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SAFETY ASPECTS OF MIDAZOLAM

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1 The LD_{50} in the rat and the mouse is about 1600 mg/kg (oral administration) and 75 mg/kg (rat) and 50 mg/kg (mouse) on intravenous administration.

2 Subchronic oral studies over 13 weeks in doses of 5, 15 and 45 mg/kg/day in the dog and 50, 100 and 200 mg/kg/day in the rat have demonstrated minimal toxicity for midazolam, as for other benzodiazepines. High doses produced increased liver weight in the rat and the expected increases in alkaline phosphatase in the dog (species-specific reaction). Detailed blood and urine analyses as well as histological examination of organs produced no indication of changes relevant for man.

3 Subchronic parenteral studies (i.v. and i.m. for five weeks) using up to 6 mg/kg/day in dogs and rats showed the compound to be not only systemically, but also locally, extremely well tolerated.

4 Reproduction toxicology studies have shown that midazolam is neither embryotoxic nor teratogenic and that it has no effect on the fertility and post-natal development of animals.

5 In the AMES test and the fluctuation test, midazolam had no mutagenic effect.

Introduction

Drugs should not only be effective but also safe, and where safety is concerned the standards set today for hypnotics are particularly high. Toxicity tests on animals are one of the main methods of ensuring safety and reducing risk in the administration of drugs to human beings although such studies do not allow any absolute or definitive predictions to be made. The purpose of this paper is to characterize midazolam by reference to the investigations that have been carried out, and to summarize the type and degree of adverse reactions as a basis for classification. The results obtained so far come mainly from subchronic tests but reproduction toxicology and mutagenic studies have also contributed information.

Acute toxicity studies

Acute toxicity tests are required by the authorities even though their scientific value is controversial. It is known that differences in the species and breed of the animals, their sex and weight, and the conditions under which they are kept are reflected in differences in the data obtained. Under our test conditions,

Table 1 Acute toxicity of midazolam

Frequency and mode of administration	*Animal LD_{50}* *Mouse (mg/kg)*	*Rat (mg/kg)*	*Extrapolated for man (50 kg)*
Oral			
Single dose	1600	1600	80 g
After 5 daily doses	1000	1000	50 g
Intravenous[a]			
Single dose	50 (10 ml/kg)	75 (15 ml/kg)	2.5/3.75 g (500/750 ml)
After 5 daily doses	50 (10 ml/kg)	75 (15 ml/kg)	2.5/3.75 g (500/750 ml)

[a] Concentration of the parenteral formulation = 15 mg/3 ml (midazolam base)

Table 2 Basic examinations carried out during a subchronic or chronic toxicity study[a]

Body weight development, feed consumption, general and local tolerance
Special investigations: eyes, ECG (dog only)
Haematology: RBC, Hgb, MCV, MCH, MCHC, PCV, WBC, WBC-differential count, reticulocytes, platelets, thromboplastin-time (dog only)
Blood chemistry: GOT, GPT, AP, bilirubin, urea, glucose, cholesterol, sodium, potassium, total protein and electrophoresis
Urinalysis: colour, SG, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, nitrite, urinary sediment
Autopsy with registration of macroscopic findings and organ weights
Histopathology of about 35 organs

[a] Basic examinations in weeks 0, 2, (4), 6, 13, 26, 52, 78

values of 1600 mg/kg were obtained with a single oral dose in the mouse and rat (Table 1); this value declined to about 1000 mg/kg when the same dose was administered on five successive days. The LD_{50} is somewhat higher than 50 mg/kg in the mouse and is about 75 mg/kg in the rat when a single intravenous injection of the definitive commercial form (15 mg in 3 ml) is given; there is no change in these values after five doses.

Subchronic tolerance studies

The results and data obtained from subchronic tolerance studies are more informative and important (Tables 2 and 3). Studies of short duration (two weeks) as well as a five-week intravenous and a 13-week oral study were carried out in the rat and the dog, the two latter being the most informative of these trials. The systemic tolerance of midazolam was also excellent and elicited the familiar symptoms of toxicity only in animals given higher doses.

In a 13-week study in rats given 50 mg/kg midazolam per day, there were no biochemical or histopathological changes nor were there any autopsy findings indicative of toxicity. In the medium-dose group of rats receiving p.o. 100 mg/kg/day, the only feature was an increase of about 30% in absolute liver weights. One hundred mg/kg/day is equivalent in man to about 400 tablets of 15 mg taken daily over a period of 13 weeks. With the highest dose of 200 mg/kg/day, there was a slight reduction in weight gain (10% in females, 3% in males) compared with the norm and an increase in liver weight of about 50% compared with the controls. In the hepatocytes of the animals receiving the highest dose, light microscopy revealed a somewhat higher content of fat and acid mucopolysaccharides (difficult to quantify microscopically). Considering that these doses are very high compared with the clinical dose in man (15 mg/day) and that we have no evidence of hepatic dysfunction (Table 4), these findings may be interpreted as being of little significance for man. At all doses blood chemistry and haematological tests invariably revealed values which were within the physiological reference range. Histological examination of some 35 organs inspected per animal showed, apart from the liver findings mentioned, no changes attributable to the compound. In this context a small comparison is enlightening: barbiturates and piperidine derivatives are fatal to small laboratory animals in doses of 400–500 mg/kg even after a single oral administration. Yet, for therapeutic purposes these compounds are given in doses which are 10 to 20 times higher than midazolam. The margin of safety between the lethal and the effective dose, i.e. the

