Prolongation of Chlorzoxazone Plasma Levels by Zoxazolamine

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The plasma levels of chlorzoxazone were determined in humans, dogs, and mice. It was shown that the co-administration of zoxazolamine and chlorzoxazone prolonged the plasma concentrations of the latter substance in mice and dogs. An interaction between the compounds under consideration, which resulted in the formation of an insoluble complex, was demonstrated by means of a solubility study. Evidence was presented to show that the prolongation of chlorzoxazone plasma levels was probably a result of a change in metabolism of this compound in the presence of the additive, zoxazolamine. It is suggested that the molecular complex formed between chlorzoxazone and zoxazolamine is directly responsible for the proposed alteration in metabolism of chlorzoxazone and the prolonged plasma levels of this compound which results when the combination of drugs is administered.

CHLORZOXAZONE (5-chlorobenzoxazolinone) is a centrally acting muscle relaxant which has been found useful in the treatment of a number of spastic diseases. The physiological disposition and metabolic fate of this compound have been reported previously by Conney and Burns (1). The present investigation is concerned with the absorption and biological life of this substance in several animal species after the administration of the drug alone and in combination with a potentiating agent.

Specifically, the animal species employed in this investigation were mice, dogs, and humans, and the potentiating agent utilized was zoxazolamine (2-amino-5-chlorobenzoxazole). The potentiating effect of the additive was demonstrated by a prolongation of chlorzoxazone plasma levels when the combination of drugs was administered.

The basis for investigating the chlorzoxazone-zoxazolamine combination was the hypothesis developed by Brodie, Hogben, Schanker, and associates (2–4) to explain the rate and extent of absorption of acidic and basic drugs from the gastrointestinal tract. It was thought that administration of chlorzoxazone (the acidic drug) and zoxazolamine (the basic compound) would demonstrate a more prolonged muscle relaxant activity than either of the compounds administered singly since the weakly acidic drug would be absorbed more readily in the acidic portion of the gastrointestinal tract, and the weakly basic drug in the more basic segments of the tract. However, it was noted that the chlorzoxazone plasma levels were appreciably prolonged when the combination of drugs was administered when compared to the levels observed after administration of chlorzoxazone alone.

The purpose of this study was to characterize the absorption pattern of chlorzoxazone in various animal species with the possibility of utilizing this information in the development of more efficient dosage forms, and to investigate the mechanism responsible for the prolongation of chlorzoxazone plasma levels noted when this compound is administered in conjunction with zoxazolamine.

EXPERIMENTAL

The estimation of chlorzoxazone levels in the biological samples was made by the method described by Conney, *et al.* (5). This procedure involved extraction of acidified biological material with an organic solvent consisting of 1.5% isoamyl alcohol in petroleum ether. The drug was then extracted from the organic phase into a 0.5 *N* sodium hydroxide solution and measured spectrophotometrically at 287 mμ where the compound exhibits an absorption maximum.

The method employed for the determination of zoxazolamine was similar to that described for chlorzoxazone. However, the drug was extracted from alkalinized biological material into the organic solvent and re-extracted into a 3 *N* hydrochloric acid solution. Measurements were made spectrophotometrically at 278 mμ.

The experimental procedure utilized to demonstrate the interaction between chlorzoxazone and zoxazolamine in distilled water and mouse plasma was similar to that reported by Higuchi and Lach (6). The analytical procedures employed were similar to those described for the biological samples.

The spectrophotometric measurements were made on a Cary model 14 recording spectrophotometer.

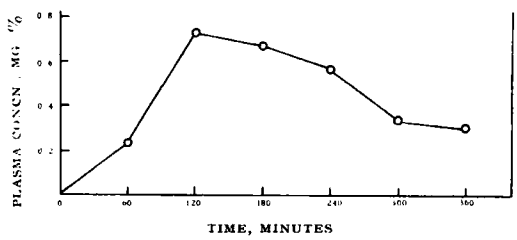

Fig. 1.—Mean plasma levels of chlorzoxazone in humans after oral administration of 0.50 Gm. of drug.

