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# Pharmacokinetic behaviour in polymorphonuclear leucocytes of *N,N*-dimethylcarbamoylmethyl α,2-dimethyl-5H-[1]benzopyrano[2,3-*b*]-pyridine-7-acetate (Y-23023), a new prodrug type of anti-inflammatory agent, and indomethacin after oral administrations in rats

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**Abstract**—*N,N*-Dimethylcarbamoylmethyl α,2-dimethyl-5H-[1]-benzopyrano[2,3-*b*]pyridine-7-acetate (Y-23023) is a prodrug developed as a new non-steroidal anti-inflammatory drug (NSAID). Y-23023 is rapidly hydrolysed to an active metabolite, α,2-dimethyl-5H-[1]benzopyrano[2,3-*b*]pyridine-7-acetic acid ($M_1$) following its absorption and then exhibits a strong anti-inflammatory activity. We have examined the pharmacokinetic behaviour in polymorphonuclear leucocytes (PMNs) of $M_1$ and of indomethacin after oral administration to rats of Y-23023 and indomethacin, respectively. Y-23023 was rapidly absorbed, producing a mean $C_{max}$ (1·13 μg $mL^{-1}$) of $M_1$ after 1 h in plasma. Indomethacin was less rapidly absorbed, producing a mean $C_{max}$ (3·38 μg $mL^{-1}$) after 3 h in plasma. The mean AUC of $M_1$ and indomethacin in plasma were 5·45 μg h $mL^{-1}$ and 22·49 μg h $mL^{-1}$, respectively. The mean $t_{max}$, $C_{max}$ and AUC of M1 in PMNs were 1 h, 11·1 ng (41 pmol)/$10^8$ cells and 58·6 ng (164 pmol) h/$10^8$ cells, respectively. The same parameters for indomethacin in the PMNs were 3 h, 15·4 ng (57 pmol)/$10^8$ cells and 95·2 ng (266 pmol) h/$10^8$ cells, respectively. The PMNs/plasma ratio of $M_1$ was about 2·8 times that of indomethacin. These results indicate that the association of $M_1$, an active metabolite of Y-23023, from blood to the PMNs is greater than that of indomethacin.

Polymorphonuclear leucocytes (PMN) are considered to be therapeutic targets of non-steroidal anti-inflammatory drugs (NSAIDs) (Baggiolini & Dewald 1985; Ragohoebar et al 1987). The interaction between NSAIDs and PMNs is of interest, because an early sign of inflammation is usually an infiltration of the PMNs and many of the mechanisms possibly involved in the action of NSAIDs are related to leucocytes (Walker et al 1976; Kaplan et al 1984).

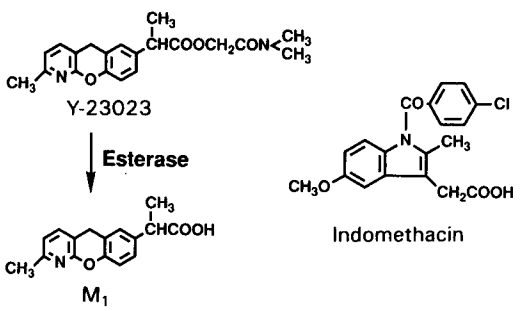


FIG. 1. Structures of Y-23023, $M_1$ and indomethacin.

*N,N*-Dimethylcarbamoylmethyl α, 2-dimethyl-5H-[1]benzopyrano[2,3-*b*]pyridine-7-acetate (Y-23023, Fig. 1) is a prodrug-type of agent developed as a new NSAID. Although Y-23023 is itself pharmacologically inactive, it is rapidly hydrolysed to an active metabolite, α,2-dimethyl-5H-[1]benzopyrano[2,3-*b*]-pyridine-7-acetic acid ($M_1$) following its absorption and subsequently exhibits a strong anti-inflammatory activity (Kawasaki et al 1989). The metabolite, $M_1$, inhibits cyclo-oxygenase in seminal vesicle microsomes of sheep in-vitro at the same potency as indomethacin, but the anti-inflammatory activity of Y-23023 in-vivo is 4–10 times that of indomethacin (Aratani et al 1989; Imayoshi et al 1989). This difference in activities between in-vitro and in-vivo activities may be due to a difference in the peripheral pharmacokinetic characteristics of $M_1$ and indomethacin.

