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The Pharmacokinetics and Pharmacodynamics of a New Thromboxane Synthetase Inhibitor, 6-(1-Imidazolylmethyl)-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid (DP-1904) in Man after Repeated Oral Doses

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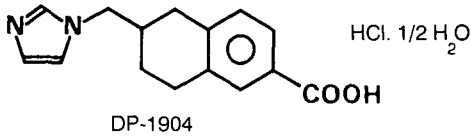
Abstract—The pharmacokinetics of DP-1904, a new potent and selective thromboxane synthetase inhibitor, and its effects on ex-vivo prostanoid formation have been studied in groups of Japanese normal male volunteers, who received repeated oral doses of 200 mg every 12 h for 4 doses, or 400 mg every 24 h for 2 doses, or 200 mg every 12 h for 14 doses. The drug was well tolerated by all subjects without evidence of adverse reactions. Repeated administration showed no significant changes in half-lives, t_{max} values, c_{max} values and AUC values. DP-1904 did not exhibit time-dependent kinetics. Its plasma levels were lower than the quantifiable level (50 ng mL^{-1}) at 12 h after each dose. These data suggest no significant accumulation of DP-1904 in normal volunteers. DP-1904 reduced the serum thromboxane B_2 by about 80% during the medication, the serum concentrations returning to about 44, 75 and 20% of the predrug control values at 36 h after the last 200 mg doses and 48 h after the last 400 mg dose.

Thromboxane A_2 (TxA_2) and prostaglandin (PG)I_2 exert opposite effects on platelet aggregation and vascular resistance, and the balance between these compounds has been proposed to be one of the factors that determine platelet reactivity, endothelial thromboresistance, and vascular tone (Moncada & Vane 1978). TxA_2 may contribute to the pathogenesis of many thrombotic disorders such as coronary heart disease (Dusting 1983) and cerebral ischaemia (Furlow & Hallenbeck 1978). Selective inhibition of thromboxane synthesis and the redirection of endoperoxide metabolism toward PGI_2 will possibly offer a new therapeutic approach to those disease states in which increased thromboxane formation is critically involved.



DP-1904

DP-1904, 6-(1-imidazolylmethyl)-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid hydrochloride hemihydrate, is a new potent and long-acting thromboxane synthetase inhibitor in animals (Kanao et al 1989). Previously, we reported the pharmacokinetics and pharmacodynamics of DP-1904 in man after single doses (Tanaka et al 1989). DP-1904 proved to be safe and effective in single oral doses, causing strong inhibition of thromboxane synthetase. Since the clinical usefulness of the drug will depend upon the safety and effect of long-term multiple dosages, which may cause a more significant change in platelet function and arachidonic acid metabolites than those observed during the single dose study, we have examined the pharmacokinetic characteristics of DP-1904 and its inhibitory effect on thromboxane synthesis in Japanese normal volunteers after repeated oral doses.

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Materials and Methods

Subjects

Eighteen volunteers (23–43 years old, 57·0–76·0 kg) participated. The subjects were judged to be in good health based on thorough prestudy physical examination and the results from haematology, urinalysis, and biochemical tests. All subjects gave written informed consent following a protocol approved by the Institutional Review Board at East Hospital of Kitasato University. On the evening before the study day the subjects reported to the hospital and vital signs were monitored at the time of check-in. All subjects were provided with standard meals and allowed to drink water freely during the study.

Dosing and sample collection for drug analysis

The 18 volunteers were assigned to groups of six, one of each group receiving a placebo. The study was conducted under a double blind design. One of three groups received 200 mg of DP-1904 every 12 h for a total of 4 doses (Group A), one received 400 mg every 24 h for a total of 2 doses (Group B), and one received 200 mg every 12 h for a total of 14 doses (Group C).

In Group A, blood samples were collected into heparinized tubes before and 0·5, 1, 2, 4, 8, 12, 13, 14, 24, 24·5, 25, 26, 28, 32, 36, 37, 38, 48 and 72 h after the initial dose. After centrifuging at 3000 rev min^{-1} for 15 min, plasma was

