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# Pharmacokinetic Assessment of the Sites of First-pass Metabolism of BMS-181101, an Antidepressant Agent, in Rats

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**Abstract**

The relative contribution of the gut and the liver to the first-pass metabolism of BMS-181101 (3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-5-fluoro-1*H*-indole dihydrochloride), a potential antidepressant agent, has been evaluated in rats.

Nine male Sprague–Dawley rats were divided into three groups of three and each rat received a single 20 mg $kg^{-1}$ dose of [$^{14}$C]BMS-181101 via a 30 min constant-rate intravenous infusion, a 30-min constant-rate intraportal infusion or oral gavage. Serial blood samples were collected for 8 h after dosing and plasma was analysed for unchanged BMS-181101 and total radioactivity. Extraction ratios for BMS-181101 by the gut and liver were calculated on the basis of ratios of the area under the plasma BMS-181101 concentration–time curve. The gut had a high intrinsic capacity for metabolizing BMS-181101–extraction ratios were 93% and 10% for the gut and liver, respectively. After oral administration BMS-181101 is sequentially exposed to the gut then the liver. As a result, the contribution of the gut to the overall first-pass effect (ca. 93%) was significantly greater than that of the liver (ca. 0·7%). The estimated total first-pass effect of 94% for BMS-181101 in rats is in excellent agreement with the observed absolute oral bioavailability of 6%.

These results clearly illustrate the importance of metabolic activity in the gut for orally administered BMS-181101.

BMS-181101, 3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-5-fluoro-1*H*-indole dihydrochloride, is a potent antidepressant agent presently under development. Its unique pharmacological profile is believed to be the result of multiple interactions with various central pre- and post-synaptic 5-HT receptors. BMS-181101 is essentially completely absorbed when administered orally to rats and monkeys (Vachharajani et al 1994). However, the absolute oral bioavailability is approximately only 9% in rats and 40% in monkeys, indicating that BMS-181101 undergoes extensive pre-systemic metabolism (Vachharajani et al 1994). Negligible amounts ($< 3\%$) of unchanged BMS-181101 were excreted in the urine of these two species suggesting that renal excretion is not a major pathway for elimination of unchanged BMS-181101.

In-vitro studies with genetically engineered cell lines from man which express specific cytochrome P450 (CYP) isozymes have revealed that BMS-181101 is metabolized by CYP3A4 and CYP2D6 (unpublished data, Bristol-Myers Squibb). Preliminary data also indicate that BMS-181101 undergoes extensive pre-systemic metabolism in man. Because CYP3A4 is present in the gut enterocyte of various species, including man (Watkins et al 1987; Kivistö et al 1996), it is likely that BMS-181101 is metabolized in the gut and its metabolites are subsequently absorbed resulting in essentially complete absorption of a radioactive dose after oral administration.

The objective of this study was to assess the relative contribution of the gut and liver as sites of first-pass metabolism of BMS-181101 after oral administration to rats. The experimental approach selected involved administration of BMS-181101 via multiple routes that are afferent to the potential sites of first-pass metabolism.

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## Materials and Methods

*Test drug and formulation*

The chemical structure of [$^{14}$C]BMS-181101 is shown in Figure 1; the radiochemical purity and

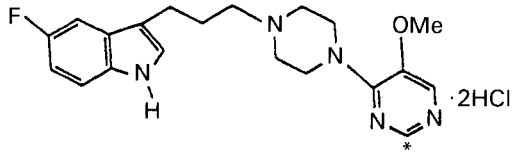


Figure 1. The chemical structure of BMS-181101. The position of the $^{14}C$ label is indicated with an asterisk.

specific activity of the material used in these experiments were 98·5% and 31·2 $\mu$Ci mg$^{-1}$, respectively. Appropriate amounts of radiolabelled BMS-1801101 and unlabelled BMS-181101 were dissolved in 5%-dextrose injection (Abbott Laboratories, Chicago, IL) to give a final concentration of 4 mg mL$^{-1}$. The dosing solution was filtered through a sterile 0·22-$\mu$m filter before administration. Each rat received approximately 50 $\mu$Ci radioactivity.

*Assessment of the extent of gut and liver metabolism*

Nine male Sprague–Dawley rats (Hilltop Laboratory Animals, Scottsdale, PA), ca. 375 g, were divided into three groups of three. Each rat received a single 20 mg kg$^{-1}$ ($\sim$50 $\mu$Ci) dose of [$^{14}$C]BMS-181101 as a 30-min constant-rate intravenous infusion (via the tail vein), or orally, or as a 30-min constant-rate intraportal infusion. All rats had their jugular and portal veins cannulated and were acclimatized for at least seven days before dosing. Serial blood samples (0·25 mL) were collected from the jugular vein 0·25, 0·5, 0·75, 1, 1·5, 2, 4, 6, and 8 h after dosing. A volume of normal saline equal to the volume of blood withdrawn was administered to rats after collection of each sample. The blood was transferred to a microvette containing potassium ethylenediaminetetraacetic acid as anticoagulant. Plasma was separated within 1 h of collection and stored at $-20$°C pending analysis for unchanged BMS-181101 and total radioactivity.