Pharmacokinetic data on NSAIDs usually have been obtained from measurements of plasma and urine concentrations. However, it is very important to investigate the pharmacokinetic behaviour in target cells. The association of NSAIDs with isolated PMNs in-vitro have been investigated extensively (Perianin et al 1990; Ragohoebar et al 1988, 1989); however, no studies have so far dealt with the transport of NSAIDs in-vivo from blood into the PMNs infiltrated in the peritoneal cavity. We have, therefore, studied the pharmacokinetic behaviour of the active metabolite following administration of the prodrug Y-23023.

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## Materials and methods

*Materials.* Y-23023, $M_1$ and pranoprofen (Yoshitomi Pharmaceutical Industries Ltd, Fukuoka, Japan), indomethacin (Wako Pure Chemical Industries Ltd, Osaka, Japan) and diazepam (Sigma Chemical Co., St Louis, MO, USA) were used. Casein sodium was purchased from Nacalai Tesque Inc. (Kyoto, Japan). All other reagents were of analytical grade.

*Animals.* Male Sprague-Dawley rats, 150–250 g, were kept at constant temperature (23 ± 2°C) under a 12:12 h light-dark cycle and were fasted for 18 h before drug administration, with free access to water.

*Preparation of plasma and PMNs.* All rats were treated intraperitoneally with 10 mL 5% casein sodium solution 16 h before administration of Y-23023 or indomethacin. These drugs were administered orally at a dose of 1 mg $kg^{-1}$ as suspensions in 0·5% methylcellulose solution at a concentration of 1 mg $mL^{-1}$. Blood and the peritoneal PMNs were collected by severing the carotid artery of five rats under light ether anaesthesia at each of the following time points: 1, 3, 5 and 8 h after oral administration. The blood samples (5 mL) were collected in heparinized tubes and were centrifuged at 1600 *g* for 15 min to obtain plasma. The PMNs were harvested from the abdominal cavity according to a modification of the method of Smith (1978). A small incision was made into the abdominal cavity and the exudate was removed. The abdominal cavity was then lavaged with saline (10 mL), which was added to the exudate. The exudate was centrifuged at 4°C for 10 min at 800 *g*. The pellet cells were washed twice with saline. The PMNs were counted and precipitated by further centrifugation at 1600 *g* for 15 min and were stored at −20°C until assay of the drugs.

*Sample analysis.* The plasma concentrations of $M_1$ were determined by high-performance liquid chromatography (HPLC). To 0·25 mL of plasma were added 1 μg pranoprofen as an internal standard, 0·25 mL 0·1 M citric acid and 2 mL toluene. After the mixture was shaken for 10 min and centrifuged, 1·6 mL organic layer was taken and evaporated at about 40°C. The residue was dissolved in 100 μL mobile phase (methanol-0·1 M ammonium acetate (5:3, v/v), adjusted to pH 3·0 with perchloric acid) and an aliquot was used for the analysis. The HPLC system used consisted of an LC-6A, an RF-530 fluorescence detector and C-R3A integrator recorder (Shimadzu, Kyoto, Japan). The detector was set at excitation and emission wavelengths of 298 and 350 nm, respectively. Samples were injected via a Rheodyne 7125 injector fitted with a 20-μL loop. Separations were performed on a Shim-pack CLC ODS column (15 cm × 4·6 mm i.d., 5 μm particle size; Shimazu, Kyoto, Japan) and a flow rate of 1·0 mL $min^{-1}$.

The plasma concentrations of indomethacin were also determined by HPLC as described by Ohnishi et al (1987) with a minor modification. A mixture of 0·25 mL plasma, 2 μg diazepam as an internal standard, 0·2 mL 0·1 M citric acid and 2 mL of a mixture of ethyl acetate-*n*-hexane (1:9, v/v) was shaken for 10 min. After centrifugation, 1·6 mL of the organic layer was evaporated at 25°C. The residue was dissolved in 50 μL mobile phase (0·1 M acetic acid-acetonitrile (2:3, v/v)) and an aliquot was used for the analysis by HPLC. The HPLC eluate was monitored by a fixed wavelength UV detector (SPD-6A; Shimadzu, Kyoto, Japan) set at 254 nm.

