METABOLISM OF LORAZEPAM

H. W. ELLIOTT

SUMMARY

The metabolism of lorazepam by man and four other species is reviewed. Lorazepam and its metabolites in blood, urine and faeces were identified by thin-layer and gas chromatography and by mass spectrometry. The principal metabolite in man, dog, pig and cat is the glucuronide, but the rat produces other metabolites after small doses of lorazepam, and significant amounts of the glucuronide only after high doses. Since all metabolites, except the glucuronide, occur in small quantities only in man, most studies in man have been confined to an estimation of free and conjugated lorazepam. Blood concentrations of unconjugated lorazepam peak at 1–4 h, significant concentrations persisting for 24 h and decreasing slowly over the next 24 h. About 95% of a dose of lorazepam was accounted for in urine and faeces over a period of 5 days; 74.5% was excreted in the urine as lorazepam glucuronide and 13.5% as minor metabolites. The excretory half-life was 12 h. The blood concentrations and excretion rates are compatible with the clinical effects of lorazepam.

Lorazepam (fig. 1), a benzodiazepine identical to oxazepam except for the presence of chlorine on the phenyl ring, is a potent sedative, hypnotic and anti-anxiety agent. Dose–response studies in humans have indicated that lorazepam 5 mg administered either orally or parenterally induces hypnosis in adult male volunteers with recovery within 6–8 h. Doses of 5 mg or less have useful sedative, anti-anxiety and hypnotic effects, but higher doses (7.5 mg) cause Stage I anaesthesia with residual depression and hangover 24 h after administration. The metabolism of lorazepam has been studied in both males and females receiving from 2.0 to 9.0 mg of the drug by the oral or parenteral route (Elliott et al., 1971; Schillings, Shrader and Ruelius, 1971; Comer et al., 1973), and in the dog, pig, cat, and rat after oral administration (Schillings, Shrader and Ruelius, 1971).

METHODS OF STUDY

The methods used for the extraction of lorazepam and its metabolites from urine and the identification of the metabolites by thin-layer chromatography and mass spectrometry were described by Schillings, Shrader and Ruelius (1971). The methods used for the determination of unconjugated lorazepam and lorazepam glucuronide in blood, urine and faeces were described by Elliott and others (1971) and Knowles, Comer and Ruelius (1971). Since the principal metabolite in humans is lorazepam glucuronide, lorazepam was determined in extracts of urine and faeces after hydrolysis with β-glucuronidase using gas–liquid chromatography. With a nickel-63 electron capture detector, concentrations of lorazepam as small as 10 ng/ml may be determined by gas–liquid chromatography. The disposition of 14C-lorazepam in eight healthy male volunteers was studied (Schillings, R. T., unpublished data). Lorazepam 2 mg, labelled with carbon-14 in the carbonyl group was given orally and urine and stool specimens were collected at intervals after administration of the drug. The radioactivity in the urine and stool specimens was determined by scintillation counting and lorazepam was determined quantitatively by gas–liquid chromatography as described above. Lorazepam glucuronide was isolated from urine and characterized by chemical analysis and mass spectrometry (Chang et al., 1973).

PATTERNS OF METABOLISM

The structural formulae of lorazepam and the five metabolites isolated from the urine of man, dog, pig, cat and rat are shown in figure 1. The principal metabolite of lorazepam in all these species, except the rat, is the glucuronide; in fact, only a small percentage of the drug is transformed to other metabolites. Species differences in the urinary excretion patterns are shown in table I. It is apparent that in all
species examined, except the rat, lorazepam glucuronide is the most important metabolite. The rat transforms low doses of lorazepam, almost completely, to metabolites II and III, but significant quantities of lorazepam glucuronide and the metabolite IV appear when the dosage is increased fourfold or more. It is of interest that the glucuronide is an important metabolite in the cat, a species which is reported not to form glucuronides easily, and is a less important metabolite in the rat, a species which forms glucuronides readily but has additional metabolic pathways at its disposal. The benzodiazepines, diazepam, oxazepam and chlorodiazepoxide also are metabolized similarly by dog and man but differently by the rat. In the rat, oxidative and methyl transfer reactions precede glucuronidation to account for the presence of metabolites II, III and IV (as glucuronides); however, the former pathways are apparently overloaded at high doses. Traces of metabolite I were found in the urine of all species studied, but the pathways leading to its formation are unknown. The addition of a methoxy group to the phenyl ring (metabolite III) appears to be a new pathway in the biotransformation of benzodiazepines.

