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Partial avoidance of first-pass elimination of azathioprine in rats by rectal dosing

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Summary

Avoidance of the hepatic first-pass elimination of an immunosuppressive drug, azathioprine (AZ), was attempted by rectal administration of AZ in rats. The mean AUC values obtained following i.v., oral and rectal dosing of AZ to three groups of rats are 103.8 ± 22.8 (S.E.), 14.3 ± 2.45 and 60.4 ± 8.27 $\mu g \cdot min/ml$, respectively. The mean systemic availabilities of AZ following oral and rectal administration expressed as the ratios of the AUCs are, $F_{oral} = 13.9\%$ and $F_{rectal} = 58.3\%$ respectively. The data showed that substantial avoidance of the hepatic first-pass elimination of AZ can be achieved via rectal administration. The fraction of AZ avoided the hepatic first-pass elimination after rectal administration is discussed with respect to the pharmacokinetics of AZ in rats by means of a circulatory transport analysis.

Introduction

Azathioprine (AZ) is a mercaptopurine derivative that suppresses T-lymphocyte delayed immune responses and is used primarily to prevent rejection of kidney transplantation (Al-Safi et al., 1984). AZ is extensively metabolized in the body (Ding and Benet, 1979). The main metabolic route is the cleavage of AZ to

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6-mercaptopurine (6-MP) by glutathione-S-transferase and hydroxylation to 8-hydroxy-AZ by aldehyde oxidase which is further cleaved to 8-hydroxy-MP (8-OHMP) (De Miranda et al., 1975). Both 6-MP and 8-OHMP are further oxidized by xanthine oxidase to thiouric acid (Breter et al., 1978; Chalmers, 1975; Lennard and Maddocks, 1982). Kaplowitz (1976) reported that the rat liver is the major in vivo site of interaction of AZs with these enzymes following large doses of AZ in the rat.

In the clinical renal transplantation, AZ is orally administered every day to the renal transplant patients at the dose range 5–15 mg/kg (Knapp et al., 1980). However, in the clinical pharmacokinetic study of AZ, no AZ was detected in the patient's plasma after oral administration of AZ (Lin et al., 1980). This is due to the considerable high hepatic first-pass elimination of this immunosuppressive drug. Furthermore, this high first-pass elimination is the most important factor for the incidence of the side-effect of this drug, namely hepatotoxicity (Gilman et al., 1980).

The possibility for the avoidance of the hepatic first-pass effect by rectal dosing was originally suggested by De Boer et al. on lidocaine (1979) and after that on nitroglycerin by Kamiya et al. (1982). Based on this information, the present investigation was undertaken to try to improve the systemic availability of AZ by avoiding of its hepatic first-pass elimination by dosage form design (i.e. rectal dosing), and the extent of avoiding the hepatic first-pass elimination of such a high hepatic clearance drug after rectal dosing was assessed with a circulatory transport pharmacokinetic analysis (Araki and Miyazaki, 1974; Miyazaki and Araki, 1978; Weiss and Foster, 1979; Van Rossum et al., 1983).

Materials and Methods

Materials

Azathioprine (AZ) was a gift from The Wellcome Foundation (London, U.K.). All the reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan). The methyl alcohol used for HPLC assay was of UV grade.

Animal experiments

Male Wistar rats, weighing 370–420 g, were used. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital, 40 mg/kg. Cannulation was performed on the animals by passing a silastic medical grade tubing (inside diameter 0.020 in.; Dow Corning, Midland, MI, U.S.A.) down through the jugular vein. Three routes of administration for AZ (i.e. intravenous (i.v.), oral and rectal) were attempted. In all experiments, AZ (10 mg) was dissolved in sufficient 1 N NaOH and then diluted with isotonic phosphate buffer, pH 7.4, to a 0.5 ml volume. The required amount of AZ (based on body weight, 5 mg/kg) in the resulting solution was administered to rats. For i.v. dosing, AZ solution was injected into rats through the cannula, and was followed by washing with a 200-μl aliquot of saline. Blood samples (0.15–0.25 ml) were withdrawn through the cannula at 2.5, 5, 10, 15, 20, 30, 45 and 60 min. For oral administration, the abdomen of the rat was opened by incision and AZ solution was injected into the duodenum. After injection, the