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RESULTS AND DISCUSSION

Ten humans received 0.50 Gm. of chlorzoxazone orally in tablet form, and the plasma levels were measured at various time intervals. Blood samples were obtained from five of the subjects at 0, 60, 180, and 300 minutes and from the remaining five subjects at 0, 120, 240, and 360 minutes. Figure 1 presents the mean plasma levels observed in this study. Peak plasma levels were attained 120–180 minutes after administration and the biological half-life was determined to be about 170 minutes. These data are consistent with the duration of action noted with this compound in clinical studies. In addition, the results noted in this investigation are in general agreement with the observations previously reported by Conney and Burns (1).

The mean plasma levels observed in dogs after oral administration of 30 mg./Kg. and 60 mg./Kg. chlorzoxazone are shown in Fig. 2. Six dogs were used at each dose level, and the drug was administered in capsule form. The biological half-life in this species was determined to be about 120 minutes with a peak level observed between 90–180 minutes. Figure 2 also shows the mean plasma levels of chlorzoxazone observed after administration of 60 mg./Kg. chlorzoxazone in conjunction with 60 mg./Kg. zoxazolamine. Four dogs were employed on this dosage schedule. Although the peak concentration of chlorzoxazone did not differ significantly from that obtained after administration of chlorzoxazone alone, there was a definite prolongation of the plasma concentration. From the limited data presented, it appears that the half-life of chlorzoxazone is prolonged to about 300 minutes when this compound is administered with an equivalent dose of zoxazolamine.

The plasma levels of chlorzoxazone determined after administration of a suspension of the drug to mice at levels of 100 mg./Kg. and 200 mg./Kg. are shown in Fig. 3. The chlorzoxazone determinations were made on a pooled blood sample from about 20 mice. The peak chlorzoxazone levels in this species were noted at 5 minutes with a half-life of only 20 minutes. The short half-life observed in this species is consistent with the data reported by Conney and Burns (1) which showed that liver homogenates prepared from mouse or guinea pig tissue metabolized chlorzoxazone three to four times as fast as a corresponding preparation using rat or rabbit tissue. The chlorzoxazone plasma levels attained in mice after oral administration of 100 mg./Kg. each of chlorzoxazone and zoxazolamine are also shown in Fig. 3. The plasma concentrations reported for the combination are the means of three separate sets of pooled blood samples. The results were similar to those observed with dogs since there was a significant prolongation of the chlorzoxazone plasma concentrations when the combination of drugs was given. The biological half-life of chlorzoxazone in this instance was about 80 minutes or four times longer than that noted when chlorzoxazone was administered singly.

Several of the possible mechanisms which could account for the prolonged chlorzoxazone plasma concentrations demonstrated after administration of the combination of drugs are (*a*) conversion of zoxazolamine to chlorzoxazone in the biological system, (*b*) prolonged dissolution, and therefore prolonged absorption due to the formation of a poorly soluble interaction product of chlorzoxazone and zoxazolamine, (*c*) an increase in absorption from the gastrointestinal tract of chlorzoxazone in the presence of zoxazolamine, (*d*) lower chlorzoxazone tissue levels (or more rapid removal of this drug from the tissues when zoxazolamine is also in the system), and (*e*) an alteration in the metabolism of chlorzoxazone when both compounds are present.

It is known that 2-aminobenzoxazoles are converted *in vitro* to 2-benzoxazolinones in dilute mineral acid. However, the *in vivo* conversion of zoxazolamine to chlorzoxazone as the mechanism responsible for the prolongation of chlorzoxazone plasma levels was readily eliminated when no detectable levels of chlorzoxazone were found after administration of 60 mg./Kg. of zoxazolamine to dogs and 100 mg./Kg. of this agent to mice.