separated and frozen at −20°C as soon as possible. Quantitative urine collections were obtained for the 0–2, 2–4, 4–8, 8–12, 12–14, 14–24, 24–26, 26–28, 28–32, 32–36, 36–38, 38–48 and 48–72 h intervals. Urine samples were stored frozen at −20°C until analysed. In Group B, blood samples were collected immediately before and 0·25, 0·5, 1, 2, 4, 6, 8, 12, 24, 24·25, 24·5, 25, 26, 28, 30, 32, 36, 48 and 72 h after the initial dose. Quantitative urine collections were obtained for the 0–2, 2–4, 4–8, 8–12, 12–24, 24–26, 26–28, 28–32, 32–36, 36–48 and 48–72 h intervals. In Group C, blood samples were collected immediately before and 1, 2, 4, 8, 12, 24, 25, 72, 73, 74, 76, 80, 84, 96, 97, 144, 145, 146, 148, 152, 156, 168 and 192 h after the initial dose. Quantitative urine collections were obtained for the 0–2, 2–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–74, 74–76, 76–80, 80–84, 84–96, 96–120, 120–144, 144–146, 146–148, 148–152, 152–156, 156–168 and 168–192 h intervals.

Drug analysis

DP-1904 in plasma and urine samples were analysed by a sensitive and selective high-performance liquid chromatographic method (Tanaka et al 1988). The method involved a solid extraction procedure with Sep-pak C_{18} cartridges. Separation was achieved on TSK-GEL ODS 80 TM (Tosoh, Tokyo, Japan) with a mobile phase of 0·5% KH_2PO_4 (pH 3·0)-tetrahydrofuran (16:1, v/v). Both DP-1904 and internal standard (DQ-2481, 6-(1-imidazolylmethyl)-5,6,7,8-tetrahydronaphthalene-3-carboxylic acid) were detected at a wavelength of 240 nm. The lower limit of quantitation was 50 ng mL^{-1} (C.V. = 3·80%) for plasma and 1·0 μg mL^{-1} (C.V. = 4·49%) for urine.

Assay of serum thromboxane B_2

Thromboxane B_2 (TxB_2) levels were measured by radioimmunoassay in sera derived from 5 mL of whole blood drawn into a glass tube. The blood was allowed to stand at 37°C for 1 h in a water bath for maximum production of thromboxane B_2. The serum was removed after centrifugation at 3000 rev min^{-1} for 15 min and stored at −20°C until analysed. RIA assays were carried out on extracted serum with a ^{125}I-RIA Kit (New England Nuclear, Mass., USA) (Kawano et al 1987).

Pharmacokinetic analysis

The plasma concentration of DP-1904 was plotted versus time and pharmacokinetic parameters for the resulting curves were calculated using a curve-fitting program for a personal computer (PC-9801, Nippon Electric Co. Ltd, Tokyo, Japan) (Yamaoka et al 1981). A one compartment open model with first-order absorption after a lag time was used to describe the plasma concentrations of DP-1904 after oral administration. The time of maximum concentration (t_{max}) and the maximum concentration in plasma (c_{max}) were observed values and not model dependent values. The area under the plasma concentration curve (AUC) from the time of administration to 12 or 24 h post dosing was calculated by the linear trapezoidal method.

Statistical analysis was carried out by Student's *t*-test.

All of the data are expressed as the mean ± the standard error (n = 5).

Results

Safety assessment

None of the subjects in the present studies developed symptoms or adverse experiences that could be related to the drug, and there were no significant changes in standard laboratory testing or platelet counts that could be drug-related. The electrocardiogram recordings did not show any changes attributable to the administration of the compound.

Pharmacokinetics of DP-1904

Group A. The pharmacokinetic parameters derived from plasma concentrations of DP-1904 after the 1st and 3rd dose are shown in Table 1. The plasma concentration versus time profiles are shown in Fig. 1. DP-1904 in plasma was not detectable at 12 h after every 200 mg oral dose. These data suggested that DP-1904 was rapidly eliminated from the body and not accumulated after repeated administration of 200 mg every 12 h for 4 doses.

The urinary excretion of DP-1904 at 12 h after each administration was approximately 50% of the dose and no change of rate due to consecutive doses was observed. Up to 36 h after the final dose it was 45·2 ± 2·83% of the dose. The

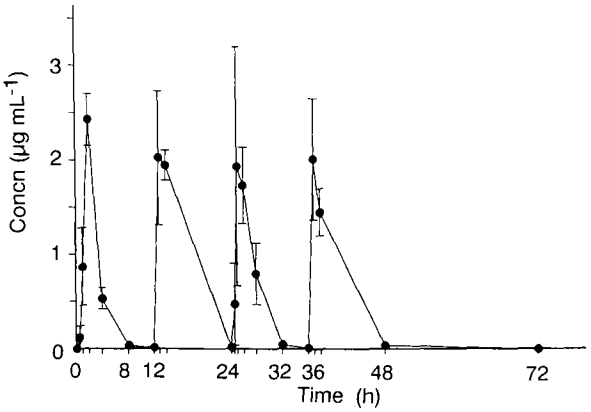

FIG. 1. Mean concentration-time profiles of DP-1904 after repeated 200 mg oral doses twice a day for two days to five normal volunteers. Bars: ±s.e. (n = 5).

