International Journal of Pharmaceutics, 78 (1992) 199–202

IJP 02622

Improved oral bioavailability of salicylamide in rabbits by a 1,3-benzoxazine-2,4-dione prodrug

Anne H. Kahns, Judi Møss and Hans Bundgaard

The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, Copenhagen (Denmark)

(Received 12 August 1991)
(Accepted 29 August 1991)

Key words: Salicylamide; 1,3-Benzoxazine-2,4-dione; First-pass metabolism; Absorption; Prodrug

Summary

The oral bioavailability of 1,3-benzoxazine-2,4-dione was investigated in rabbits and compared with that of salicylamide. The benzoxazinedione was shown to be hydrolyzed to salicylamide both in vitro and in vivo. The oral bioavailability of salicylamide was increased 2.5-fold following administration as 1,3-benzoxazinedione compared to oral dosing of an equimolar amount of salicylamide per se. This increased bioavailability of salicylamide was ascribed to a diminished first-pass metabolism achieved by protecting the vulnerable phenolic group in the form of a benzoxazinedione ring structure.

Introduction

Salicylamide (**I**) has both analgetic, antipyretic and hypnotic activities but its clinical value is limited due to extensive presystemic biotransformation after oral as well as rectal administration (Levy and Matsuzawa, 1967; Gugler et al., 1975; De Boer et al., 1983; Xu et al., 1989). This first-pass metabolism primarily involves conjugation of the phenolic group with glucuronic and sulfuric acid (Morris and Levy, 1983; Xu et al., 1989) and therefore, protection of this group in the form of prodrugs may be a potentially useful means to minimize the metabolic inactivation and hence increase the oral or rectal bioavailability.

Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

Several years ago a salicylamide derivative named Carsalam was described as having a much higher antipyretic activity than salicylamide after peroral administration to rabbits (Baker et al., 1963). Carsalam is 2H-1,3-benzoxazine-2,4(3H)-dione (**II**), a compound in which the phenolic and amide groups of salicylamide are linked together in the form of a carbamate bond. It has recently been shown to be a major metabolite of ethyl 2-carbamoyloxybenzoate, a new anti-inflammatory and analgetic drug (Kamal, 1990). We have recently studied the hydrolytic degradation of this benzoxazinedione (Kahns and Bundgaard, 1991). At pH 7.4 and 37 °C the compound is quantitatively hydrolyzed to salicylamide and carbon dioxide (Scheme 1) with a half-life of 16.9 h. A slight enzymatic catalysis of this hydrolysis was observed with human plasma and rat liver homogenate (Kahns and Bundgaard, 1991). These results suggested that compound **II** at least partly

may function as a prodrug of salicylamide and that its greater antipyretic activity relative to salicylamide may be due to a reduced first-pass metabolism. We have now examined the oral absorption of compound **II** in rabbits and compared it with that of salicylamide. The extent of first-pass metabolism of salicylamide in rabbits following peroral administration has previously been reported to be about 95% (Shibasaki et al., 1981) so that the rabbit should be a useful animal model.

Materials and Methods

Chemicals

Salicylamide was obtained from E. Merck, Darmstadt. 2H-1,3-Benzoxazine-2,4(3H)-dione (**II**) and its sodium salt were prepared as previously described (Hoback et al., 1955; Crum and Francks, 1965).

HPLC assay

2H-1,3-Benzoxazine-2,4(3H)-dione (**II**) and salicylamide (**I**) were determined by reversed-phase HPLC procedures with a Shimadzu system consisting of an LC-6A pump, an SPD-6A variable-wavelength UV detector and a 20 μl loop injection valve (Rheodyne 7125). A Chrompack column (100 × 3 mm) packed with CP Spher C-8 (5 μm particles) was eluted at ambient temperature with a mobile phase of 0.02 M acetate buffer of pH 4.0 containing triethylamine at a concentration of 10^{-3} M to improve peak shape. The column effluent was monitored at 240 nm and the flow rate was 1.5 ml min^{-1}. Quantitation of the compounds was performed by measuring the peak heights in relation to those of standards chromatographed under the same conditions. Salicylamide showed a retention time of 3.6 min whereas compound **II** eluted after 4.8 min.

Bioavailability studies in rabbits

Three male albino rabbits weighing 2.0–2.5 kg were fasted overnight prior to oral drug administration. The rabbits received 100 mg of salicylamide or an equimolar amount of compound **II** (in the form of sodium salts). Furthermore, the compounds were given by intravenous injection in the marginal ear vein. The sodium salt of compound **II** was dissolved in 5 ml of 0.1 M sodium hydrogen carbonate of pH 8.5 whereas 1.0 ml of an alkaline salicylamide sodium solution was diluted to 5 ml with the carbonate solution.

After administration of the compounds, blood samples were taken from the ear vein at appropriate intervals in heparinized test tubes. The plasma was separated by centrifugation at 3000 rpm for 10 min and samples of 125 μl were withdrawn and deproteinized by mixing with 250 μl of a 2% solution of zinc sulphate in methanol-water (1:1 v/v). After centrifugation at 13000 rpm for 3 min, 20 μl of the clear supernatant was analyzed by the HPLC procedure described above.