Although it is possible that prolonged dissolution and absorption occur when chlorzoxazone and

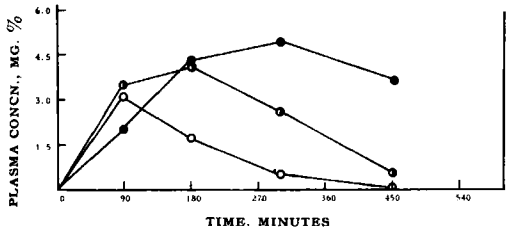


Fig. 2.—Mean plasma levels of chlorzoxazone in dogs after oral administration of 30 mg./Kg. (O) and 60 mg./Kg. (◑) of the drug alone and after administration of 60 mg./Kg. each of chlorzoxazone and zoxazolamine (●).

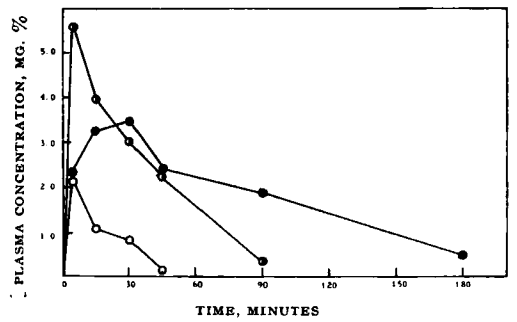


Fig. 3.—Plasma levels of chlorzoxazone in mice after oral administration of 100 mg./Kg. (O) and 200 mg./Kg. (◑) of the drug alone and after administration of 100 mg./Kg. each of chlorzoxazone and zoxazolamine (●).

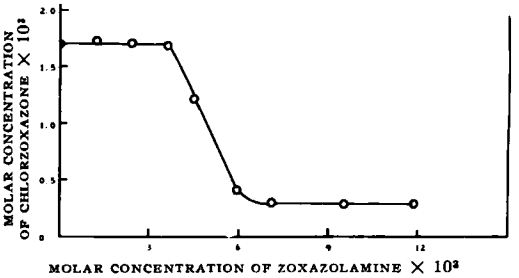


Fig. 4.—Phase diagram showing the effect of zoxazolamine on the apparent solubility of chlorzoxazone in water at 30°C.

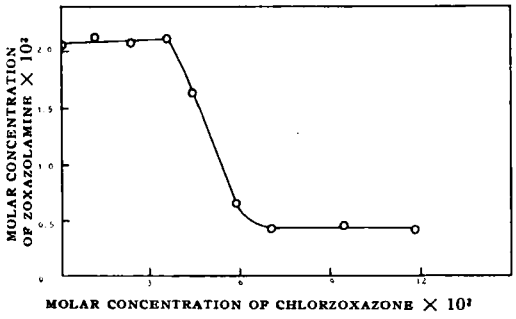


Fig. 5.—Phase diagram showing the effect of chlorzoxazone on the apparent solubility of zoxazolamine in water at 30°C.

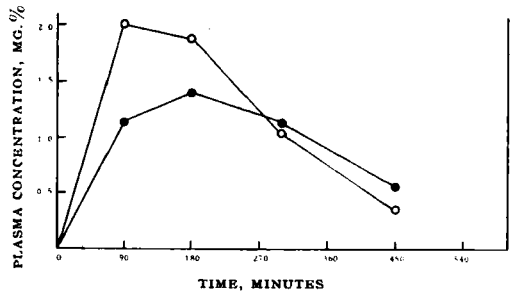
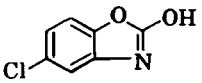


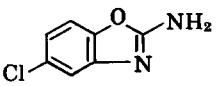
Fig. 6.—Mean plasma levels of zoxazolamine in dogs after oral administration of 60 mg./Kg. (O) of the drug alone and after administration of 60 mg./Kg. each of zoxazolamine and chlorzoxazone (●).

zoxazolamine are administered simultaneously (due to their interaction at the absorption site), this mechanism alone would not seem to explain adequately the large areas under the time-concentration curves observed for the drug combination.