Table 3 Midazolam: 13-week, oral, subchronic toxicity studies

Animal	*Dosage/mode of administration*	*Extrapolated for man (50 kg)*	
Rat (24 males and 24 females/group)	0, 50, 100 and 200 mg/kg/day administered as feed admixture	Low dose Mid-dose Top dose	2.5 g/day 5.0 g/day 10.0 g/day
Dog (3 males and 3 females/group)	0, 5, 15 and 45 mg/kg/day administered in gelatine capsule	Low dose Mid-dose Top dose	250 mg/day 750 mg/day 2250 mg/day

Table 4 Plasma-GPT, plasma-AP, plasma-total bilirubin and serum-total protein with midazolam in rats (p.o.) after 13 weeks of treatment (mean ± s.d.)

	GPT (U/l)	*AP (U/l)*	*Bilirubin (mg/100 ml)*	*Protein (g/100 ml)*
Males				
control	16 ± 8	279 ± 43	0.08 ± 0.02	6.8 ± 0.3
50 mg/kg/day	13 ± 7	260 ± 45	0.06 ± 0.02	7.2 ± 0.2
100 mg/kg/day	12 ± 3	257 ± 39	0.08 ± 0.02	6.8 ± 0.3
200 mg/kg/day	12 ± 7	260 ± 57	0.07 ± 0.00	7.3 ± 0.03[a]
Females				
control	16 ± 3	230 ± 54	0.07 ± 0.02	6.8 ± 0.3
50 mg/kg/day	13 ± 5	161 ± 27	0.06 ± 0.02	7.1 ± 0.3
100 mg/kg/day	11 ± 2[a]	176 ± 38	0.08 ± 0.01	7.3 ± 0.02[a]
200 mg/kg/day	15 ± 3	154 ± 29	0.08 ± 0.02	7.6 ± 0.3[b]

Statistics: *t*-test, significant difference (versus control) with error rate of 0.05^a and 0.01_b

therapeutic index, is thus many times greater in the case of midazolam than for the reference compounds mentioned.

In the dog, the pharmacological effect, namely muscle relaxation, ataxia and sedation, seemed to be more rapid in onset and of shorter duration in all dosage groups than with the other benzodiazepines we have tested. The only noteworthy features were a marked proteinuria (300 mg/100 ml) in two out of six dogs in the top dose group after 13 study-weeks (with no detectable morphological kidney lesions) and a dose-related increase in alkaline phosphatase (Figure 1). A renal function test was not carried out; the urine parameters examined are listed in Table 2. This increase in alkaline phosphatase, always accompanied by normal plasma bilirubin and transaminases, represents a reaction of the dog to oral or parenteral doses of benzodiazepine that are undoubtedly high by clinical standards. This reaction is not known to us in any other species or in man; it can be immediately reversed by discontinuation of the compound. In occasional cases very high doses can also provoke, in addition to this enzyme increase, a focal intrahepatic cholestasis, accompanied in rare cases by a mild bile duct proliferation (Figure 2). There were almost no signs of cholestasis with midazolam.

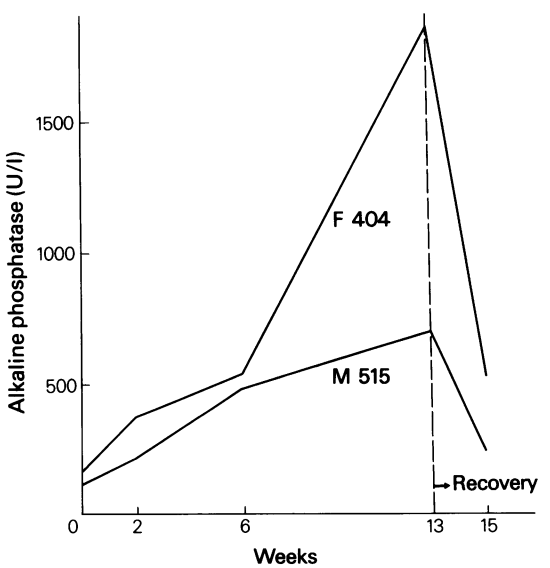

Figure 1 Alkaline phosphatase levels in two dogs which received an oral dose of 45 mg/kg/day midazolam (Ro 21-3981/001) for 13 weeks.

Parenteral administration: special considerations

With respect to parenteral toxicity tests, registration authorities prescribe that the dosage form tested on the animal must correspond to the definitive commercial form in regard to composition and concentration and that the mode of administration should also be the same as that used in clinical medicine.

