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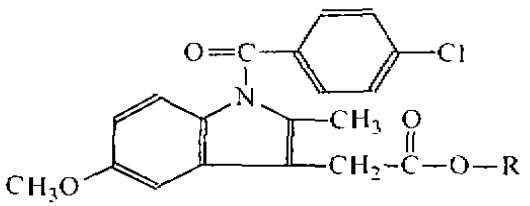
Anti-inflammatory and ulcerogenic effects of 3-(*N*, *N*-diethylamino) propylindometacin HCl

HUANG Zhi-Li[1], KAGOSHIMA Masatoyo, KAGAWA Eiichiro, WANG Wei-Qing, SHIMADA Hideyo
(*Department of Clinical Pharmacology, School of Pharmaceutical Sciences, Kitasato University, Tokyo 108, Japan*)

KEY WORDS 3-(*N*,*N*-diethylamino) propylindomethacin; indomethacin; carrageenan; edema; stomach ulcer

AIM: To study anti-inflammatory effects of a novel indometacin ester, 3-(*N*, *N*-diethylamino) propylindometacin HCl (prodrug) and its ulcerogenicity in rats. **METHODS**: Carrageenin (Car)-induced paw edema and ulcer index were examined. **RESULTS**: Car-induced paw edema was inhibited by 36.6 % ($P<0.01$) at 3 h and 34.6 % ($P<0.01$) at 5 h after a single ip injection of the prodrug 7.09 mg·kg^{-1}. On the same molar basis, indometacin (Ind) 5 mg·kg^{-1} ip inhibited edema by 45.6 % at 3 h and 39.2 % at 5 h, however, there was no statistical significant difference ($P>0.05$) between the edema-inhibitory effect of the prodrug and that of Ind. The dose 10 μg/paw exhibited 64 % inhibition of the swelling, the prodrug >10 μg/paw showed no additional inhibition of swelling; the acute gastric lesion properties of the prodrug were much lower than those of Ind 6 h after *po*. **CONCLUSION**: The prodrug is a potent anti-inflammatory agent with lower ulcerogenicity in the stomach.

Indometacin (Ind) is widely used as a non-steroidal anti-inflammatory agent. But gastric toxicity is a side-effect that is common to all non-steroidal anti-inflammatory drugs including Ind[1,2], therefore numerous efforts of drug designers to develop derivatives of Ind which would yield adequate anti-inflammatory potency without adverse reaction of gastric mucosa, had led to the synthesis of the Ind ester, 3-(*N*,*N*-diethylamino) propylindometacin HCl (prodrug). The present study was to investigate the anti-inflammatory effect of the compound, and estimate ulcerogenicities of Ind and its prodrug in rats.



	R
Indometacin	**-H**
Ind prodrug	**$-CH_2CH_2CH_2-N(C_2H_5)_2\cdot HCl$**

MATERIALS AND METHODS

Chemicals Ind prodrug was donated by Prof A A Hussain, University of Kentucky, Lexington KY 40536-0082, USA. Ind (Lot № 60H0448) and carrageenin (Car) (Lambda № C-3889) were purchased from Sigma Chemical Co. All other chemicals used were AR.

Preparation of prodrug and Ind Ind 50 mg, put in 3.5 mL of 2.5 % Na_2CO_3 solution, was immediately mixed with 1.5 mL of HCl 0.5 mol·L^{-1}, and continuously shaken for about 20 min until completely dissolved. The pH of the solution was 7.0 and Ind in the solution was stable for at least 48 h, 7.09 mg prodrug was dissolved in 5 mL saline. All the solutions were immediately used after preparation.

Car-induced edema test Male SD rats weighing 145-175 g were obtained from Japanese Laboratory Animal Institute, the rats caged individually were deprived of food for 16 h (water *ad lib*).

The change in edema volume of the rat hind paw was measured on unanesthetised rats[3], rats were injected ip with Ind 5 mg·kg^{-1} and prodrug 7.09 mg·kg^{-1}, respectively, 0.5 h before the injection of Car, the control group received an equivalent amount of saline. A 0.1 mL of freshly prepared 1 % Car solution in pyretic-free saline was subplantarly injected into rat hind paw and the volume of the paw was immediately measured, 0.5, 1, 3, 5 h after the Car injection, the volume of the paw was also determined. The % swelling of the paw was calculated using the following equation:

[1] Now in *Department of Pharmacology, Wannan Medical College, Wuhu 241001, China*.

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% Swelling = V_t/V_i (1)

Where V_t = mean increase in paw volume at a certain time after Car injection, V_i = initial paw volume.

