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European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 143–146
European Journal of Pharmaceutics and Biopharmaceutics
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Note

Impact of Solutol HS 15 on the pharmacokinetic behavior of midazolam upon intravenous administration to male Wistar rats

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Received 29 July 2002; accepted in revised form 4 March 2003

Abstract

The pharmacokinetic profile of midazolam (MDZ) and its major metabolites 1′-OH-midazolam (1′OH-MDZ) and 4-OH-midazolam (4OH-MDZ) was investigated in rats. MDZ was administered intravenously at 5 mg/kg either in the absence (NaCl 0.9%, control group) or in the presence of the surfactant Solutol HS 15, a weak inhibitor of cytochrome P450 3A (CYP3A) activity in vitro (Solutol HS 15-treated group).

It was found that the pharmacokinetic profiles of MDZ, 1′OH-MDZ and 4OH MDZ did not differ significantly in the two dosing vehicles (P values above 0.2). MDZ exhibited a high plasma clearance (Cl) of 79 and 92 ml/min/kg (corresponding to a blood Cl of 64 and 75 ml/min/kg), a high volume of distribution (V_d) of 4.0 and 3.6 l/kg, and an area under the plasma concentration–time curve ($AUC_{t0-tinf}$) of 1062 and 932 h.ng/ml in the control group and in the Solutol HS 15-treated group, respectively. The amount of MDZ excreted unchanged into urine was below 0.01% with both dosing vehicles. $AUC_{t0-tinf}$ in the control group was 12.3 h.ng/ml for 1′OH-MDZ and 38.8 h.ng/ml 4OH-MDZ. In the Solutol HS 15-treated group, $AUC_{t0-tinf}$ was 14 h.ng/ml for 1′OH-MDZ and 35.4 h.ng/ml for 4OH-MDZ. The metabolite concentrations excreted into urine were below the limit of quantification.

In the rat, MDZ has a high blood clearance that is limited by liver blood flow. Therefore, weak CYP3A inhibitors like Solutol HS 15 are not likely to affect the hepatic blood clearance of MDZ in vivo.

Keywords: Midazolam; Solutol HS 15; Rat; Pharmacokinetics; Cytochrome; P450 3A

1. Introduction

Solutol HS 15, the main component of which is the polyethylene glycol 660 ester of 12-hydroxy stearic acid [1], is recommended as non-ionic solubilizing agent to be added to injection solutions [2]. In recent experiments in our laboratory, the *p*-glycoprotein (*p*-gp) and cytochrome P450 3A (CYP3A) substrate colchicine [3–5] was administered intravenously to male Wistar rats both in the presence and in the absence of Solutol HS 15. Co-administration of Solutol HS 15 resulted in a 2-fold decrease in the plasma clearance and in a more than 2-fold increase in the maximum plasma concentration of colchicine as compared to a solution of colchicine in NaCl 0.9%. In vitro, in rat hepatocytes, the intrinsic clearance (Cl_{int}) of colchicine (1.5 μl/min/10^6 cells) was significantly reduced already at 0.003% Solutol HS 15 present in the incubation medium [6]. At this concentration, Solutol HS 15 had no toxic effect on the cell integrity as shown light microscopically, by measuring the lactate dehydrogenase release into the culture medium, or the adenosine triphosphate content of the cells [7].

Similar to colchicine, Cl_{int} of midazolam (MDZ) in rat hepatocytes was decreased in the presence of Solutol HS 15 [7]. MZD is a 1, 4-benzodiazepine that is widely used as short-acting hypnotic agent in newborn infants requiring prolonged mechanical ventilation or need to undergo invasive procedures [8]. MDZ is poorly water-soluble (120 μg/ml)

Abbreviations: 1′OH-MDz, 1′-hydroxy-midazolam; 4OH-MDZ, 4-hydroxy-midazolam; $AUC_{t0-tinf}$, area under the plasma concentration–time curve; $AUMC_{t0-tinf}$, area under the moment curve from time 0 to time infinity; Cl, clearance; c_{max}, maximum plasma concentration; CYP3A, cytochrome P450 3A; MDZ, midazolam; MRT, mean residence time; NaCl, 0.9%, isotonic sodium chloride solution; $t_{1/2\_2nd\_phase}$, half-life for the first elimination phase; $t_{1/2\_2nd\_phase}$, half-life for the second elimination phase; V_d, volume of distributions.