pore in the duodenal part was closed with a drop of tissue cement. Blood samples (0.15–0.25 ml) were collected at 2.5, 5, 10, 15, 20, 30, 40, 50 and 60 min. For rectal dosing, a restricted rectal infusion device was modified for use (Kamiya et al., 1982). The device was constructed with the connected two septum plugs at a fixed distance (2.0 cm) with a stainless needle. This device was inserted into the rat rectum from the anus. The upper septum plug (8.0 mm diameter) was used to prevent upward spreading of AZ solution. The lower septum plug (10.0 mm diameter) was glued to the anus. AZ solution was injected into the rectum following the withdrawal of a volume of air from the rectum identical to that of the rectal AZ solution. Blood samples (0.15–0.25 ml) were collected at 2.5, 5, 10, 15, 20, 30, 40, 50 and 60 min. All of the blood samples were centrifuged at $10{,}000 \times g$ for 2 min and plasma fractions were removed immediately. The plasma samples were stored in a freezer at $-20°C$ until analyzed.

Assay of AZ

To 100 μl of rat plasma sample was added 5 μl of 70% perchloric acid followed by being well mixed with a vortex mixer for 1 min. After centrifugation, the supernate was neutralized with 4 μl of 6 N KOH. The mixture was centrifuged to remove the precipitate and 50 μl of the supernate were injected directly into the HPLC system as reported (Lin et al., 1980). Separation of the biological samples was performed with a reversed phase ODS-C18 column packed in our laboratory. Mobile phase consisted of $H_2O : CH_3CN : CH_3COOH$ (400 : 75 : 0.1). The UV detector was set at 280 nm, corresponding to the absorption peak of AZ. Levels were estimated by the chromatographic technique of comparing peaks obtained from test plasma with curves obtained from rat plasma to which was added known amounts of AZ. The standard curve of AZ added to the rat plasma was linear over the range 0.1–20 μg/ml and passed through the origin.

Pharmacokinetic calculations

Plasma AZ concentration–time curves obtained after i.v. injection were best-fit to a two-exponential function:

$$C(t) = A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t)$$

where A and B are coefficients and α and β are exponents of the two-exponential equation. The method of residuals was used to determine initial kinetic parameter estimates (Gibaldi and Perrier, 1982), and final parameter values were determined by a weighted, non-linear least-squares regression analysis. The program used was 'KINONITE/BAS' which was developed by Nielsen-Kudsk (1983). Convergence was defined as a relative change in the residual sum of squares less than 1×10^{-4}. Compartmental modeling was not possible for oral and rectal AZ plasma concentration–time curves because of the extensively rapid increase of plasma AZ concentrations after administration. The elimination rate constant for the AZ concentration–time curves after oral or rectal dosing was obtained by a linear-regression fit of the terminal portion of AZ plasma disappearance curve. Elimination

$T_{1/2}$ after oral or rectal AZ dosing was determined by dividing 0.693 by thus obtained elimination rate constant. Area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule up to the last measured plasma concentration, $C_{p(last)}$, and extrapolated to infinity by addition of the term $C_{p'(last)}/\beta$, were $C_{p'(last)}$ is the calculated value based on the best-fit curve and β is the elimination rate constant. Plasma clearance was determined by non-compartmental approach (Benet and Galeazzi, 1979). Namely, $V_{d(ss)} = \text{Dose} \cdot (\text{AUMC})/(\text{AUC})$, where AUMC is the area under the product of time and concentration ($t \cdot C$) versus time (t) curve (Cutler, 1981). Total body clearance (CL) was determined by dividing the i.v.-dose by the $AUC_{i.v.}$. Mean residence time (MRT) was calculated with the equation, MRT = (AUMC)/(AUC). Bioavailability of oral or rectal AZ, a measure of the amount of oral or rectal drug that gains access to the systemic circulation as parent compound, was calculated by dividing the mean AUCs after oral or rectal AZ by the mean AUC after i.v. AZ. Statistical analysis was performed using the non-parametric test, Mann-Whitney U-test (Bruning and Kintz, 1977).

Results

When AZ was i.v. injected into 6 rats, biexponential decay curve was seen in the systemic plasma (Fig. 1). As shown in Fig. 1, AZ disappeared rapidly from the plasma, with an α-half-life of 2.50 ± 0.831 (S.E.) min and a β-half-life of 36.2 ± 9.43 (S.E.) min (Table 1). The mean clearance of AZ after i.v. injection was 31.8 ± 3.63 (S.E.) ml/min.