The average paw swelling in the group of drug-treated rats was compared with that in the group of control and the anti-inflammatory activities of these drugs were calculated with following formula:

% Inhibition = 1 − % Swelling of drug-treated group/% Swelling of control group (2)

Statistical analyses were carried out with the t-test, comparing the % inhibition of the drug-treated group with that of control group.

Method of bioassay and dose-response correlation The method of bioassay of the anti-inflammatory effect of prodrug using Car-induced model was employed by dose-response correlation. After 1 % Car solution was prepared by dissolving in the pyretic-free saline containing appropriate amounts of prodrug, 0.1 mL of the solution was subplantarly injected into left hind paws of 6 rats in each group, the doses of prodrug used were 0.1, 0.3, 1, 3, 10, 30 μg/paw.

The percent swelling in individual rats and the average % of inhibition of edema formation were calculated as mentioned above.

Gastric ulcerogenicity study Male SD rats, weighing 185−215 g, were kept in individual cages with a raised mesh bottom, deprived of food for 24 h (water *ad lib*). Ind 10 mg·kg^{-1} and prodrug 14.17 mg·kg^{-1} were *po* given to rats, before the rats were killed, 1 mL of 10 % pontamine was *iv* administered to each rat in order to observe lesions easily. After 6 h, the rats were sacrificed, and the stomachs were removed, opened along the greater curvature, and examined for lesions developed in the glandular portion under a stereo zoom microscope with an eyepiece micrometer, the length and width of every lesion was measured, each lesion area (length × width) was calculated, and total of the areas (mm^2) of all lesions for each rat was used as the ulcer index[4].

RESULTS AND DISCUSSION

The % of inhibition of Car-induced edema formation by Ind and prodrug was shown in Tab 1.

The prodrug inhibited edema formation by 36.6 % ($P<0.01$) at 3 h and 34.6 % ($P<0.01$) at 5 h, comparing to the control after a single ip of prodrug 7.09 mg·kg^{-1}. On the same molar basis, Ind 5 mg·kg^{-1} ip inhibited the edema by 45.6 % ($P<0.01$) and 39.2 % ($P<0.01$) at 3, 5 h, respectively. There was no significant difference ($P>0.05$) on % of inhibition between the prodrug and Ind. However, neither Ind nor prodrug inhibited the edema formation 1 h after Car injection, that was the first phase of edema formation induced by Car. The development of edema in rat paw after Car injection had been described as a biphase event. The initial phase (1st h) of the edema was attributed to the leakage of histamine and serotonin, the edema maintenance during the plateau phase to kinin-like substances, and the second phase (after 1st h) of swelling to the release of prostaglandin-like substance[5]. Neither Ind nor prodrug had influence on the first phase, but they could significantly inhibit the second phase and prevent the development of edema. Pharmacokinetic study showed that most of the prodrug after dosing became Ind[6]. It was inferred that the anti-inflammatory activity of prodrug was probably related to Ind which inhibited the biosynthesis of prostaglandins at the second phase of edema formation.

Tab 1. Effects of Ind and prodrug ip on carrageenin-induced paw edema. $n = 6$ rats, $\bar{x} \pm s$. $^cP<0.01$ *vs* control; $^dP>0.05$ *vs* Ind.

Group/ mg·kg^{-1}		Edema/μL 3 h	Edema/μL 5 h	Inhibition/% 3 h	Inhibition/% 5 h
Control	−	497 ± 25	603 ± 23	−	−
Ind	5	286 ± 26^c	388 ± 35^c	45.6	39.2
Prodrug	7.09	310 ± 21^{cd}	407 ± 16^{cd}	36.6	34.6

The % of inhibition (in probit scale) of rat paws measured 3 h after the subplantar injection of the prodrug-Car solution plotted against the log dose of prodrug was shown in Fig 1. A good linear

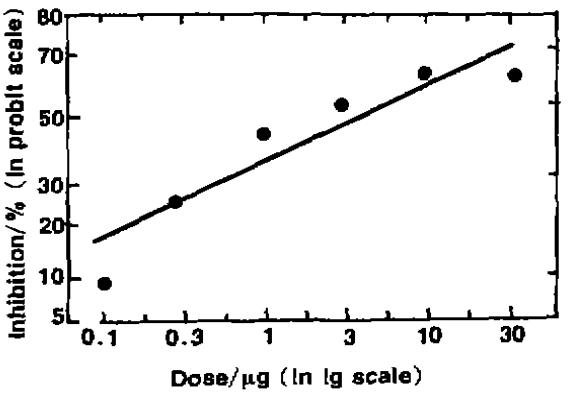

Fig 1. Dose-response of the prodrug 3 h after subplantar injection with carrageenin. $n = 8$ rats, $\bar{x} \pm s$.

