The effect of urethane and pentobarbital anaesthesia and hepatic portal vein catheterization on liver blood flow in the rat[1]

C.R. Hiley, M.S. Yates and D.J. Back

Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX (England), 24 January 1978

Summary. The effect of urethane and pentobarbital anaesthesia and hepatic portal vein catheterization on liver blood flow was investigated in the rat. Liver blood flow with pentobarbital anaesthesia was 40% greater than with urethane. Hepatic portal vein catheterization had no effect under pentobarbital anaesthesia whereas it produced an 18% fall in liver blood flow with urethane.

In recent years there has been an increased awareness of the role of liver blood flow in modifying the pharmacokinetics of drug disposition[2,3]. Since a number of pharmacokinetic studies involve anaesthesia with the use of different general anaesthetics[4-6] and sometimes employing hepatic portal vein catheterization[6,7], we have compared the effects of these procedures on liver blood flow. Portal vein catheterization is a technique used in pharmacokinetic studies for an estimate of the hepatic 'first pass effect'[6]. In this paper we present the results of experiments with urethane and pentobarbital anaesthesia in which liver blood flow has been measured using radioactive microspheres.

Method. Male Wistar rats (250-350 g) were anaesthetized with pentobarbital sodium (50 mg kg^{-1}) or urethane (14% w/v in 0.9% saline; 10.0 ml kg^{-1}) both given i.p.

The right carotid artery was cannulated and with the aid of pressure monitoring, the tip of the cannula was manipulated into the left ventricle. 60,000-80,000 carbonized microspheres (15±5 µm diameter; 3M Company, St. Paul, Minn.) labelled with ^{85}Sr were injected into the left ventricle over 20 sec in a total volume of 0.6 ml 0.9% saline/0.02% Tween 80. Simultaneously, blood was withdrawn from a femoral artery at 0.6 ml min^{-1} for 90 sec with a syringe withdrawal pump (Perfusor IV, Braun, Melsungen). Arterial blood pressure was recorded from the other femoral artery by means of a pressure transducer (Bell and Howell type 4 - 327 - 221) and a pen recorder (Grass model 79).

In some experiments a broken 23 gauge needle attached to a cannula was inserted into the portal vein before the injection of microspheres.

Cardiac output and liver blood flow were determined by the method of McDevitt and Nies[8]. In this method, hepatic arterial flow is determined from the microspheres trapped in the liver, and portal venous return is obtained indirectly by adding together the flows to the spleen, pancreas and gastrointestinal tract. Throughout this paper, liver blood flow refers to the sum of hepatic arterial and portal venous flows.

Statistical comparisons were performed by means of a non-paired Student's t-test.

Results. The results of experiments with urethane and pentobarbital anaesthesia and the effect of portal vein catheterization on cardiac output and liver blood flow are shown in the table.

Although there was no difference in cardiac output between rats under urethane and pentobarbital anaesthesia, liver blood flow (ml · min^{-1} 100 g b.wt^{-1}) was 47% greater with pentobarbital. This difference in liver blood flow was highly significant ($p<0.001$). Since there was no difference in liver weight between the 2 groups of animals, liver perfusion (ml · min^{-1} g $liver^{-1}$) was 40% greater with pentobarbital compared to urethane anaesthesia ($p<0.01$).

Portal vein catheterization produced no haemodynamic changes in rats under pentobarbital anaesthesia. In contrast this procedure evoked a 13% reduction in cardiac output and a reduction of approximately 18% in both indices of liver blood flow in animals under urethane anaesthesia.

Discussion. It has been previously reported that when compared to other anaesthetics urethane produces a reduction in the distribution of cardiac output to the gastrointestinal tract[9]. This is confirmed by the present findings of a lower liver blood flow in rats anaesthetized with urethane rather than pentobarbital. This is not unexpected since there has been a report of hypersecretion of adrenalin during urethane anaesthesia[10]. An important consequence of the lower liver blood flow in rats under urethane anaesthesia may be differences in the 'first pass effect' after oral administration of drugs which are highly cleared by the liver[3].

The fundamental question arises, however, as to which anaesthetic produces a liver blood flow which corresponds to that in conscious animals. An analysis of cardiac output distribution in conscious rats revealed that the distribution to the hepato-splanchnic tissues is even lower than that occurring with urethane anaesthesia[9,11]. However, anaesthesia depresses cardiac output in the rat[12]. In the conscious rat cardiac output was found to be 28.6 ml · min^{-1} 100 g

Cardiac output and liver blood flow under urethane and pentobarbital anaesthesia and the effect of hepatic portal vein catheterization catheterization

	Pentobarbital		Urethane	
	1 Control (n=6)	2 P.V. Cath (n=6)	3 Control (n=6)	4 P.V. Cath (n=6)
Body weight (g)	288 ±20	285 ±10	277 ±16	291 ±11
Cardiac output (ml · min^{-1} 100 g b.wt^{-1})	22.7 ± 0.2	22.1 ± 0.6	22.1 ± 1.0	19.3 ± 0.3*
Mean arterial pressure (mm Hg)	115 ± 2	113 ± 3	108 ± 5	111 ± 4
Liver weight (g · 100 g b.wt^{-1})	3.86± 0.21	4.00± 0.07	3.88± 0.12	3.96± 0.10
Liver blood flow (ml · min^{-1} 100 g b.wt^{-1})	5.22± 0.17	5.33± 0.25	3.55± 0.25***	2.87± 0.40
Liver blood flow (ml · min^{-1} g $liver^{-1}$)	1.33± 0.12	1.31± 0.05	0.95± 0.06**	0.80± 0.11