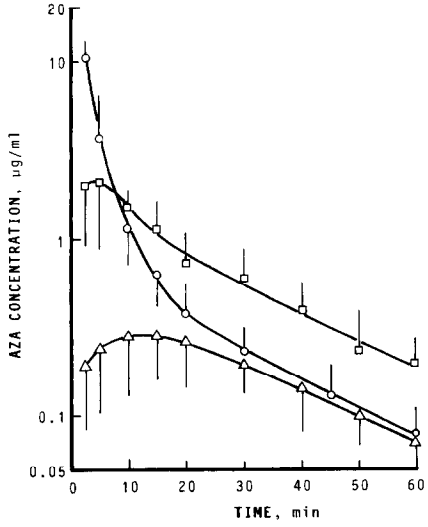

Fig. 1. Mean plasma concentrations of AZ after i.v., oral and rectal administration of 5 mg/kg dose of AZ to rats. Points and bars represent means and standard errors of values in 6 rats.

TABLE 1

PHARMACOKINETIC PARAMETER FOR AZATHIOPRINE IN RATS FOLLOWING 5 mg/kg i.v., ORAL AND RECTAL ADMINISTRATION

Route of administration	AUC ($\mu g \cdot min/ml$)	Clearance [a] (ml/min)	α-half-life (min)	β-half-life (min)	Vd_{ss} (mg/kg)	MRT (min)
i.v.	103.8 ± 22.8	31.8 ± 3.63	2.50 ± 0.831	36.2 ± 9.43	533 ± 91.2	17.3 ± 3.43
Oral	14.3 ± 2.45	224.6 ± 44.6	–	33.2 ± 2.34 [b]	–	40.5 ± 3.62
Rectal	60.4 ± 8.27	52.2 ± 5.13	–	29.1 ± 6.27 [b]	–	37.0 ± 5.5

Each value shows the mean ± S.E.

[a] Apparent clearance was determined by dividing the oral or rectal dose by AUC (oral or rectal).

[b] Not significantly different from the β-half-life following i.v. administration.

After AZ was administered orally to 6 rats, the plasma AZ concentration increased gradually and reached the maximum at about 15 min after dosing. After that, plasma AZ concentration declined rapidly with a β-half-life almost equal to that following the i.v. injection of AZ. Despite the fact that the oral AZ dose was equal to the i.v. dose, plasma concentrations were considerably lower, namely about one-tenth. The apparent clearance of AZ, given by the relationship: $CL_{app(oral)} = Dose/AUC_{oral}$ was 224.6 ± 44.6 (S.E.) ml/min.

Fig. 1 also shows the mean plasma concentration curves of AZ after rectal administration into 6 rats. The absorption of AZ was very rapid; maximum concentration was reached within 5 min. The β-half-life was almost equal to that obtained after the i.v. injection of AZ. The plasma AZ concentrations were about two times higher than that following the oral administration of AZ, though the AZ doses were identical in all of the experiments. The apparent clearance of AZ $CL_{app(rect)}$ was 52.2 ± 5.13 (S.E.) ml/min.

By comparing the plasma AZ concentration–time curves shown in Fig. 1, it is clear that the rank order of the AUCs of AZ are i.v. > rectal > oral, whereas the β-half-life ($T_{1/2}$ s) are of the same order of magnitude for all. The various systemic availability of AZ after the different routes of administration, expressed as the ratios of the AUCs based on plasma concentration was determined by the following relationship: $F_{oral\ or\ rectal} = AUC_{oral\ or\ rect.}/AUC_{i.v.}$. Namely, the appropriate oral bioavailability was 13.9%, whereas the rectal bioavailability was 58.3%, which differs from the oral value ($P < 0.05$). Thus, rectal bioavailability of AZ is more than twice as high as oral bioavailability.