Table 1. Pharmacokinetic parameters derived from plasma concentrations of DP-1904 after the 1st and 3rd dose of twice daily dosing of 200 mg DP-1904 orally to volunteers (n = 5).

	t_0 min	$t_{\frac{1}{2}}$ min	K_a h^{-1}	V_d L	t_{max} min	c_{max} μg mL^{-1}	AUC (0–12 h) μg h mL^{-1}
1st dose	39·5 ± 11·5	46·6 ± 4·10	1·25 ± 0·20	35·7 ± 2·76	108 ± 12·0	2·50 ± 0·26	6·04 ± 0·31
3rd dose	48·0 ± 19·3	46·0 ± 9·90	6·67 ± 4·20	30·0 ± 4·63	120 ± 32·9	3·25 ± 0·85	6·83 ± 0·72

(Mean ± s.e.)

Table 2. Pharmacokinetic parameters derived from plasma concentrations of DP-1904 after the 1st and 2nd dose of once daily dosing of 400 mg DP-1904 orally to volunteers (n = 5).

	t_0 min	$t_{\frac{1}{2}}$ min	K_a h^{-1}	V_d L	t_{max} min	c_{max} $\mu g\ mL^{-1}$	AUC (0–24 h) $\mu g\ h\ mL^{-1}$
1st dose	29·3 ± 9·46	43·1 ± 5·39	4·63 ± 2·48	39·4 ± 5·71	84·0 ± 14·7	5·58 ± 1·20	9·73 ± 0·70
2nd dose	35·5 ± 5·97	50·8 ± 4·61	4·57 ± 3·10	50·6 ± 5·10	96·0 ± 14·7	4·29 ± 0·47	9·10 ± 0·41

(Mean ± s.e.)

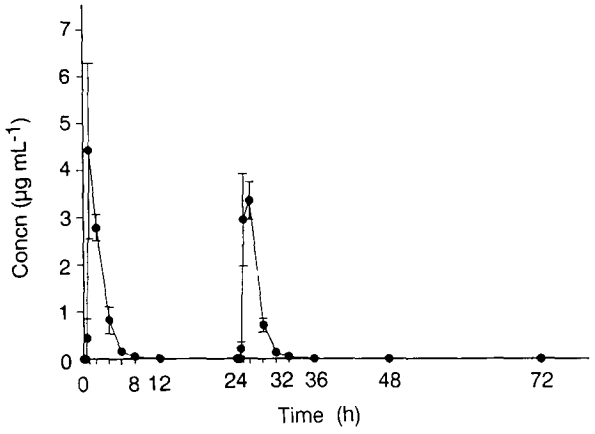

FIG. 2. Mean concentration-time profiles of DP-1904 after repeated 400 mg oral doses once a day for two days to five normal volunteers. Bars: ± s.e. (n = 5).

urinary excretion rate in this multiple dose study was similar to that in the single dose study (Tanaka et al 1989).

Group B. The pharmacokinetic parameters derived from plasma levels of DP-1904 after the 1st and 2nd dose are shown in Table 2. The plasma concentration versus time profiles are shown in Fig. 2. The concentration of DP-1904 in plasma was lower than the quantifiable level (50 ng mL^{-1}) at 24 h after each 400 mg oral dose and the drug was not detectable 48 h after the final dose. These data suggest a similar conclusion to that made about Group A.

The urinary excretion of DP-1904 at 24 h after each administration was also approximately 50% of the given dose with no change due to repeated doses. Up to 48 h after the final dose it was 50·1 ± 2·07% of the dose. The rate in this study was also similar to that in the single dose study (Tanaka et al 1989).