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0939-6411/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0939-6411(03)00041-9

and highly lipophilic (log $P = 4.26$). The benzodiazepine is mainly metabolized by hepatic and gastrointestinal CYP3A isoenzymes [9]. In contrast to colchicine, MDZ has a high clearance in rat hepatocytes (23 μl/min/10^6 cells). The current investigation was undertaken to find out whether the in vitro effect of Solutol HS 15 on Cl_{int} of MDZ in rat hepatocytes could be translated into the in vivo situation. Therefore, the impact of Solutol HS 15 on the pharmacokinetic behavior of MDZ was studied in male Wistar rats.

2. Materials and methods

2.1. Chemicals

MDZ was purchased from Fluka (Buchs, Switzerland) and Solutol HS 15 was obtained from BASF (Ludwigshafen, Germany). All other materials used were of analytical grade or will be described separately in the this section.

2.2. In vivo experiment

Male Wistar rats (BRL, Füllinsdorf, Switzerland) weighing circa 250 g were used for in vivo studies. The animals were housed in standard cages and maintained under a 12 h light/dark cycle with access to standard laboratory chow and water ad libitum. For kinetic studies, all rats had an indwelling cannula (silicone rubber/polyethylene) implanted in the right jugular vein for blood sampling. Surgery was performed under 10 mg/kg xylazin (Ketasol-100, Graeub, Bern, Switzerland) and 90 mg/kg ketamin (Rampun®, Bayer, Lyssach, Switzerland) anesthesia two days before the experiment. During the study, all animals were housed individually in plastic metabolism cages and were unrestrained throughout the experimental time period. All rat experiments were performed in accordance to current legislation on the welfare of experimental animals. Rats received a single dose of MDZ at 5 mg/kg, formulated either as solution in isotonic sodium chloride solution ($n = 3$, control group) or as solution in isotonic sodium chloride solution in the presence of 5% (w/v) of Solutol HS 15 ($n = 3$, Solutol HS 15 treated group). The injection volume was 2 ml/kg and the concentration of MDZ in the formulations was 2.5 mg/ml. All dosing vehicles were well tolerated by the animals. The rats did not show any unusual behavior after the treatment. Blood samples (0.3 ml) were collected from the jugular vein at defined intervals. Collection tubes contained ethylene-diamin-tetra-acetic acid (EDTA, Fluka, Buchs, Switzerland) and sodium fluoride (Fluka, Buchs, Switzerland) as anticoagulant and stabilizer, respectively. Plasma samples were obtained by immediate centrifugation of blood samples. Urine samples were collected from both groups during 0–8 h. All samples were kept frozen at −20°C until assayed by high performance liquid chromatography (HPLC) combined with tandem mass spectrometry (LC-MS/MS) as described previously [10].

2.3. Data analysis

The area under the plasma concentration–time profile (AUC) was calculated using a log-linear trapezoidal method (WinNonlin 1.5, Scientific Consulting Inc., USA). Systemic plasma clearance (Cl) was calculated as dose/$AUC_{t0-tinf}$. Blood clearance was calculated as plasma Cl divided by the blood/plasma concentration ratio. The volume of distribution (V_d) was calculated as mean residence time (MRT) multiplied with Cl. MRT was derived from the equation $AUMC_{t0-tinf}/AUC_{t0-tinf}$. P values were calculated with a two-sample t-test assuming equal variances. P values of below 0.05 were considered as being statistically significant.