The PMN concentrations of $M_1$ and indomethacin were also determined by the HPLC method as described above. The PMNs were resuspended in 0·5 mL 1 M $Na_2HPO_4$-0·5 M citric acid buffer (pH 3·0) and were lysed by sonication. These lysed cells were used for the analysis. The same procedures as for the plasma sample were applied for $M_1$ and indomethacin.

*Pharmacokinetic analysis.* The plasma and PMNs concentrations were plotted against time, and the peak concentration ($C_{max}$) and the time to attain the peak concentration ($t_{max}$) were determined. The total area under the plasma concentration–time curve from time 0 to 8 h after dosing (AUC) was calculated according to the trapezoidal rule. The PMNs/plasma ratio (R) of $M_1$ and indomethacin was defined by the following equation:

$$R = AUC_{PMNs}/AUC_{plasma} \quad (1)$$

*Measurement of the octanol–water partition coefficient.* The partition between *n*-octanol and Tris-HCl buffer (pH 7·4) was determined for $M_1$ and indomethacin as described by Ritschel (1980). Each drug was dissolved in an aqueous solution at a concentration of 1 μg $mL^{-1}$. Ten millilitres buffer and *n*-octanol were placed in an Erlenmeyer flask fitted with a glass stopper and mechanically shaken for 5 h at 37°C. The concentration of drug in the aqueous phase was determined by HPLC as described above. The partition coefficient (P) was calculated as the following equation:

$$P = (C_1 - C_2)/C_2 \quad (2)$$

where $C_1$ and $C_2$ are the drug concentrations in the aqueous phase before and after equilibration, respectively.

*Equilibrium dialysis.* Acrylic resin plates with five compartments of 25 mm diameter were used for the dialysis studies. Two plates were clamped together with a semipermeable membrane (Spectropor 2, mol. wt cut-off 12–14 kDa, Spectrum Medical Industries Inc.) between them; the dialysis membrane was prepared for use by washing twice with purified water and then soaking for 24 h in the phosphate buffer. An aliquot of rat serum was put into one compartment and, unless otherwise specified, 0·75 mL pH 7·4 isotonic phosphate buffer into the other. The drug was added to the buffer (1·4 and 0·15 μg $mL^{-1}$ for $M_1$ and 1·8 and 0·18 μg $mL^{-1}$ for indomethacin). Preliminary studies indicated that equilibrium was attained by 2 h and that there was no loss of drug to the cell or membrane. The dialysis was performed in triplicate for 2 h at 37°C. No significant volume shifts have been noted for this system and conditions in our laboratory. The free concentrations of drug were determined by HPLC. The percentage of binding was calculated according to the equation:

$$\% \text{ Bound} = (1 - B/(A - B)) \times 100 \quad (3)$$

where A and B are the drug concentrations in the buffer phase before and after equilibration, respectively.

## Results and discussion

PMNs were successfully obtained from peritoneal fluid when 5% casein solution was administered intraperitoneally 16 h before oral administration of drugs. Purity was determined by a microscopic count after staining of cytocentrifuge slides with May–Grünwald–Giemsa (85% PMNs, 7% macrophages).

The time courses of the drug concentration in plasma and PMNs after oral administrations of Y-23023 or indomethacin to rats are shown in Fig. 2. Y-23023 was rapidly absorbed, producing a mean $C_{max}$ of 1·13 μg $mL^{-1}$ of $M_1$ after 1 h. Indomethacin was less rapidly absorbed, producing a mean $C_{max}$ of 3·38 μg $mL^{-1}$ after 3 h. The mean AUC of $M_1$ and indomethacin in plasma were 5·45 and 22·49 μg h $mL^{-1}$, respectively.