Group C. The pharmacokinetic parameters derived from plasma levels of DP-1904 after the 1st, 7th and 13th dose are shown in Table 3. The plasma concentration versus time profiles are shown in Fig. 3. As only a limited number of sampling times was available it was not always possible to characterize the plasma concentration versus time profiles of DP-1904 nor to assess the observed C_{max} values and T_{max} values. Again, the concentration of DP-1904 in plasma was lower than the quantifiable level (50 ng mL^{-1}) at 12 h after each 200 mg oral dose and the drug was not detectable 36 h

Table 3. Pharmacokinetic parameters derived from plasma concentrations of DP-1904 after the 1st, 7th and 13th dose of twice daily dosing of 200 mg DP-1904 orally to volunteers (n = 5).

	t_0 min	$t_{\frac{1}{2}}$ min	K_a h^{-1}	V_d L	t_{max} min	c_{max} $\mu g\ mL^{-1}$	AUC (0–12 h) $\mu g\ h\ mL^{-1}$
1st dose	23·7 ± 14·5	39·4 ± 6·86	6·00 ± 1·99	29·4 ± 8·02	96·0 ± 14·7	3·17 ± 0·52	5·44 ± 0·32
7th dose	9·86 ± 9·86	37·9 ± 4·16	1·40 ± 0·10	40·1 ± 4·94	72·0 ± 12·0	2·03 ± 0·27	4·61 ± 0·44
13th dose	11·8 ± 11·8	65·5 ± 27·9	4·69 ± 2·09	52·8 ± 20·7	72·0 ± 12·0	2·20 ± 0·38	4·55 ± 0·59

(Mean ± s.e.)

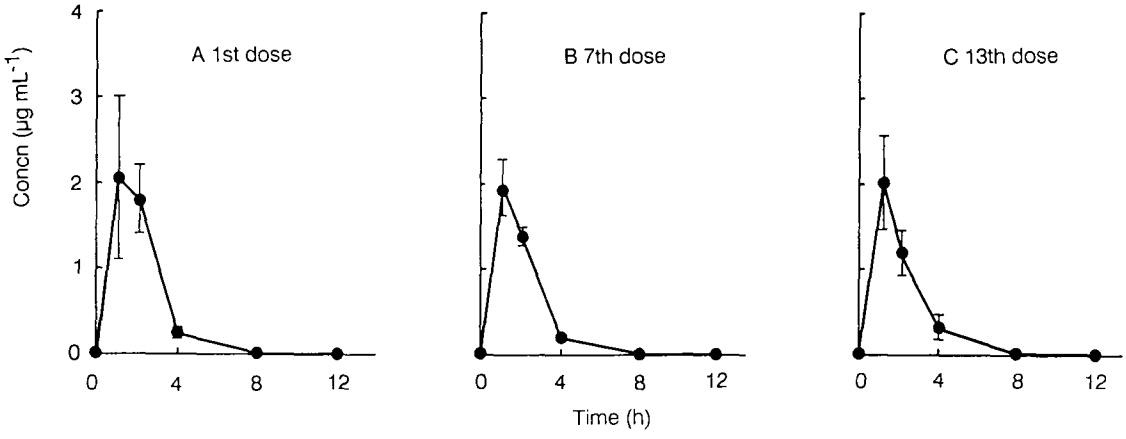

FIG. 3. Mean concentration-time profiles of DP-1904 after repeated 200 mg oral doses twice a day for seven days to five normal volunteers. Bars: ± s.e. (n = 5).

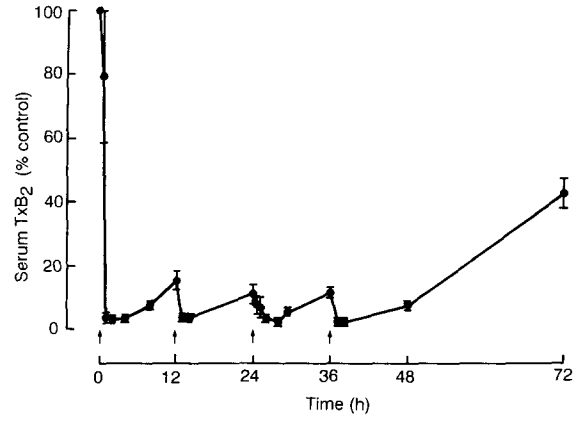

FIG. 4. Profiles of serum TxB_2 during and after repeated 200 mg oral doses twice a day for two days. Times of administrations are indicated by arrows. Bars: ±s.e. (n = 5).

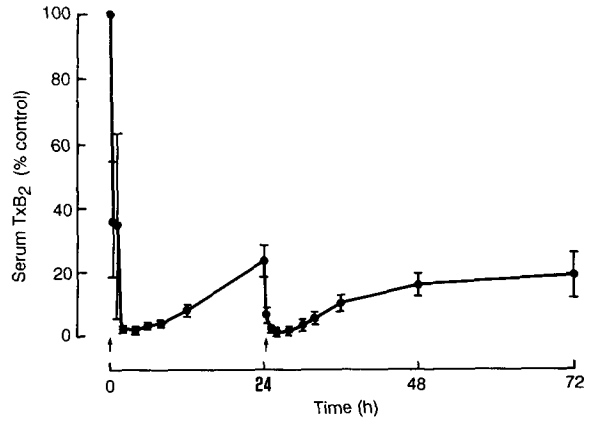

FIG. 5. Profiles of serum TxB_2 during and after repeated 400 mg oral doses once a day for two days. Times of administrations are indicated by arrows. Bars: ±s.e. (n = 5).