The relative importance of the several metabolic pathways in man are shown in figure 2. Lorazepam and metabolite II appear in urine exclusively as the glucuronides, but metabolite I and other minor metabolites are found in both the unconjugated and conjugated forms. Since relatively little lorazepam is transformed to metabolites other than the glucuronide in man, the majority of the metabolic studies in humans were confined to determination of unconjugated and conjugated lorazepam. The plasma concentrations of unconjugated, conjugated and total lorazepam in four subjects who received lorazepam 5 mg i.m. are shown in figure 3. The plasma lorazepam...
Fig. 2. The relative importance of the various urinary metabolic pathways for lorazepam in man.

Fig. 3. Human plasma concentrations of unconjugated (active), conjugated and total lorazepam for 48 h following a single i.m. dose of lorazepam 5 mg (n = 4).
concentrations are approximately twice those in the erythrocytes. The highest plasma concentrations of unconjugated lorazepam correspond to the period of most intense drug action—1-4 h following administration. Clinically, these subjects appeared to have recovered by 7-8 h although plasma concentrations at this time were very close to peak concentrations. Significant concentrations persisted at 24 h and decreased slowly over the following 24 h. The concentration of conjugated lorazepam increased steadily for 4 h following administration, then remained at essentially the same value for the following 8 h, after which it decreased over the period from 12 to 48 h. Following the oral administration of lorazepam 5 mg, the concentration of unconjugated lorazepam in serum increased to 45 ng/ml at 2 h (about half the value attained after i.m. administration) and changed very little over the next 4 h. Thereafter, the concentration decreased very slowly so that after 24 h the concentration remained at 25 ng/ml. The blood concentrations of lorazepam following the administration of a 5-mg dose i.v. are compared with the blood concentrations following the same doses i.m. or orally in figure 4. The initial high concentration achieved after i.v. administration decreased quickly to approximately the concentration achieved eventually following i.m. administration. Peak concentrations following i.m. administration were not achieved until 2 h after drug administration. Thereafter the concentrations approximated those seen after i.v. injection and declined at approximately equal rates during the 48-h period of study. Blood concentrations following oral administration were significantly less than after i.m. or i.v. administration, but the rate of decrease in the serum concentration was slower after oral administration. It is noteworthy that the subjects who had received the drug orally appeared to be as sedated as those who had received the drug i.m. or i.v. and that, although there was e.e.g. evidence of drug action within 10 min following i.v. administration, peak drug effects did not occur for 45-60 min regardless of the route of administration.

The urinary excretion of lorazepam was determined in all metabolic studies, but is summarized best in a study of 14C-labelled lorazepam. Most of the administered carbon-14 was accounted for in urine (94.4%) and faeces (6.6%) collected over a period of 5 days: 74.5% of the radioactivity in urine represented lorazepam glucuronide and 13.5% represented minor metabolites.