3. Results and discussion

The mean plasma concentration–time curves for MDZ and its major metabolites 1′OH- and 4OH-MDZ upon oral administration of MDZ to male Wistar rats are depicted in Fig. 1. In rat liver, there is a preferential formation of 4OH-MDZ over 1′OH-MDZ [11]. The pharmacokinetic parameters of MDZ in the two different dosing vehicles did not differ significantly as shown in Table 1. MDZ exhibited a high plasma clearance (control group: 78.7 ± 3.3 ml/min/kg; Solutol HS 15-treated group: 91.8 ± 10.1 ml/min/kg, $P = 0.29$) and a high volume of distribution (V_d) (control group: 4.0 ± 0.3 l/kg, Solutol HS 15-treated group: 3.6 ± 0.4 l/kg, $P = 0.42$). When considering the blood/plasma concentration ratio of 1.23 [12], blood Cl was 64.0 ± 2.6 and 74.6 ± 8.2 ml/min/kg in the control and in the Solutol HS 15-treated groups ($P = 0.29$). The area under the plasma concentration–time curve ($AUC_{t0-tinf}$) was similar in both groups with 1062.1 ± 44.7 h.ng/ml for the control group and 931.9 ± 110.1 h.ng/ml for the Solutol HS 15-treated group ($P = 0.33$). Further, the average maximum

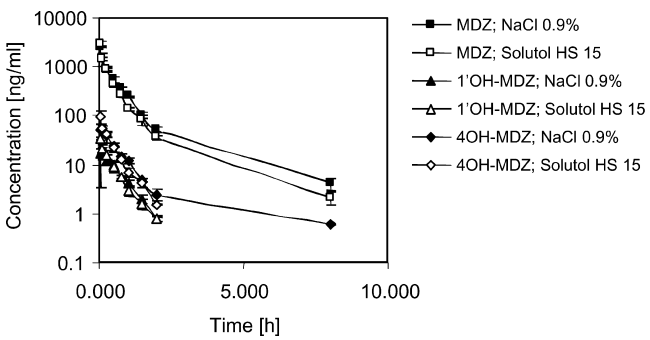

Fig. 1. Plasma concentration–time profile of MDZ, 1′OH-MDZ and 4OH-MDZ upon oral administration of MDZ to male Wistar rats at 5 mg/kg. Formulations: solution in isotonic sodium chloride (NaCl 0.9%); solution in isotonic sodium chloride: Solutol HS 15, 95:5 (Solutol HS 15); $n = 3$; mean ± SEM.

Table 1
Pharmacokinetic parameters of MDZ, 1′OH-MDZ and 4OH-MDZ upon oral administration of MDZ to male Wistar rats at 5 mg/kg

Formulation	Compound	$AUC_{t0-tinf}$ (h.ng/ml)	c_{max} (ng/ml)	$t_{1/2\_1st\_phase}$ (h)	V_d (l/kg)	Cl (ml/min/kg)	Excretion into urine (0–8 h) (% of dose)
1	MDZ	1062.1 ± 44.7	3850.5 ± 456.5	0.27 ± 0.02	4.0 ± 0.3	78.7 ± 3.3	0.003 ± 0.001
2	MDZ	931.9 ± 110.1	4713.9 ± 494.8	0.24 ± 0.01	3.6 ± 0.4	91.8 ± 10.1	0.006 ± 0.003
1	1′OH-MDZ	12.3 ± 3.0	21.1 ± 7.5	NA	NA	NA	BLQ
2	1′OH-MDZ	14.0 ± 2.8	51.3 ± 23.3	NA	NA	NA	BLQ
1	4OH-MDZ	38.8 ± 8.7	63.6 ± 17.7	NA	NA	NA	BLQ
2	4OH-MDZ	35.4 ± 3.5	134.5 ± 49.8	NA	NA	NA	BLQ

Formulations: solution in isotonic sodium chloride (formulation 1); solution in isotonic sodium chloride: Solutol HS 15, 95:5 (formulation 2); $n = 3$; c_{max}, maximum plasma concentration; $t_{1/2}$, half-life; V_d, volume of distribution; Cl, plasma clearance; NA, not available; BLQ, below limit of quantification; mean ± SEM; significance: $^*P \leq 0.05$.

plasma level (c_{max}) was 3850.5 ± 456.5 ng/ml in the control group and 4713.9 ± 494.8 ng/ml in the Solutol HS 15-treated group ($P = 0.27$). The half-live for the first phase ($t_{1/2\_1st\_phase}$) was 0.27 ± 0.02 h for the control group and 0.24 ± 0.01 h for the Solutol HS 15-treated group ($P = 0.27$). The half-life for the second phase ($t_{1/2\_2nd\_phase}$) was not calculated with the data generated in the current investigation, as the terminal phase consists of one data point only. Thus, the accuracy of the estimate would be rather limited. The amount of MDZ excreted unchanged into urine was below 0.01% with both dosing vehicles.

