3.79	87.6	43.4				
XIV	70.1	12.85	3.60	115.5	32.6				
XV	72.1	11.18	3.71	105.2	21.3				
XVI	80.0	6.80	3.71	138.6	23.9				

* Quantitation done in solvent system of acetonitrile : water(1:1) for I - XIII and absolute ethanol for XIV-XVI, at 220 nm. Coefficient of corraelation for calibratoon curves, r^2, was between 0.996 and 0.971 (n=6).

** - sign indicates absence of ibuprofen spot on the TLC

Activity in Acetic Acid Induced Writhing Reflex Assay- An intraperitoneal injection (10 ml/kg) of 0.6 % acetic acid was used to induce writhing in mice. One hour prior to irritant injection, 0.146 m mol/kg, of I or its esters (II-XVI) was administered p.o., suspended/emulsified in 0.5 % acacia solution. Number of writhings in a 20 minute period proceeding the irritant injection, were counted.

ED_{50}, the dose providing 50 % protection was calculated by graphical method, after administering drug at dose levels of 0.097, 0.146, 0.218 and 0.290 mmol/kg.

RESULTS AND DISCUSSION

The percent yield, aqueous solubility, log P, hydrolysis half-lives in aqueous buffer, human plasma and hydrolysis in simulated gastric fluid are reported in Table - 1.
The results of ulcerogenic studies are reported in Table -2.
Figure 1,2,3 and 4 depict the activity profile of I and its esters II-XVI in carrageenan induced oedema assay.
The analgesic activity of different compounds is depicted in Figure - 5.

A useful prodrug of ibuprofen should possess significantly less gastroulcerogenicity while retaining the

TABLE - 2

Shows the effect of various ibuprofen prodrugs on gastric ulcerogenicity when administered as a single oral dose of 1.213 m mol/kg of the body weight, in rats

S.No.	Group	n	Av.wt. of rat g.	Total ulcer length m.m.	No.of Pete-chiae	Ulcer Index ^ m.m.	Ulcer %
1.	Control	6	192	0	1	0.03(0.03)	0
2.	I	7	177	71	92	12.43(2.07)	100
3.	II	6	179	71	41	13.16(3.62)	100
4.	III	6	180	50	35	9.12(4.67)	80
5.	IV	6	189	31	33	6.26(1.30) *	83.3
6.	V	6	173	43	20	8.00(3.75)	66.6
7.	VI	6	186	5	25	1.64(0.63)**	40
8.	VII	6	179	54	24	9.80(5.02)	80
9.	VIII	6	173	5	11	1.16(0.60)**	20
10.	IX	6	198	42	29	8.00(1.91)	100
11.	X	6	181	15	24	3.40(1.66)**	60
12.	XI	6	190	9	13	2.00(1.22)**	40
13.	XII	6	211	4	14	1.12(0.59)**	40
14.	XIII	6	148	2	4	0.44(0.44)**	20
15.	XIV	6	128	0	4	0.11(0.08)**	0
16.	XV	6	148	1	1	0.20(0.20)**	0
17.	XVI	6	168	0	5	0.16(0.12)**	0

^ Five petechiae were taken equivalent to 1 mm ulcer length. Ulcer index is the mean ulcer score for a group.

* p value < 0.05

** p value < 0.01

analgesic and anti-inflammatory activity. A spectrum of activity profile was observed for alkyl ester prodrugs of ibuprofen. The analgesic activity was retained by nearly all the prodrugs (except compound XIII), but the ulcerogenic and anti-inflammatory properties showed a wide variation. On the basis of these results the synthesised prodrugs can be divided into four groups -

GROUP -1 , containing compound III, maintains the activity versus side-effects, when compared to that of the parent drug on the molar basis. For drugs administered

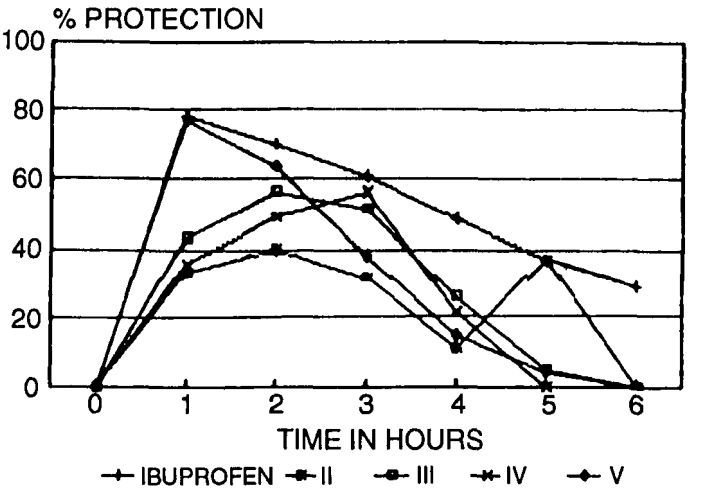

FIGURE 1

Percentage protection provided by oral administration of ibuprofen and its esters II-V against carrageenan induced rat paw oedema