Faecal excretion of carbon-14 was highest during the 3rd day but was not complete until the 5th day, in contrast to urinary excretion, which was virtually complete by the 4th day. This delayed faecal excretion may indicate that some metabolites of lorazepam undergo entero-hepatic circulation. The cumulative urinary and faecal excretions of carbon-14 are shown...
in figure 5, which illustrates the delay in excretion of lorazepam and its metabolites in the faeces. Nearly half of the total drug and carbon-14 recovery has occurred by 12 h, about 80% by 48 h and 94.4% by 120 h. From a semi-log plot of percent of carbon-14 and lorazepam unexcreted in urine vs. time, a urinary half-life of 12 h and an elimination rate constant of 6% of the remaining carbon-14 per hour were calculated. Changes in the rate of excretion possibly related to absorption and redistribution of lorazepam and its metabolites in the body compartments are shown in figure 6. The highest rate of excretion occurred at 5 h. The curvature of the carbon-14 curve at about 30 h is significant and, like the delayed faecal excretion, is suggestive of an entero-hepatic circulation of lorazepam metabolites or possibly of diurnal variations in excretion. The concentrations of free and conjugated lorazepam in plasma of eight volunteers who received daily 5-mg doses of drug i.m. for 4 days are presented in figure 7 (Langlois, A. B., unpublished data). The maximum concentrations of both free and conjugated lorazepam were reached on the 2nd day, after which a steady state prevailed. The average maximum concentrations of free lorazepam were 62.5, 62.3 and 62.2 ng/ml and of lorazepam glucuronide were 108.6, 102.3 and 107.3 ng/ml on days 2, 3 and 4 respectively. These constant values indicate that no accumulation of the drug in plasma occurred after the second dose.

DISCUSSION

The comparative metabolism of lorazepam is of theoretical interest. Its unexpected ready glucuronidation by the cat and the use of alternate metabolic pathways by the rat should be useful in future studies of the mechanisms of drug metabolism. It is apparent that predictions regarding biotransformation based on chemical structure and known routes of metabolism should be verified in several species to obtain the complete story of biotransformation. On the other hand, the qualitatively similar metabolic pattern found in three of four animal species made it likely that lorazepam glucuronide would be the major metabolite in man also.

Glucuronide formation is considered an efficient detoxification mechanism since it produces an easily excreted water-soluble metabolite. However, not all glucuronides are excreted at the same rate. Obviously, binding to plasma and tissue constituents, entero-hepatic circulation, rate of metabolism and other factors must account for the differences observed in studies of drug metabolism.

The effects of lorazepam on behaviour in man may be related to drug metabolism, since both were studied in the same subjects. Behaviour was similar regardless of the route of drug administration—oral, i.m. or i.v. A dose of 5 mg caused c.n.s. depression similar to anaesthesia with the onset of action apparent at 30 min and maximum depression noted from 1 to 4 h after drug administration. During this...
Fig. 7. Plasma concentrations of unconjugated (active), conjugated and total lorazepam in humans who received lorazepam 5 mg i.m. daily for 4 days (n = 8).

period all subjects showed markedly impaired balance, required assistance in walking and slept when not stimulated (Elliott et al., 1971). Serum concentrations of free drug during the period of maximal drug action approximated 45 ng/ml after oral and 70–90 ng/ml after i.m. or i.v. administration. Considering any route of administration, the degree of c.n.s. depression correlated well with the serum lorazepam concentration but after oral administration the serum concentration required to cause the same degree of c.n.s. depression was less than that required after i.m. or i.v. administration. The reason for this discrepancy is unknown.

A slow onset of action is expected after oral drug administration, but i.m. or i.v. administration did not hasten the effect appreciably. Delay in absorption is suggested by the low increase in the serum concentration of unconjugated lorazepam after i.m. administration but belied by the rapid attainment of an equilibrium concentration after i.v. administration. It is more likely that the explanation of the slow onset of action lies in slow penetration of the blood–brain barrier by lorazepam, although it should be noted that sleep spindles appeared in the e.c.g. of some subjects 2–4 min after drug administration. It is likely that early high brain concentrations were not maintained as blood concentrations decreased to equilibrium values.

The duration of action correlated well with peak blood concentrations of unconjugated lorazepam in the subjects from whom these metabolic data were obtained who, possibly because they had abused drugs in the past, recovered when blood concentrations were still relatively high. Subsequent studies using populations less accustomed to drug abuse indicated that the long persistence of high serum concentrations of lorazepam correlated with prolonged drug action.