The mean $t_{max}$, $C_{max}$ and AUC of $M_1$ in PMNs were 1 h, 11·1 ng (41 pmol)/$10^8$ cells and 58·6 ng (164 pmol) h/$10^8$ cells, respectively. The same parameters for indomethacin in the PMNs were 3 h, 15·4 ng (57 pmol)/$10^8$ cells and 95·2 ng (266 pmol) h/$10^8$ cells, respectively. The $C_{max}$ and AUC of indomethacin in the PMNs were 1·4 and 1·6 times greater than those of $M_1$. There was considerable variability in the association of indomethacin from plasma to the PMNs, compared with that of $M_1$. The PMNs/plasma ratios of $M_1$ and indomethacin were 0·011 and 0·004, respectively. Although $M_1$ (log P = 0·41) is less lipophilic than indomethacin (log P = 1·29), the PMNs/plasma ratio of $M_1$ was about 2·8 times higher than that of indomethacin (Table 1). The partition coefficients were not related to the association of these drugs to the PMNs. Protein binding is considered to be a major determinant of the pharmacokinetic

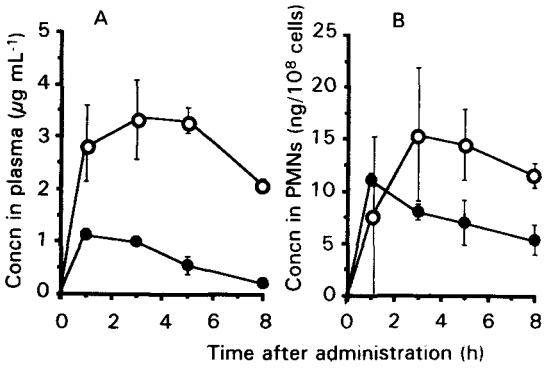


FIG. 2. Plasma (A) and PMNs (B) concentrations of $M_1$ (●) and indomethacin (○) in rats. The prodrug Y-23023 or indomethacin were administered orally at a dose of 1 mg $kg^{-1}$. Each value represents the mean ± s.d. of five rats.

Table 1. PMNs/plasma ratios (R) and partition coefficient (log P).

| Compound | R[a] | log P[b] |
|---|---|---|
| $M_1$ | 0.011 | 0·41 |
| Indomethacin | 0.004 | 1·29 |

[a] The PMNs/plasma ratio (R) was calculated by the following equation: $R = AUC_{PMN}/AUC_{plasma} \times 10^3$. The value represents the mean of five rats. [b] The P is partition coefficient between *n*-octanol and 0·05 M Tris-HCl buffer (pH 7·4) at 37°C.

behaviour at the site of action of NSAIDs (Lin et al 1987). Generally, NSAIDs bind strongly to serum proteins (> 90% in most cases) (Verbeeck et al 1983). The in-vitro mean serum-protein binding of indomethacin was 92·3% over the concentration range 0·18–1·8 $\mu g\ mL^{-1}$ and that of $M_1$ was 92·8% over the concentration range 0·14–1·4 $\mu g\ mL^{-1}$. Thus, the free concentration of indomethacin in plasma was almost the same as that of $M_1$. These results suggest that $M_1$ associates from blood to the PMNs by a specialized mechanism, rather than by passive diffusion.

Ragohoebar et al (1987, 1988) reported that acetylsalicylic acid and sodium salicylate associated with the human peripheral PMNs by facilitated diffusion of the un-ionized species across the lipid interior of the membrane and by association with the outer membrane or cytosolic matrix. The characteristics of diclofenac uptake by the PMNs were different from those of the salicylic acid derivatives. Cell association with diclofenac in-vitro occurs through various processes including absorptive or fluid-phase endocytosis, passive diffusion, carrier-mediated uptake, ligand trapping or permeation, and the activation of sodium/hydrogen antiport (Perianin et al 1990). Similarly, the mechanisms of association of indomethacin to the PMNs appear to be more complex than a simple one or two binding-sites model that obeys principles of mass action (Ragohoebar et al 1989). However, the detailed mechanisms whereby $M_1$ associates to PMNs can not be clarified in this study.

The results presented in this report show that the association of $M_1$, an active metabolite of Y-23023, from blood to the PMNs is greater than that of indomethacin. The differences between in-vitro (seminal vesicle microsomes) and in-vivo pharmacological activity for $M_1$ and indomethacin should be at least due to the differential abilities of the two drugs to associate with the inflammatory cells such as PMNs. Further studies are necessary to clarify the mechanisms of association of $M_1$ to PMNs.

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