All values are given as mean ± SEM. P.V. Cath: portal vein catheterization; * $p<0.05$ compared to columns 1, 2 and 3; ** $p<0.01$ between columns 1 and 3; *** $p<0.001$ between columns 1 and 3.

b.wt.$^{-1}$ [12], some 30% higher than the values recorded in our experiments, with a distribution of 13.1% to the hepatosplanchnic tissue[11]. Thus, simple calculation reveals that liver blood flow should be 3.75 ml · min^{-1} 100 g b.wt^{-1}. This closely resembles the value of 3.55 ml · min^{-1} 100 g b.wt^{-1} obtained with urethane in the present study and consequently urethane anaesthesia in rats may produce a liver blood flow which corresponds most closely to that in conscious animals.

However, in contrast to pentobarbital anaesthesia, urethane in rats appears to produce a reduction in cardiac output and liver blood flow as a result of portal vein catheterization. Although cardiac output is reduced, mean arterial pressure remains unchanged, indicating that the animal is in a state of compensatory shock[13]. The resulting vasoconstriction in the splanchnic vascular bed[13] may explain the greater reduction in liver blood flow than in cardiac output. Liver blood flow fell by 18% whereas cardiac output was decreased by only 13%.

In conclusion, it appears that although urethane anaesthesia may produce a liver blood flow which is similar to that in conscious animals, the trauma of portal vein catheterization may induce circulatory shock. Thus anaesthesia itself, the choice of general anaesthetic and hepatic portal vein catheterization may have profound effects in pharmacokinetic studies involving drugs which are highly cleared by the liver.

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Chemical and photooxidation of thiothixene (Navane®): Structure of the thiothixene fluorophor[1]

L.E. Eiden and J.A. Ruth

Department of Pharmacology and Toxicology, School of Pharmacy, The University of Kansas, Lawrence (Kansas 66045 USA), 6 February 1978

Summary. The spontaneous photooxidation of thiothixene (Navane®) to 2-(N,N-dimethyl-sulfonamido)-9-thioxanthone is described. The corresponding sulfoxide is demonstrated to be the fluorescent species obtained upon permanganate oxidation of thiothixene in the fluorometric assay procedure of Mjörndal and Oreland.

In the course of investigating plasma levels of the thioxanthene neuroleptic thiothixene (**1**) (Navane®), we had occasion to detail 2 previously unreported aspects of the chemistry of the drug which we report herein. These concern the chemical oxidation and the spontaneous photooxidation of thiothixene, both processes which influence the successful assay of low concentrations of the drug.

Although reasonable care was taken to prevent undue exposure of assay solutions to sunlight, we nonetheless noticed on several occasions a photodecomposition occuring in millimolar neutral or dilute acid stock solutions, resulting in the formation of a light yellow precipitate. Isolation of the material by extraction with ethyl acetate, followed by recrystallization from hexane/ethyl acetate afforded a light yellow crystalline substance of m.p. 172–174 °C (uncorrected). On the basis of the following spectral data we assigned the thioxanthone structure **2** to the decomposition product. Mass spectrometry demonstrated a molecular ion at m/e 319, and major fragments at m/e 275 (-NMe_2), 211 (-SO_2NMe_2) and 183 (-CO, -SO_2NMe_2). Infrared spectroscopy (20% solution in $CHCl_3$) revealed a carbonyl absorption at 1630 cm^{-1}, and proton NMR spectrometry ($CDCl_3$) showed absorptions at δ (ppm downfield from TMS standard) 2.8 (s, 6H, SO_2NMe_2), 7.3–8.1 (m, 5H), 8.6 (m, 1H) and 8.9 (d, 1H). Satisfactory microcombustion analyses were obtained for $C_{15}H_{13}S_2O_3N$. These data appear to be the same as those reported earlier for this compound[2].

The formation of **2**, prevented in the absence of direct irradiation or by degassing the solutions with nitrogen, is most likely attributable to the addition of singlet oxygen to the olefin, resulting in an intermediate adduct such as the dioxetane **1***[3,4], which then collapses to **2**. Once initiated, the reaction would be autocatalytic, the thioxanthone formed being an excellent triplet sensitizer[5]. Alternatively, since phenothiazines are known to form charge transfer complexes with oxygen which then rearrange to hydroperoxides[6], it is possible that a similar mechanism might be operative in the case of thioxanthenes.

Aside from the possible source of error such an anomalous oxidation could introduce into the assay procedure, we were also concerned about the reaction from another standpoint. We were employing the thiothixene assay procedure of Mjörndal and Oreland[7] which, like phenothiazine assay procedures[8,9] involves permanganate oxidation to generate a fluorescent species, the structure of which has not been reported. In our own hands the permanganate oxidation sometimes resulted in variable calibration curves. We felt that by establishing the structure of the fluorophor we might be able to more carefully control conditions so that the oxidation was always reproducible. We were