An interval of at least 7 days was allowed prior to the next experiment in the same rabbit.

The area under the plasma concentration vs time curves (AUC) was calculated by the trapezoidal rule (0–420 min after administration).

Stability in rabbit tissues

The hydrolysis of compound **II** was studied at 37 °C in rabbit plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.4 as well as in 20% rabbit gut and 10% rabbit liver homogenate. The gut and liver homogenates were prepared as previously described (Buur and Bundgaard, 1985;

II I

Scheme 1.

Møss et al., 1990). The initial concentration of the compound was 10^{-4} M. The mixtures were kept in a water bath at 37 °C and at appropriate intervals samples were withdrawn, deproteinized and analyzed as described above. Pseudo-first-order rate constants for the hydrolysis were obtained from the slopes of linear plots of the logarithm of remaining compound **II** against time.

Results and Discussion

The absorption characteristics of salicylamide and the benzoxazinedione (**II**) following peroral administration were assessed in three rabbits each receiving an equimolar dose of the compounds corresponding to 100 mg salicylamide. The compounds were given in the form of aqueous solutions of their sodium salts. The pK_a of the acidic NH group in compound **II** is 7.3 and the compound is readily soluble at pH > 8 (Kahns and Bundgaard, 1991).

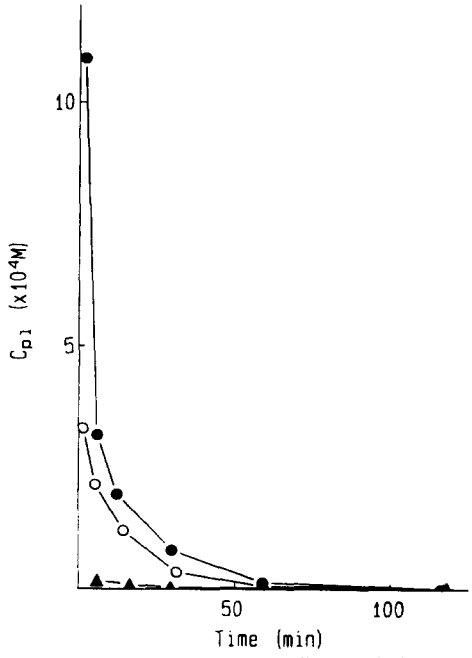

Fig. 1. Plasma concentrations of salicylamide in three rabbits following peroral administration of salicylamide sodium in amounts corresponding to 100 mg salicylamide.

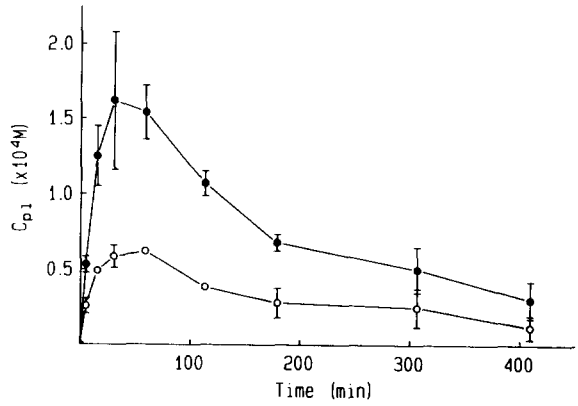

Fig. 2. Mean plasma concentrations of 2H-1,3-benzoxazine-2,4(3H)-dione (**II**) (●) and salicylamide (○) in three rabbits following peroral administration of compound **II** (sodium salt) in an amount corresponding to 100 mg salicylamide. Error bars are ±S.D. Where not shown, the symbol is larger than the error bar.

The results of the oral absorption studies are shown in Figs 1 and 2 and Table 1. As seen from Fig. 1, salicylamide sodium is very rapidly absorbed, the peak plasma concentration occurring before 2 min. The compound is also rapidly eliminated from plasma. The bioavailability of salicylamide was highly variable within the three rabbits as seen from Fig. 1 and the AUC values listed in Table 1. Attempts to determine the absolute bioavailability of orally given salicylamide in rabbits by using an i.v. dosing as reference were not successful because the elimination of salicylamide from plasma occurred so fast following i.v. injec-

TABLE 1

*Area under the plasma concentration versus time curves (AUC) of salicylamide (**I**) and 2H-1,3-benzoxazine-2,4(3H)-dione (**II**) following peroral administration of a single 100 mg dose of salicylamide or the molar equivalent amount of compound **II** to rabbits*

Rabbit no.	AUC (μmol min ml^{-1})		
	I after administration of compound **I**	**I** after administration of compound **II**	**II**
1	0.8	10.2	26.2
2	9.7	15.6	33.1
3	4.9	11.5	31.9
Mean ± SD	5.1 ± 4.5	12.4 ± 2.8	30.5 ± 3.8

tion that no reliable plasma concentration values could be obtained in the early phase. This finding contrasted with that reported by Shibasaki et al. (1981) in a similar study of salicylamide in rabbits.