Glucuronide formation was detected 2–5 min after i.v. administration and as early as 10 min after i.m. administration. Peak concentrations which occurred 4 h after i.m. or i.v. administration were maintained until at least 12 h and then decreased more rapidly than the serum concentrations of the free drug. The results are compatible with firm binding of lorazepam to plasma and tissue components. This could lead to cumulative drug action following multiple doses. However, as reported above, plasma concentrations of free and conjugated lorazepam increased only
following the second daily 5-mg i.m. dose of lorazepam in a 4-day study.

The results of excretion studies in man might have been predicted from the metabolite identification and blood studies. About 90% of administered lorazepam is excreted in the urine largely as the glucuronide and urinary excretion is virtually complete in 4 days. Small amounts of other metabolites in urine and possibly some free lorazepam in the feces were detected also. The excretory half-life and excretion rates are compatible with the principal pharmacological actions of lorazepam determined in a non-tolerant population—long-acting anesthetic-type c.n.s. depression with minimal depression of respiration and cardiovascular function.

REFERENCES


METABOLISMO DEL LORAZEPAM

SUMARIO
Se considera el metabolismo del lorazepam en el Hombre y otras cuatro especies. Mediante cromatografía en fase gaseosa y en capa fina y utilizando espectrometría de masa se identificaron el lorazepam y sus metabolitos en la sangre, orina y heces. El metabolito principal en el Hombre, perro, cerdo y gato es el glucuronido, pero la rata produce otros metabolitos tras la administración de pequeñas dosis de lorazepam, y cantidades significantes del glucuronido sólo tras dosis elevadas. Dado que todos los metabolitos, excepto el glucuronido, ocurren en pequeñas cantidades solamente en el Hombre, la mayoría de los estudios en esta especie se han limitado a calcular el lorazepam libre y el conjugado. Las concentraciones hemáticas de lorazepam no-conjugado alcanzan la máxima a 1-4 h, persistiendo concentraciones significativas durante 24 h y disminuyendo lentamente durante las siguientes 24 h. El 95% aproxim. de una dosis de lorazepam fue detectada en orina y heces durante un período de 5 días; el 74,5% fue excretado en la orina como lorazepam glucuronido, y el 13,5% como metabolitos menores. La media vida excretoria fue de 12 h. Las concentraciones en sangre y los índices de excreción son compatibles con los efectos clínicos del lorazepam.

STOFFWECHSEL VON LORAZEPAM

ZUSAMMENFASSUNG

METABOLISME DU LORAZEPAM

RESUME
L'auteur passe en revue le métabolisme du lorazepam par l'homme et quatre autres espèces animales. On a identifié le lorazepam et ses métabolites dans le sang, l'urine et les matières fécales, à l'aide de la chromatographie en couches minces et en phase gazeuse ainsi que par la spectrométrie de masse. Le principal métabolite chez l'homme, le chien, le cochon et le chat est la glucuronide, mais le råd produit d'autres métabolites après de faibles doses de lorazepam, et d'importantes quantités de glucuronide seulement après des doses élevées. Étant donné que tous les métabolites, à l'exception de la glucuronide n'apparaissent chez l'homme qu'en petites quantités, la plupart des études faites chez l'homme se sont limitées à une estimation de lorazepam libre et conjugué. Les concentrations dans le sang de lorazepam non-conjugué atteignent un maximum de 1 à 4 h, tandis que d'importantes concentrations persistent pendant 24 h et diminuent lentement pendant les 24 h suivantes. Environ 95% d'une dose de lorazepam ont été retrouvés dans l'urine et les matières fécales sur une période de 5 jours; 74,5% ont été expulsés dans l'urine sous forme de glucuronide de lorazepam et 13,5% sous forme de métabolites mineurs. La demi-vie excrétory a été de 12 h. Les concentrations dans le sang et les taux d'excrétion sont compatibles avec les effets cliniques du lorazepam.

We regret to record that Dr Elliott died on 1st August 1976.