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ACETAMINOPHEN–DIPHENHYDRAMINE INTERACTION IN RABBITS

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SUMMARY

Acetaminophen–diphenhydramine interaction was investigated by oral administration of acetaminophen to rabbits, alone or in combination with diphenhydramine. Blood samples were collected before and 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5,0 and 6.0 h after acetaminophen administration. Assays of acetaminophen in plasma samples were carried out using a HPLC method. Diphenhydramine significantly reduced the maximum plasma concentration (C_{max}) of acetaminophen. Diphenhydramine, however, had little effect on the time taken to reach the maximum plasma concentration (T_{max}), the area under the plasma concentration–time curve (AUC_0^∞) and the elimination half-life ($t_{1/2}$) of acetaminophen. Gastric emptying experiment was performed by injecting into the rabbit stomach phenol-red containing solution with and without diphenhydramine. The gastric content recovered at 0.5 h was analyzed for phenol-red remaining to determine the rate of gastric emptying. Diphenhydramine significantly increased the weight percentage of phenol-red remaining in the rabbit stomach 0.5 h post-injection. It was concluded that diphenhydramine affected the rate but not the extent of acetaminophen absorption by delaying the gastric emptying.

INTRODUCTION

Acetaminophen is often used in combination with diphenhydramine and chlorpheniramine in man with little understanding of the potential interactions between acetaminophen and these antihistamines. Lavigne and Marchand (1973) reported that diphenhydramine markedly inhibited the gastrointestinal absorption of *p*-aminosalicylate in rats

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and humans. Feldman and Putcha (1977) found that diphenhydramine caused a marked decrease in gastric emptying and intestinal transition in rats. Recently, Imamura et al. (1980) demonstrated that chlorpromazine and atropine, which have been shown to delay gastric emptying, decreased the rate of acetaminophen absorption in rabbits. The objective of this study is to investigate whether diphenhydramine would have any effect on the gastrointestinal absorption of acetaminophen in rabbits.

MATERIALS AND METHODS

Materials

Acetaminophen and vanillin internal standard were obtained from the Aldrich Chemicals (Milwaukee, Mich.). Diphenhydramine hydrochloride was kindly supplied by Parke-Davis (Detroit, Mich.). Ether and sodium chloride were analytical grade and were supplied by Mallinckrodt (St. Louis, Mo.). Methanol, HPLC grade, was obtained from Burdick and Jackson (Muskegon, Mich.).

Methods

Acetaminophen–diphenhydramine interaction

Male New Zealand white rabbits weighing 2.8–3.5 kg were fasted for 38–42 h prior to the experiment, but water was allowed ad libitum. Food and water were withheld during the experiment. Acetaminophen (100 mg/kg) alone or in combination with diphenhydramine (10 and 20 mg/kg) was dissolved in 70 ml warm deionized water (35–37°C) and was orally administered. Blood samples (0.5 ml) were collected from the ear vein in heparinized vacutainers before and 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5 and 6 h after oral administration. Blood samples were immediately centrifuged and the plasma aliquots obtained were refrigerated until analysis.

Assays of acetaminophen were performed using a high-pressure liquid chromatographic method (Wang and Lee, 1980). To a 0.2-ml aliquot of the rabbit plasma to be assayed was added 2.5 μg of the vanillin internal standard and 0.8 g of sodium chloride. The mixture was vortexed for 30 s followed by extraction with 8 ml of ether for 15 min on a test tube rotator. After centrifugation, the ether layer was transferred to another test tube and evaporated to dryness at room temperature under a stream of nitrogen. The residue was reconstituted in 2 ml of the mobile phase and a 10–50-μl aliquot injected onto the column. The column used was a μBondapak C-18 (30 cm × 4 mm i.d.; Waters Associates). The mobile phase consisted of methanol/water (35%, v/v). The flow-rate was set at 2.5 ml/min with an operating pressure of 2000 psi. The effluent was monitored at 254 nm with a full detection scale of 0.01 A. This procedure provided a low detection limit of 0.25 μg for 1 ml of plasma.

The area under the plasma concentration–time curve (AUC_0^∞) was estimated according to the trapezoidal rule. The elimination half-life ($t_{1/2}$) was calculated from the regression slope of the log-linear portion of the plasma concentration–time curve, assuming first-order kinetics.

Gastric emptying experiment

Gastric emptying was determined by a slightly modified method of Goto et al. (1972). Male rabbits in the same weight range were fed soybean crude refuse for at least 1 week and were fasted for 24 h prior to the gastric emptying experiment. In our experience, this fasting condition is equivalent to feeding of commercial solid diet followed by 38–42 h exemption from food with ad libitum water as previously described in the interaction experiment. Since Maeda et al. (1977) reported coprophagy in rabbits, the incident was kept minimal by housing the animal in the metabolic cage with a feces collecting pan underneath.