correlation was presented between the % of inhibition and the log dose of prodrug over the dose range 0.1 – 10 μg/paw. The dose of 10 μg/paw exhibited 64 % inhibition of the swelling found in the control. An increase of prodrug beyond 10 μg/paw showed no additional inhibition of the swelling. A regression was performed by bliss probit analysis on the log dose of prodrug injected subplantarly and the % of inhibition of the edema formation, the regression equation was as follows:

$$\%\ \text{Inhibition (probit)} = 0.597 \log (\text{dose}) + 4.633 \quad (3)$$

A good linear relationship ($r = 0.923$, $P < 0.01$) existed between the log dose of prodrug and edema inhibition % of measured in the study. The median inhibitory dose (its 95 % confidence limits) was 4.114 μg/paw (1.814 – 9.328). It was revealed that the bioassay method for identifying anti-inflammatory activity of new drug was practicable and valuable.

The gastric ulcerogenicity of prodrug 14.17 mg·kg^{-1} and Ind 10 mg·kg^{-1} *po* was shown in Tab 2, both Ind and prodrug induced visible lesion in the corpus mucosa, but the severity and incidence were significant difference ($P < 0.01$). After 6 h, the acute gastric lesion properties of the prodrug were 1/60 of Ind. On the other hand, ulcer index and incidence induced by prodrug were much lower than those by Ind.

Tab 2. Ulcerogenic action of prodrug and Ind 6 h after *po* dosing. $n = 8$ rats, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control; $^fP < 0.01$ *vs* Ind.

Group/mg·kg^{-1}		Ulcer index/mm^2	Incidence
Ind	10	7.70 ± 1.14^c	8/8
Prodrug	14.17	0.13 ± 0.06^{cf}	5/8
Control	–	0	0

In conclusion, Ind prodrug is a potentially useful anti-inflammatory agent which activity was equal to that of Ind, and have much lower ulcerogenic effects of inflammatory drugs on the stomachs. At the present time, pharmacokinetics is under study and other biological studies confirming the clinical uses have being carried out in our laboratory.

REFERENCES

1 Balaa MA. Nonsteroidal anti-inflammatory gastropathy: from theory to practice.
Am J Med Sci 1991; **301**: 272 – 6.

2 *Graham DY. The relationship between nonsteroidal anti-*inflammatory drug use and peptic ulcer disease
Gastroenterol Clin North Am 1990; **19**: 171 – 82.

3 Vinegar R.
Quantitative studies concerning kaolin edema formation in rats.
J Pharmacol Exp Ther 1968; **161**: 389 – 95.

4 Suzuki T, Kagoshima M, Shimada H. Effects of various psychotropic drugs on indomethacin-induced ulcer in rats.
Folia Pharmacol Jpn 1992; **100**: 20 – 3.

5 Swingle KF. Indomethacin, sulindac and analogs. In: Scherrer RA, Whitehouse MW, editors. Anti-inflammatory agents; vol II. New York: Academic Press, 1974: 43.

6 Kagoshima M, Kagawa E, Huang ZL, Shimada H.
How to reduce indomethacin-induced gastric mucosal lesions — a new hydrosoluble indomethacin derivative and intra-nasal administration of indomethacin.
Jpn J Pharmacol 1994; **64** Suppl I: 284.

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盐酸 3-(*N*,*N*-二乙胺)丙基吲哚美辛抗炎及致溃疡作用

黄志力，鹿兒島正豐，香川榮一郎，王尉青，島田英世

(北里大学药学部临床药理学教室，东京 108，日本国)

关键词 3-(*N*,*N*-二乙胺)丙基吲哚美辛；吲哚美辛；角叉菜胶；水肿；胃溃疡

目的：考察盐酸 3-(*N*,*N*-二乙胺)丙基吲哚美辛(prodrug)抗炎及致溃疡作用. **方法**：观察大鼠角叉菜胶(Car)性炎症反应及口服给药胃溃疡指数的变化. **结果**：腹腔给药显著抑制 Car 性趾水肿，3 h 及 5 h 的抑制率分别为 36.57 %和34.56 %，与等摩尔浓度的吲哚美辛(Ind)差异无显著性；足趾 10 μg/paw 给药达到 64 %的抑制率，再增加剂量不能增加效果；14.18 mg·kg^{-1}的 prodrug 灌胃 6 小时后溃疡指数显著低于同摩尔数 Ind. **结论**：该药为一抗炎作用强，致溃疡性低的药物.