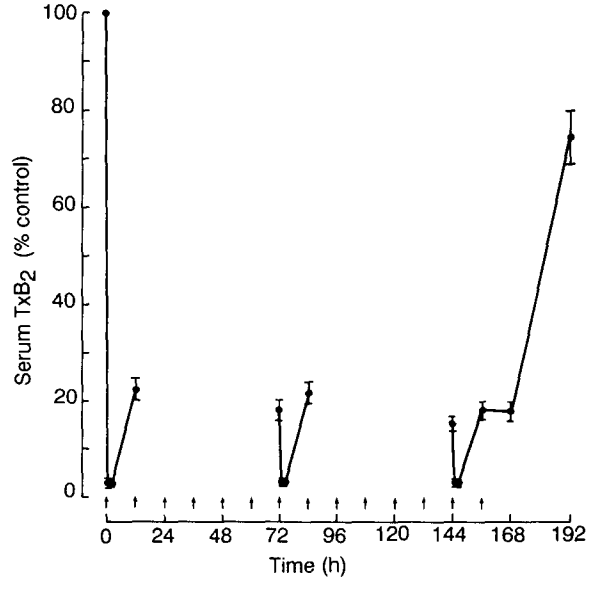

FIG. 6. Profiles of serum TxB_2 during and after repeated 200 mg oral doses twice a day for seven days. Times of administrations are indicated by arrows. Bars: ±s.e. (n = 5).

after the final dose, also confirming the rapid elimination of the drug with no significant accumulation.

The urinary excretion pattern for this group was similar to Group A.

Effect of DP-1904 on thromboxane synthesis

The inhibition of TxB_2 was almost complete 1 h after the first dose (Figs 4–6), and remained greater than 91, 82 and 83% 12 h after the last 200 mg doses (Groups A, C) and 24 h after the last 400 mg dose (Group B) (Figs 4–6). TxB_2 serum concentrations returned to about 44, 75 and 20% of the predrug control values 36 h after the last 200 mg doses (Groups A, C) and 48 h after the last 400 mg dose (Group B).

Discussion

DP-1904 is a potent and long-acting thromboxane synthetase inhibitor now under clinical trial in patients with cardiovascular diseases such as angina pectoris where long-term multiple dosages are essential to manage the disease. Previously we reported pharmacokinetics and pharmacodynamics of DP-1904 after single oral doses (Tanaka et al 1989).

In the present study, multiple-dose pharmacokinetics and pharmacodynamics of DP-1904 in normal volunteers were investigated to assess its safety and efficacy.

There were no adverse reactions, subjective symptoms, or cardioavascular changes in multiple dose studies.

DP-1904 had a short half-life and was rapidly eliminated from the body. The plasma concentrations were less than the quantifiable level (50 ng mL^{-1}) 12 h after every dosing. These data suggested that there should be no significant accumulation of the drug on multiple dosing to normal volunteers.

The pharmacokinetic parameters did not change significantly even after seven days' dosing of 200 mg twice a day (Group C) indicating that DP-1904 did not exhibit time-dependent kinetics.

DP-1904 effectively and reversibly blocked ex-vivo serum thromboxane formation during repeated doses. The serum levels of TxB_2 were reduced by about 80% 12 h after each dose in all groups.

The IC50 (concentrations required for 50% inhibition of TxB_2 production) in-vitro in rat platelet-rich plasma is 1·1 μM (330 ng mL^{-1}) (Kanao et al 1989). This suggests that the plasma levels of DP-1904 12 h after dosing is much less than those required for 80% reduction in serum TxB_2 levels in man. The biological effect outlasted the plasma drug levels. The same phenomenon was observed in the single dose study (Tanaka et al 1989). A possible explanation for this finding could be the high affinity of DP-1904 for thromboxane synthetase in platelets or other tissues.

No accumulation or waning of the action of DP-1904 was evident during medication; however, twice daily dosing rather than an increased single dose was required for smooth suppression of serum thromboxane B_2 formation.

The absence so far of clinically relevant side effects of this drug in conjunction with its attractive therapeutic potential in those clinical conditions that may result from elevated intrinsic thromboxane formation should encourage its further clinical study.

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