*Assay procedures*

Unchanged BMS-181101 was analysed by the method previously described by Shah et al (1996) with minor modifications. Detection was by UV at 254 nm and the lower limit of quantification was 10 ng mL$^{-1}$. Quality-control samples were prepared before initiation of the study and these samples were stored with the study samples and analysed to confirm the accuracy and precision of the method, and the stability of study samples during storage.

For determination of total radioactivity a sample of plasma (20 $\mu$L) was mixed with scintillation fluid (10 mL) and samples were counted for 30 min by means of a Packard Instruments (CT) LS3900 counter.

*Estimation of pharmacokinetic parameters*

Plasma concentration–time (C–t) data were analysed by non-compartmental pharmacokinetic methods (Riegelman & Collier 1980; Gibaldi & Perrier 1982). The highest observed concentration and the corresponding sampling time were defined as $C_{max}$ and $t_{max}$, respectively. The elimination half-life ($t_{1/2}$) was estimated from $t_{1/2} = \ln 2/\lambda$ where $\lambda$ is the the slope of the regression line that best fit the terminal portion of the log-linear concentration–time curve. The area under the concentration–time curve ($AUC_{0-\infty}$) was calculated by a combination of the trapezoidal and log-trapezoidal rules, and then extrapolated to infinity. The total plasma clearance (CL), the volume of distribution at steady state ($Vd_{SS}$) and the absolute bioavailability were estimated by standard methods.

Extraction ratios (E) of BMS-181101 by the gut ($E_{gut}$) and liver ($E_{liver}$) were calculated on the basis of the method described by Cassidy & Houston (1980). Because BMS-181101 is completely absorbed after oral administration (Vachharajani et al 1994), $E_{gut}$ and $E_{liver}$ were calculated by use of the equations:

$$E_{gut}\ (\%) = [1 - (AUC_{p.o.})/(AUC_{i.p.})] \times 100 \quad (1)$$

$$E_{liver}\ (\%) = [1 - (AUC_{i.p.})/(AUC_{i.v.})] \times 100 \quad (2)$$

where $AUC_{p.o.}$, $AUC_{i.p.}$ and $AUC_{i.v.}$ are the oral, intraportal and intravenous AUC values.

After oral administration, BMS-181101 is exposed first to the gut then to the liver before entering the systemic circulation. The extraction ratios for the gut and liver represent the relative contribution of the gut and liver as sequential first-pass sites (Morrison et al 1991). Because the gut is the first site of extraction, its contribution is simply the E value for the gut.

$$E_{gut\ first\text{-}pass}\ (\%) = E_{gut}(\%) \quad (3)$$

The contribution of the liver to the metabolism of BMS-181101 was calculated as:

$$E_{liver\ first\text{-}pass}\ (\%) = [100 - E_{gut\ first\text{-}pass}\ (\%)] \times E_{liver} \quad (4)$$

## Results

*Assay procedure*

Analytical quality-control samples were included in each run to assess the accuracy and precision of the assay procedure. The between- and within-day variability of plasma quality-control samples was $< 10\%$. The predicted concentrations deviated by

< 8% from the corresponding nominal concentrations. These results indicated that BMS-181101 was stable in rat plasma and the assay was accurate, precise and reproducible.

*Extent of gut and liver metabolism*

The mean plasma concentration–time profiles of unchanged BMS-181101 and total radioactivity after intravenous, intraportal and oral administration are shown in Figure 2. The estimated pharmacokinetic parameters are listed in Table 1. The mean $AUC_{0-\infty}$ of unchanged BMS-181101 after intravenous and intraportal administration were 12·2 $\mu g\ mL^{-1}$ and 10·9 $\mu g\ mL^{-1}$, respectively. However, $AUC_{0-\infty}$ of unchanged BMS-181101 after oral administration was approximately one-fifteenth the intravenous or intraportal values. The absolute bioavailabilities of BMS-181101 after intraportal and oral administration were approximately 90% and 6%, respectively. Both unchanged BMS-181101 and total radioactivity levels at the end of 30-min infusion were comparable for the intravenous and intraportal routes.

The extraction ratios are a reflection of the intrinsic capacity of the gut and liver to metabolize BMS-181101 (Table 2); the intrinsic capacity of the gut was approximately nine times greater than that of the liver. The relative contribution as first-pass effect sites for orally administered BMS-181101 was 93% in the gut and 0·7% in the liver.