Discussion

The purpose for the rectal route of drug administration is: (1) to diminish or decrease the side-effect of drugs on the gastrointestinal tract; or (2) to deliver drugs into the systemic circulation rapidly where intramuscular injection of drugs is difficult from the standpoint of security of the patients. However, attention has been focused on the rectal administration of drugs to increase the systemic availability of drugs which undergo the hepatic first-pass elimination after oral administration. In practice, the avoidance of the hepatic first-pass effect of lidocaine (De Boer et al., 1979) and nitroglycerin (Kamiya et al., 1982) have been achieved by the rectal administration. In this study, an immunosuppressive drug, AZ, was used to improve its systemic availability by rectal dosing. To avoid the other factors such as dissolution and liberation from the suppository base, AZ was administered into rat rectum as solution. Recently, De Leede et al. (1983) suggested that the avoidance of first-pass elimination of lidocaine is sensitive to the site of absorption in the rectum of rats. When the restricted rectal area (0.2–2 cm distance from the anus) was used for absorption, systemic availability of lidocaine was considerably increased. In this study, the rectal area for AZ was limited to 2 cm distance from the anus. However, when AZ solution was administered into the rectum of three rats under the condition that the area for absorption was not restricted (i.e. the upper septum plug

was not used), significance was not detected with respect to the plasma AZ concentration–time curve.

To estimate the fraction of the administered AZ dose which avoided the first-pass elimination of the liver, we used a circulatory transport model analysis (Araki and Miyazaki, 1974; Miyazaki and Araki, 1978; Weiss and Foster, 1979; Van Rossum et al., 1983). The following assumptions were made here: (1) eliminating organs are treated as black-boxes characterized by the input-output behavior under the assumption of linear condition; (2) the distribution of AZ to blood constituents are negligible (i.e. plasma AZ concentration is almost equal to whole blood AZ concentration); (3) AZ is eliminated from both the liver and other disposition organs but not eliminated from the heart–lung subsystem; and (4) in the case of oral or rectal administration, AZ is completely absorbed unchanged. After oral AZ administration, all of the absorbed amount of AZ enters the liver where AZ is eliminated by metabolism. However, in the case of rectal administration, some parts of the administered dose directly enters the mixed venous pool (f_{nh} = the fraction of dose directly enters the mixed venous pool) though the remains go to the liver (f_h = the fraction of dose goes to the liver).

Based on the above assumptions, we derived the equation which relates the AUCs obtained after three different routes of administration. Namely,

$$AUC_{rect} = f_h \cdot AUC_{oral} + f_{nh} \cdot AUC_{i.v.} \tag{1}$$

By introducing the relationship, $f_h = 1 - f_{nh}$ into Eqn. 1, we can obtain the following equation which is basically the same as given by De Boer et al. (1979).

$$f_{nh} = \frac{AUC_{rect} - AUC_{oral}}{AUC_{i.v.} - AUC_{oral}} \tag{2}$$

Putting the AUC values shown in Table 1 into Eqn. 2, the fraction (f_{nh}) of the administered dose which bypasses the liver following rectal administration of AZ can be determined, $f_{nh} = 51.5\%$. This evidence that AZ avoided the first-pass elimination after rectal administration is also supported by the MRT values shown in Table 1. The MRT after oral or rectal AZ dosing is related to the MRT after i.v. dosing as follows:

$$MRT_{oral} = MRT_{i.v.} + \bar{t}_h \tag{3}$$

$$MRT_{rect} = MRT_{i.v.} + \frac{F \cdot f_h}{F \cdot f_h + f_{nh}} \cdot \bar{t}_h \tag{4}$$

where $\bar{t}_h$ is the mean hepatic transit time (Chiou, 1983). In addition, $F = 1 - E$, where E is the hepatic extraction ratio. By representing the fractional term as f'_h, Eqn. 4 will be reduced to:

$$MRT_{rect} = MRT_{i.v.} + f'_h \cdot \bar{t}_h \tag{5}$$

As shown in Eqn. 3, the difference between the MRT_{oral} and $MRT_{i.v.}$ is the mean hepatic transit time ($\bar{t}_h$) for AZ. From Table 1, we can estimate the value of $\bar{t}_h$ as 23.2 min. On the other hand, the difference between the MRT_{rect} and $MRT_{i.v.}$ is the product of $\bar{t}_h$ and the fraction decreased by the direct entrance into the mixed venous pool, f'_h. Namely, $MRT_{rect} - MRT_{i.v.} = 19.7$ min. Then, we may estimate the values of f'_h as 0.849. This means that the apparent mean hepatic transit time of AZ was about 15% decreased by the rectal administration of AZ.

Based on these results, we are making AZ as a suppository and trying to administer AZ to renal transplant patients who are now receiving AZ only as a tablet, though systemic availability is a serious problem in the clinical immunosuppressive therapy.

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