The remaining possible mechanisms listed above also may be rationalized on the basis of an interaction between these muscle relaxant compounds. An examination of the chemical structure of the compounds under consideration shows that the structural requirements necessary for the formation of a molecular complex in aqueous solution similar to those described in previous communications (6–8) are present in these substances. The expected interaction was illustrated by means of a solubility study. The phase diagrams describing this interaction are



Chlorzoxazone



Zoxazolamine

shown in Figs. 4 and 5. Analysis of the phase diagrams and chemical analysis of the relatively insoluble complex formed showed the addition product to be composed of one molecule of chlorzoxazone to one of zoxazolamine.

It is unlikely that the complex demonstrated in aqueous solution would be stable in the acidic medium of the stomach. However, if the formation of a complex between these compounds was responsible for an increase in absorption of chlorzoxazone from the gastrointestinal tract, a corresponding increase in zoxazolamine plasma levels would also be observed. That this does not occur is demonstrated in Fig. 6 which shows the mean plasma levels of zoxazolamine after oral administration to dogs of 60 mg./Kg. zoxazolamine and 60 mg./Kg. zoxazolamine in combination with a like amount of chlorzoxazone. Similar results were noted with the plasma levels attained in mice after oral administration of 100 mg./Kg. zoxazolamine alone and in combination with 100 mg./Kg. chlorzoxazone. The early levels noted in both species were lower when the combination of drugs was administered than those observed when zoxazolamine was given alone. This may be a result of a lowering of the solubility or a slower dissolution rate of the zoxazolamine in the presence of chlorzoxazone in the gastrointestinal tract.

If an increase in gastrointestinal absorption is not

Table I.—Plasma Levels of Chlorzoxazone in Mice After Intraperitoneal Administration (mg. per cent)

	Time, Minutes					
	5	15	30	45	90	180
Chlorzoxazone, 100 mg./Kg.	6.80	3.66	0.73	0.81	0	0
Chlorzoxazone, 100 mg./Kg. Zoxazolamine, 100 mg./Kg.	6.22	3.25	3.96	2.31	1.65	0

Table II.—Brain and Plasma Levels of Chlorzoxazone in Mice After Oral Administration (mg. per cent)

		Time, Minutes				
		15	30	45	90	180
Chlorzoxazone alone	Brain	1.67	1.26	1.23	0	0
	Plasma	3.97	2.98	2.24	0.33	
Chlorzoxazone in combination	Brain	1.95	3.16	1.87	1.20	0
	Plasma	5.37	4.82	3.69	2.21	0.33

Table III.—Brain and Plasma Levels of Zoxazolamine in Mice After Oral Administration (mg. per cent)

		Time, Minutes				
		15	30	45	90	180
Zoxazolamine alone	Brain	2.05	1.24	0.99	0.35	0
	Plasma	0.69	0.36	0.15	0.06	0
Zoxazolamine in combination	Brain	0.73	0.60	0.60	0.20	0
	Plasma	0.54	0.36	0.32	0.22	0

responsible for the prolongation of the chlorzoxazone levels noted, then administration of the combination by other than the oral route should also result in a prolongation of chlorzoxazone plasma levels. Table I shows the chlorzoxazone plasma levels determined after intraperitoneal administration of 100 mg./Kg. chlorzoxazone alone and in combination with 100 mg./Kg. zoxazolamine. It is apparent from these results that this route of administration affects the chlorzoxazone levels in a manner similar to that observed after oral administration of the drug combination.

A comparison of Table I with Fig. 3 shows that at times greater than 5 minutes post administration the plasma levels resulting from the intraperitoneal route are similar to those observed when the same drug combination was administered orally to mice.