1′OH-MDZ and 4OH-MDZ were detectable in the plasma samples already at the first sampling time point, 2 min after intravenous dosing of MDZ. Comparable to parent MDZ, $AUC_{t0-tinf}$ and c_{max} of the metabolites did not differ significantly in the two dosing vehicles (1′OH-MDZ, control group: $AUC_{t0-tinf} = 12.3 \pm 3.0$ h.ng/ml, $c_{max} = 21.1 \pm 7.5$ ng/ml; 1′OH-MDZ, Solutol HS 15-treated group: $AUC_{t0-tinf} = 14.0 \pm 2.8$ h.ng/ml ($P = 0.70$), $c_{max} = 51.3 \pm 23.3$ ng/ml ($P = 0.29$); 4OH-MDZ, control group: $AUC_{t0-tinf} = 38.8 \pm 8.7$ h.ng/ml, $c_{max} = 63.6 \pm 17.7$ ng/ml; 4OH-MDZ, Solutol HS 15-treated group: $AUC_{t0-tinf} = 35.4 \pm 3.5$ h.ng/ml ($P = 0.74$), $c_{max} = 134.5 \pm 49.8$ ng/ml ($P = 0.25$)). The amount of metabolites excreted into urine was below the limit of quantification of the LC-MS/MS method.

In previous experiments using microsomes prepared from human lymphoblastoid cells co-expressing recombinant human NADPH-cytochrome P450 reductase and human cytochrome P450 3A4, Solutol HS 15 was found to be a weak inhibitor of CYP3A activity (IC_{50} of 5 μM). Moreover, Solutol HS 15 was found to significantly reduce Cl_{int} of the CYP3A substrates colchicine and MDZ in rat hepatocytes already at concentrations below its critical micellar concentration of 0.021% [7,13]. In vivo, intravenous co-administration of Solutol HS 15 and colchicine to rats resulted in a significant decrease in colchicine Cl as well as in an increase in c_{max} [6].

With 1.5 μl/min/10^6 cells, the intrinsic clearance of colchicine in rat hepatocytes is low. The capacity of the liver to remove the compound is rather limited. Thus, weak CYP3A inhibitors like Solutol HS 15 can significantly affect the clearance of colchicine in vivo. In contrast to colchicine, with 23 μl/min/10^6 cells, MDZ exhibits a high intrinsic clearance in rat hepatocytes. The enzymes are so active that the liver removes nearly all the compound presented to it. The hepatic clearance of MDZ is, therefore, determined by the rate of compound supply to the liver. Changing the capacity of the enzymes by the weak CYP3A inhibitor Solutol HS 15 is not likely to affect the overall clearance of MDZ in the rat. This suggestion is supported by our findings, as with more than 64 ml/min/kg, blood clearance slightly exceeds liver blood flow of 60 ml/min/kg [14].

It can be concluded that co-administration of Solutol HS 15 did not alter the overall pharmacokinetics of MDZ and its major metabolites 1′OH-MDZ and 4OH-MDZ in the rat. The most likely reasons for this lack of effect are the high hepatic extraction ratio of MDZ and the comparatively weak inhibitory potential of Solutol HS 15 on CYP3A. In man, however, the situation could be different, since MDZ clearance is more than 10-fold lower as compared to the rat [14].

Even though this study has been performed with a limited number of animals, from the data generated it can be seen that a dramatic impact of the excipient Solutol HS 15 on the pharmacokinetic fate of MDZ would be unlikely to occur. To confirm our data larger studies will have to be performed.

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