The data in Fig. 2 show that peroral administration of compound **II** results in much prolonged plasma levels of both salicylamide and parent compound **II**. Since salicylamide is rapidly eliminated from plasma when given as such both orally and intravenously, the prolonged plasma levels of the compound observed after administration of compound **II** must be due to a relatively slow conversion of the latter in vivo to salicylamide. The AUC values of salicylamide following oral dosing of compound **II** vary only slightly within the three rabbits and are 2.5-fold higher than those observed after oral dosing of salicylamide itself (Table 1). Hydrolysis experiments showed that whereas compound **II** is rather stable in rabbit gut homogenate and thus should be absorbed in intact form, its hydrolysis to salicylamide is catalyzed by enzymes present in plasma and, in particular, in the liver (Table 2). This catalysis is stronger than that seen with human plasma and rat liver homogenate (Kahns and Bundgaard, 1991).

In conclusion, this study shows that 2H-1,3-benzoxazine-2,4(3H)-dione (**II**) behaves as a prodrug of salicylamide and that the pronounced first-pass metabolism of salicylamide can be depressed by a factor of about 2.5 with this prodrug as assessed in rabbits. The benzoxazinedione is, however, only partly converted to salicylamide and therefore, its marked antipyretic activity previously observed following oral administration to rabbits (Baker et al., 1963) may be due to the compound itself in addition to salicylamide formed by hydrolysis in vivo.

TABLE 2

Half-lives of hydrolysis of 2H-1,3-benzoxazine-2,4(3H)-dione (II) in various media (pH 7.4) at 37 °C

Medium	Half-life (h)
Buffer pH 7.4	16.9
20% rabbit gut homogenate	19.6
80% rabbit plasma	4.6
20% rabbit liver homogenate	0.4

References

Baker, J.A., Hayden, J., Marshall, P.G., Palmer, C.H.R. and Whittet, T.D., Some antipyretics related to aspirin and phenacetin. *J. Pharm. Pharmacol.*, 15 (1963) 97T–100T.

Buur, A. and Bundgaard, H., Prodrugs of 5-fluorouracil. III. Hydrolysis kinetics in aqueous solution and biological media, lipophilicity and solubility of various 1-carbamoyl derivatives of 5-fluorouracil. *Int. J. Pharm.*, 23 (1985) 209–222.

Crum, J.D. and Franks, J.A., Jr., The chemistry of heterocycles. III. 2H-1,3-benzoxazine-2,4(3H)-dione and some 3-substituted derivatives. *J. Heterocycl. Chem.*, 2 (1965) 37–40.

De Boer, A.G., Breimer, D.D., Gubbens-Stibbe, J.M. and Bosma, A., First-pass elimination of salicylamide in man following oral and rectal administration. *Biopharm. Drug Disp.*, 4 (1983) 321–330.

Hoback, J.H., Crum, J.D. and Carrol, D.W., Some *N*-substituted 2,4-dioxo-dihydro-5,6-benzo-1,3-oxazines. *Proc. West. Virg. Acad. Sci.*, 27 (1955) 40–43.

Gugler, R., Lain, P. and Azarnoff, D.L., Effect of portacaval shunt on the disposition of drugs with and without first-pass effect. *J. Pharmacol. Exp. Ther.*, 195 (1975) 416–423.

Kahns, A.H. and Bundgaard, H., Hydrolysis of 1,3-benzoxazine-2,4-dione (a potential salicylamide prodrug) and various *N*-substituted derivatives. *Acta Pharm. Nord.*, 3 (1991) 45–50.

Kamal, A., Metabolism of ethyl 2-carbamoyloxybenzoate (4003/2), a prodrug of salicylic acid, carsalam and salicylamide. *Biochem. Pharmacol.*, 40 (1990) 1669–1671.

Levy, G. and Matsuzawa, T., Pharmacokinetics of salicylamide elimination in man. *J. Pharmacol. Exp. Ther.*, 156 (1967) 285–293.

Morris, M.E. and Levy, G., Determination of salicylamide and five metabolites in biological fluids by high-performance liquid chromatography. *J. Pharm. Sci.*, 72 (1983) 612–617.

Møss, J., Buur, A. and Bundgaard, H., Prodrugs of peptides. 8. In vitro study of intestinal metabolism and penetration of thyrotropin-releasing hormone (TRH) and its prodrugs. *Int. J. Pharm.*, 66 (1990) 183–191.

Shibasaki, J., Konishi, R., Koike, M., Imamura, A. and Sueyasu, M., Some quantitative evaluation of first-pass metabolism of salicylamide in rabbit and rat. *J. Pharm. Dyn.*, 4 (1981) 91–100.

Xu, X., Hirayama, H. and Pang, K.S., First-pass metabolism of salicylamide. Studies in the once-through vascularly perfused rat intestine-liver preparation. *Drug Metab. Disp.*, 17 (1989) 556–563.