In order to obtain evidence for the *in vivo* formation of a complex between the compounds under consideration, a solubility study was conducted utilizing mouse plasma as the solvent and maintaining the temperature at 37.5°. Because of the limited number of experimental points available, it was not possible to analyze the phase diagram as was the case for the aqueous study described previously. However, evidence of complex formation in this system was obtained since (in each instance) the presence of one of the molecules in the system significantly decreased the solubility of the other in the biological fluid.

The effect of administration of the combination of drugs on the chlorzoxazone tissue levels was made by determining chlorzoxazone levels in the brains of mice. The dosage regimen utilized for this part of the study was 200 mg./Kg. chlorzoxazone alone and 200 mg./Kg. chlorzoxazone in combination with 50 mg./Kg. zoxazolamine. The brains of ten mice were pooled and homogenized with distilled water and an aliquot of the final preparation was used for the drug determination.

The results of this experiment, along with the chlorzoxazone plasma levels attained employing the same dosing schedule, are presented in Table II.

The data indicate that the brain levels of chlorzoxazone are proportional to the plasma levels of this drug, and that the prolongation of plasma concentrations is reflected in prolonged tissue concentrations. Therefore, the prolonged chlorzoxazone plasma levels noted in this study are probably not the result of a lowering or more rapid depletion of tissue chlorzoxazone in the presence of zoxazolamine.

The brain levels of zoxazolamine observed after oral administration of 50 mg./Kg. of this compound alone and in combination with 200 mg./Kg. chlorzoxazone are shown in Table III along with the plasma concentrations of zoxazolamine determined at these same dosage levels.

It is interesting to note that the brain levels of zoxazolamine are considerably lower after administration of the drug combination than those observed after administration of the zoxazolamine alone. However, there was no significant difference in the plasma levels noted after either dosage regimen was used. These results may be rationalized on the basis of complex formation between the muscle relaxant compounds. Since a large excess of chlorzoxazone molecules (in relation to zoxazolamine) is available in the system, the tissue distribution of this material would not be expected to be influenced significantly by the formation of the proposed complex. However, because of the relatively low concentration of zoxazolamine present, the distribution of this compound in the various biological compartments could be considerably altered by an interaction of the type suggested.

The data indicate that the prolongation of chlorzoxazone plasma levels demonstrated when this agent is administered in combination with zoxazolamine is probably due mainly to an alteration in the metabolism of chlorzoxazone. In addition, evidence has been presented to support the theory that this modification in the metabolism of chlorzoxazone may be mediated through the formation of a molecular complex between the muscle relaxant compounds.

Prolongation of drug activity by mechanisms similar to that described in this study would be especially useful for those drugs which are absorbed in a limited portion of the gastrointestinal tract and therefore cannot be prolonged by a delayed release mechanism. Furthermore, many of the problems encountered in controlling the release of drugs and assuring their complete availability from the various sustained action forms would be greatly simplified in a system of this type.

SUMMARY AND CONCLUSIONS

Chlorzoxazone plasma levels were determined in humans, mice, and dogs after the oral administration of this compound alone, and in mice and dogs after administration of this agent in conjunction with zoxazolamine. In addition, the chlorzoxazone plasma levels in mice after intraperitoneal administration of the drug alone and in combination were determined.

The zoxazolamine plasma levels in mice and dogs were observed after oral administration of this compound alone and in combination with chlorzoxazone.

The brain levels of these compounds attained in mice after the oral administration of the drugs alone and in combination were also determined.

Formation of a molecular complex between the agents under study was demonstrated by means of solubility studies.

A prolongation of chlorzoxazone plasma levels was noted when this compound was administered simultaneously with zoxazolamine to mice or dogs. Evidence was presented to show that this prolongation probably results mainly from an alteration in the metabolism of chlorzoxazone. Furthermore, it is suggested that the proposed change in metabolism is mediated through the formation of a molecular complex *in vivo* between these muscle relaxant compounds.

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