## Discussion

First-pass metabolism is a phenomenon characterized by significant pre-systemic removal of a drug after oral administration. Drugs that undergo extensive first-pass metabolism often have low and variable bioavailability. The importance of metabolic activity in the gut has recently been recognized (Benet et al 1996). Metabolism in the gut could be beneficial for prodrugs which are bioactivated in the gut or for conjugated drugs which are re-absorbed after biliary excretion and, in turn, prolong the pharmacological activity (Ilett et al 1990). However, more than 50% of the drugs on the market are metabolized by the cytochrome P450 3A subfamily (Wacher et al 1995), which is found in abundance in the gut (Watkins et al 1987; Kivistö et al 1996). Thus, metabolic deactivation or reduction of the

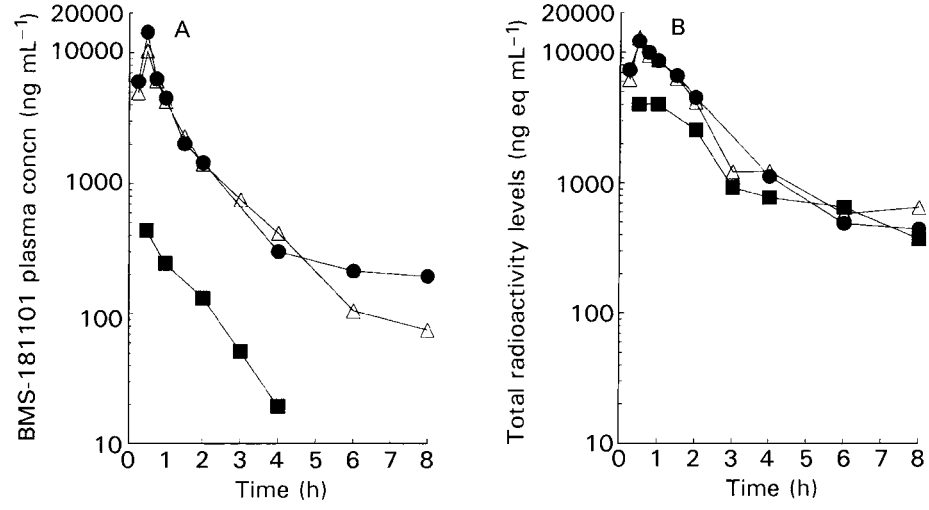


Figure 2. Mean plasma concentration–time profiles of BMS-181101 (A) and total radioactivity (B) after a single 20 mg $kg^{-1}$ dose of radiolabelled BMS-181101 administered intravenously (●), intraportally (△) and orally (■) to rats (n = 3 for each route).

Table 1. Mean (± s.d.) pharmacokinetic parameters of BMS-181101 in rats after single 20 mg $kg^{-1}$ intravenous, intraportal or oral doses.

| Parameter | Intravenous infusion | Intraportal infusion | Oral |
|---|---|---|---|
| Maximum concentration (ng $mL^{-1}$) | 14371 (4947) | 10355 (3258) | 536 (31·0) |
| Time of maximum concentration (h) | 0·50 | 0·50 | 0·50 (0·25) |
| Area under the plasma concentration–time curve (ng h $mL^{-1}$) | 12227 (2883) | 10961 (1956) | 732 (216) |
| Total plasma clearance (mL $min^{-1}$) | 28·5 (7·3) | 31·1 (6·12) | – |
| Volume of distribution at steady state (L) | 1·80 (0·25) | 2·38 (0·80) | – |
| Elimination half-life (h) | 1·19 (0·76) | 1·63 (0·49) | 1·02 (0·31) |
| Absolute bioavailability (%) | – | 89·6 | 6·0 |

n = 3 per group.

Table 2. Relative contributions of the gut and liver as sites of first-pass metabolism for BMS-181101 in rats after oral administration.

| Parameter | Estimate |
|---|---|
| Extraction ratio for gut (%) | 93·3 |
| Extraction ratio for liver (%) | 10·4 |
| First-pass effect due to gut (%) | 93·3 |
| First-pass effect due to liver (%) | 0·69 |
| Total first-pass effect (%) | 94·0 |

absolute bioavailability pose a greater challenge for orally administered drugs which are metabolized by CYP3A4.

BMS-181101 is a substrate for CYP3A4, and undergoes extensive first-pass metabolism resulting in low oral bioavailability despite complete absorption. Similar findings of low oral bioavailability as a result of gut metabolism have also been reported for cyclosporin (Kolars et al 1991). Various experimental techniques using multiple routes of administration to evaluate the extent of metabolism by different organs have been reported in the literature (Conway et al 1973; Iwamoto & Klaassen 1977; Cassidy & Houston 1980; Brewster et al 1981; Chanoine et al 1991). However, most used either anaesthetized rats with intraportal administration or conscious rats with intraperitoneal administration to assess hepatic metabolism of a xenobiotic. The experimental approach described herein involved administration of BMS-181101 by multiple routes (intravenous, intraportal and oral) to conscious rats as reported by Zhong et al (1994) to determine the relative contribution of the gut and the liver as sites of first-pass metabolism.

The results obtained in this study clearly indicated that the gut was the main site of metabolism of BMS-181101 in rats. Approximately 93% of the orally administered dose was metabolized by the gut and approximately 10% of the intraportally administered dose appeared to be metabolized in the liver. The relative contribution as first-pass-effect sites for orally administered BMS-181101 were 93% for the gut and only 0·7% for the liver. The total first-pass effect ($E_{gut+liver\ first\text{-}pass}$) of 94% and the observed absolute bioavailability of approximately 6% were in excellent agreement. Thus these results clearly illustrate the importance of metabolic activity in the gut for orally administered BMS-181101 and for CYP3A4 substrates in general.

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