This theoretically correct requirement has the disadvantage that, with compounds of low toxicity the systemic toxicity threshold can often hardly be reached because technical and physiological limits are set to the volumes that can be administered. Midazolam, which is also intended for use as premedication in anaesthesiology, is barely toxic and was, as expected, very well tolerated on parenteral administration to the rat and the dog in three doses, although as much as 1.2 ml/kg was given daily for five weeks intravenously, and 0.5 ml/kg/day intramuscularly in an additional group of dogs (Table 5). This 1.2 ml/kg is the theoretical equivalent of the daily injection of 20 ampoules (3 ml/15 mg) in a human subject weighing 50 kg. As an additional remark on the local tolerance of intravenous injections, it might be mentioned that throughout the test it was possible to inject the solution daily intravenously in all dogs and, with a few exceptions, in the rat; this is not something that can be taken for granted and is evidence of good local tolerance. It must also be remembered that, particu-

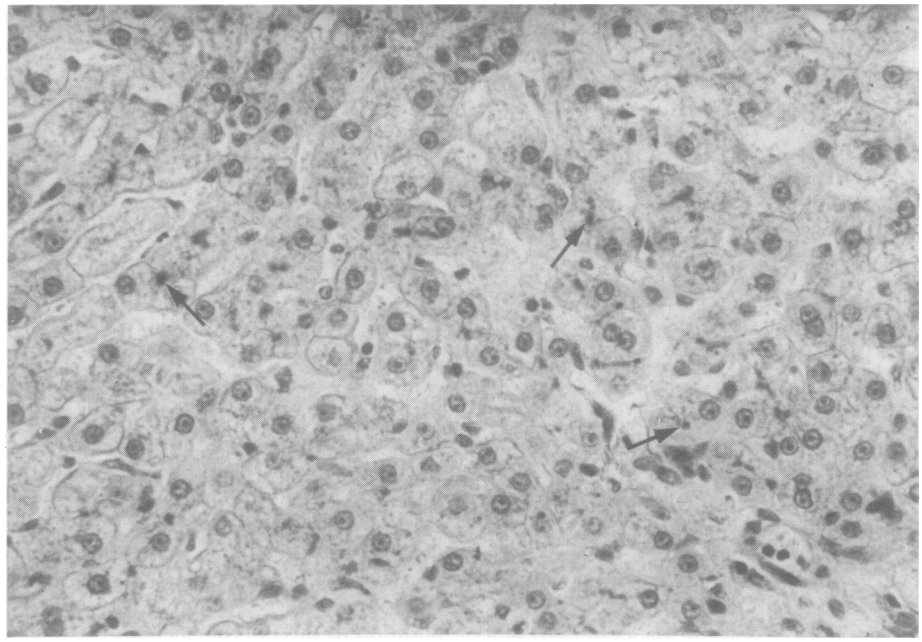

Figure 2 Magnification (250×) of liver from dog (HE-staining) after subchronic oral benzodiazepine administration. Slight swelling of liver cells and mild intrahepatic, intracanalicular cholestasis (arrowed).

larly in the groups receiving the highest dosages, enormous volumes had to be injected into sometimes very small vessels—in the rat, for example, into a vessel of perhaps 0.3 mm diameter with a needle about 0.5 mm thick. Midazolam was also excellently tolerated when administered by the intramuscular route. There was at no time any increase in the GOT, which is a good indicator of muscle fibre damage.

Reproduction toxicology, teratological and cancerogenicity studies

Reproduction toxicology and teratological studies have shown that midazolam is neither embryotoxic nor teratogenic and exerts no influence on the fertility and post-natal development of animals.

The mutagenicity tests carried out in microbial test systems (AMES test and fluctuation test) proved to be negative. Cancerogenicity studies are in progress.

Concluding remarks

No one will deny that an absolutely safe drug is an impossibility. This is a fact which cannot be altered by even the best animal tests and the most detailed investigations. One reason for this is that certain limits are set on our ability to detect side-effects in animals—i.e. the primarily neurological and psychical side-effects which are very difficult, or even impossible, to

Table 5 Parenteral administration of midazolam (15 mg/3 ml): five-week toxicity studies

Animal	*Dosage/mode of administration*	*Extrapolated for man (50 kg)*
Rat (12 males and 12 females/group)	Intravenous administration: control, 0.2, 0.5 and 1.2 ml/kg/day	
Dog		Low dose 10 ml/day (50 mg/day)
Group A; 3 males and 3 females/ dose	Intravenous administration: control, 0.2 and 1.2 ml/kg/day	Mid-dose 25 ml/day (125 mg/day) Top dose 60 ml/day (300 mg/day)
Group B: 2 males and 2 females	Intravenous administration: 0.5 ml/kg/day	
Group C: 2 males and 2 females	Intramuscular administration: 0.5 ml/kg/day	

register in large-scale toxicological tests—and another is that it is certainly not legitimate to extrapolate the unadjusted results of animal experiments to human beings. Considering, however, that the metabolism and kinetics of midazolam are very similar in the rat, the dog and man—thus favouring the extrapolation of the results from animals to man—and that it has a very high therapeutic index, this compound can be regarded as satisfying high safety standards.