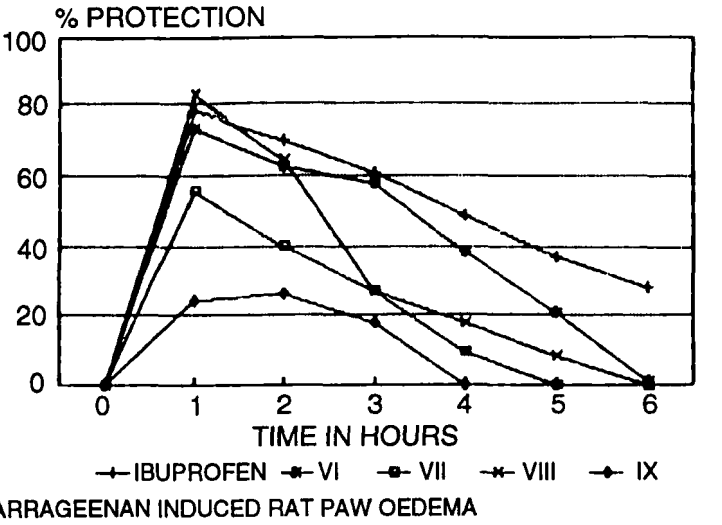

FIGURE 2

Percentage protection provided by oral administration of ibuprofen and its esters VI-IX against carrageenan induced rat paw oedema

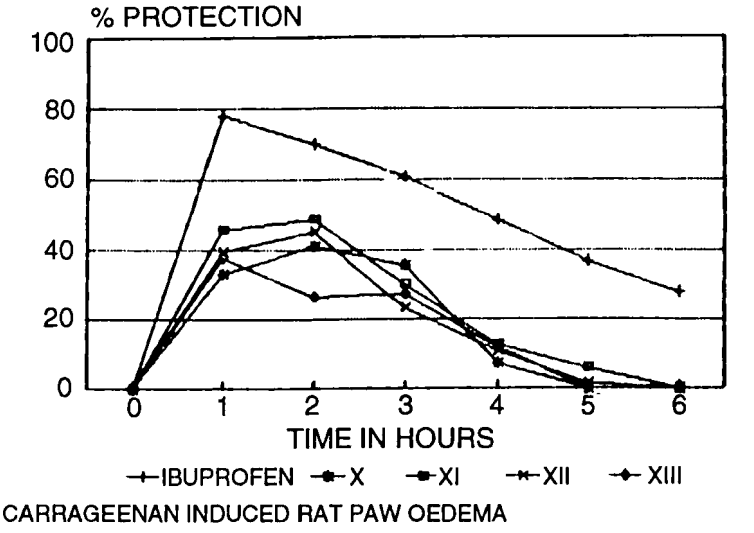

FIGURE 3

Percentage protection provided by oral administration of ibuprofen and its esters X-XIII against carrageenan induced rat paw oedema

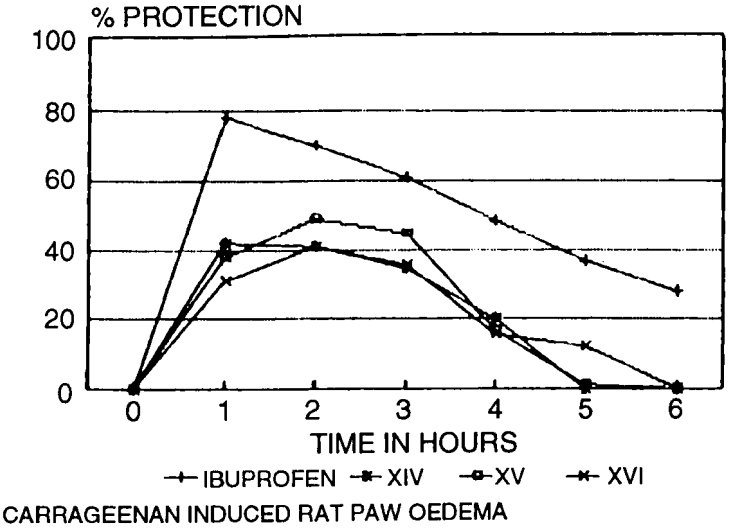

FIGURE 4

Percentage protection provided by oral administration of ibuprofen and its esters XIV-XVI against carrageenan induced rat paw oedema

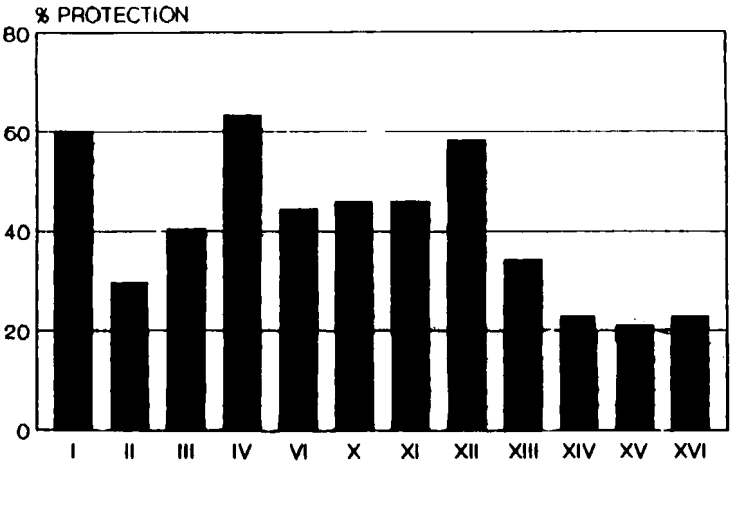

FIGURE 5

Percentage protection provided by ibuprofen (I) and some of its prodrugs, against acetic acid induced writhing reflex in mouse.

orally, esterases in the gut wall have been indicated as the main source of hydrolysis (11,12). Also, accumulation of NSAID within mucosal cells a priori is a principal factor associated with their damage (13).

GROUP - 2, has compounds which exhibit significantly reduced activities in all assays (Compound X , XI XII, XIII, XIV, XV and XVI). This can be attributed to poor absorption, inefficient hydrolysis of the prodrug to parent drug or alternative modes of metabolism. The hydrolysis kinetics, do not allow concentration of ibuprofen in gastric mucosal cells hence preventing ulcerogenicity but this also prevents building up of therapeutically effective drug concentrations in plasma.

GROUP - 3, has compounds II, V, VII and VIII which show no reduction in ulceration, but significantly less anti-inflammatory activity. These compounds do not show signs of hydrolysis in simulated gastric fluid over a period of 2 h, pointing towards absence of pre-absorption hydrolysis. These prodrugs might themselves be having gastric irritant properties. This also indicates that it is not sufficient only to mask the free carboxylic group but the masking should be done with the right type of chemical substitution. The role of site specific ester hydrolysis in biological tissues might also come into picture. Interestingly all the compounds

except II are esters of branched chain alcohols with ibuprofen.

GROUP - 4 has compound IV and VI which exhibit an exploitable improvement in therapeutic ratio. These can be potential prodrugs of ibuprofen as they offer significant reduction in ulcerogenicity coupled with rertainment of anti-inflammatory and analgesic activity. The profile can be attributed to the rate of hydrolysis in the stomach, gut and plasma, changes in locus of absorption of the prodrug and altered pharmacokinetics resulting from these changes.

Quantitation of Alkyl Ester Prodrugs of Ibuprofen- A mathematical relationship was established between hydrophobicity, hydrolysis kinetics and biological activity of the ester prodrugs of ibuprofen, using log P, t 1/2 in plasma and ED_{50} in analgesic activity assay as parameters. Linear and multiple linear regression was applied using commercially available computer software to obtain the following equations -

$$ED_{50} = 0.0607 \log P - 0.052 \quad (r^2 = 0.386)$$

$$ED_{50} = 0.00042\ t\ 1/2 + 0.118 \quad (r^2 = 0.0023)$$

$$ED_{50} = -0.008 \log P + 0.103 \log P^2 + 0.014\ t\ 1/2 \quad (r^2 = 0.9298)$$

Biological activity is poorly related to log P and t 1/2 alone but an excellent correlatioship is obtained when both the parameters are used, simultaneously.

CONCLUSIONS

The fact that prostaglandin synthesis inhibition is implicated both in pharmacological and ulcerogenic activity of NSAIDs, makes prodrug designing a very tricky affair. The prodrug should hydrolyse in a manner which prevents accumulation of active drug in gastric mucosa but maintain the pharmacological activty. The difference in susceptibility of the prodrug hydrolysis to enzymes present in gut, plasma and other biological tissues further complicate the problem. A very rapid hydrolysis kinetics in biological system is certainly not the answer, as build up of active drug in gastric mucosa is still there. A controlled hydrolysis kinetics which helps the prodrug to cross the mucosa in intact form, followed by elicitation of pharmacological activity without build up of systemic toxic levels of the drug, provides the answer. Here hydrolysis kinetics modulates the heterogeneous pharmacokinetics of the drug, preventing build of toxic drug levels. The role of n-propyl and n-butyl ester in this context calls for further extensive studies.

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