To empty the solid residuals in the stomach, a vinyl tube was inserted into the rabbit stomach and 50–100 ml of warm washing fluid (0.2% NaCl solution at pH 1.2 and 37°C) was injected. The fluid in the stomach was rapidly withdrawn by suction with a syringe. This procedure was repeated until the fluid withdrawn was virtually free of solid material. For the determination of gastric emptying, 80 ml of phenol-red containing solution (10% w/v in the washing fluid) with and without diphenhydramine was injected into the rabbit stomach through the vinyl tube. After the gastric contents were thoroughly mixed, 1 ml of the gastric fluid was collected for the analysis of initial phenol-red concentration (C_0). The initial volume of the gastric content (V_0) was calculated from the initial phenol-red concentration. Half-hour post-introduction of the non-absorbable marker, the gastric content was emptyed as completely as possible. The volume of this gastric content (V_1) was measured and its phenol-red concentration (C_1) subsequently analyzed. In order to remove any residual gastric content, a second aliquot of 80 ml of washing fluid was injected. The washing fluid was recovered and the concentration of phenol-red in the washing (C_2) determined. The volume of the residual gastric content (V_2) was calculated as follows:

$$V_2 = \frac{80\ C_2}{C_1 - C_2}$$

The weight percentage of phenol-red remaining in the rabbit stomach, PR%, was calculated at 0.5 h post-injection.

$$PR\% = \frac{C_1(V_1 + V_2)}{C_0 V_0} \times 100$$

Assays of phenol-red were performed spectrophotmetrically after alkalization of samples by adding 1 N NaOH.

Statistical analysis

The significance of difference among the 3 treatments was evaluated using two-way analysis of variance (ANOVA). In addition, the significance of difference between any two treatments was evaluated using the multiple-range test of Duncan (1955). A probability value (P) of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

The mean concentrations of plasma acetaminophen after oral administration of acetaminophen alone or in combination with diphenhydramine at 10 and 20 mg/kg doses are shown in Table 1 and depicted in Fig. 1. At the low dose of 10 mg/kg, diphenhydramine significantly reduced the mean plasma acetaminophen concentrations at 0.25 and 0.5 h from 54.5 ± 3.1 to 39.3 ± 8.6 and from 41.7 ± 2.8 to 33.3 ± 4.3 μg/ml, respectively (Duncan's multiple-range test, $P < 0.05$). Similarly, diphenhydramine at 20 mg/kg dose significantly reduced the mean plasma acetaminophen concentrations at 0.25 and 0.5 h from 54.5 ± 3.1 to 32.9 ± 4.4 and from 41.7 ± 2.8 to 33.0 ± 2.3 μg/ml, respectively ($P < 0.05$). However, the degree of concentration reduction did not appear to be proportional to diphenhydramine dose. On the other hand, diphenhydramine at high dose significantly elevated the mean plasma acetaminophen concentrations at 2.0, 3.0 and 4.0 h from 8.4 ± 0.5 to 10.9 ± 0.9, from 4.0 ± 0.2 to 6.2 ± 0.5 and from 2.2 ± 0.2 to 3.6 ± 0.2 μg/ml, respectively ($P < 0.05$). However, no significant elevation of plasma acetaminophen was observed at low dose of diphenhydramine at the same time intervals.

The maximum plasma concentration (C_{max}) and the time taken to reach C_{max} (T_{max}) of acetaminophen after oral administration of acetaminophen alone or in combination with diphenhydramine are shown in Table 2. Diphenhydramine at 10 and 20 mg/kg doses significantly reduced C_{max} of acetaminophen from 54.5 ± 3.1 to 40.7 ± 7.9 and 36.3 ± 3.2 μg/ml, respectively ($P < 0.05$). However, no significant difference was found in T_{max} of acetaminophen among treatments, although 3 of the 6 rabbits demonstrated an increased T_{max} of acetaminophen at 20 mg/kg dose of diphenhydramine. A significant

TABLE 1

PLASMA ACETAMINOPHEN CONCENTRATIONS, EXPRESSED AS MEAN ± S.D., AFTER ORAL ADMINISTRATION OF ACETAMINOPHEN ALONE OR IN COMBINATION WITH DIPHENHYDRAMINE IN RABBITS

Time (h)	Mean plasma acetaminophen concentrations (μg/ml)			Statistics (ANOVA)
	Control	Diphenhydramine		
		10 mg/kg	20 mg/kg	
0.25	54.5 ± 3.1	39.3 ± 8.6 *	32.9 ± 4.4 *	$P < 0.05$
0.50	41.7 ± 2.8	33.3 ± 4.3 *	33.0 ± 2.3 *	$P < 0.05$
0.75	29.5 ± 1.9	26.6 ± 3.4	27.2 ± 2.5	NS
1.0	21.7 ± 1.2	20.8 ± 2.7	24.4 ± 1.5	NS
1.5	13.3 ± 0.7	14.3 ± 1.9	15.4 ± 1.0	NS
2.0	8.4 ± 0.5	9.5 ± 1.2	10.9 ± 0.9 *	$P < 0.05$
3.0	4.0 ± 0.2	5.2 ± 0.5	6.2 ± 0.5 *	$P < 0.05$
4.0	2.2 ± 0.2	2.4 ± 0.5	3.6 ± 0.2 *	$P < 0.05$
5.0	1.3 ± 0.2	1.6 ± 0.4	1.7 ± 0.1	NS
6.0	0.8 ± 0.2	0.9 ± 0.2	1.2 ± 0.1	NS

* Significantly different from control, $P < 0.05$ (Duncan's multiple-range test).

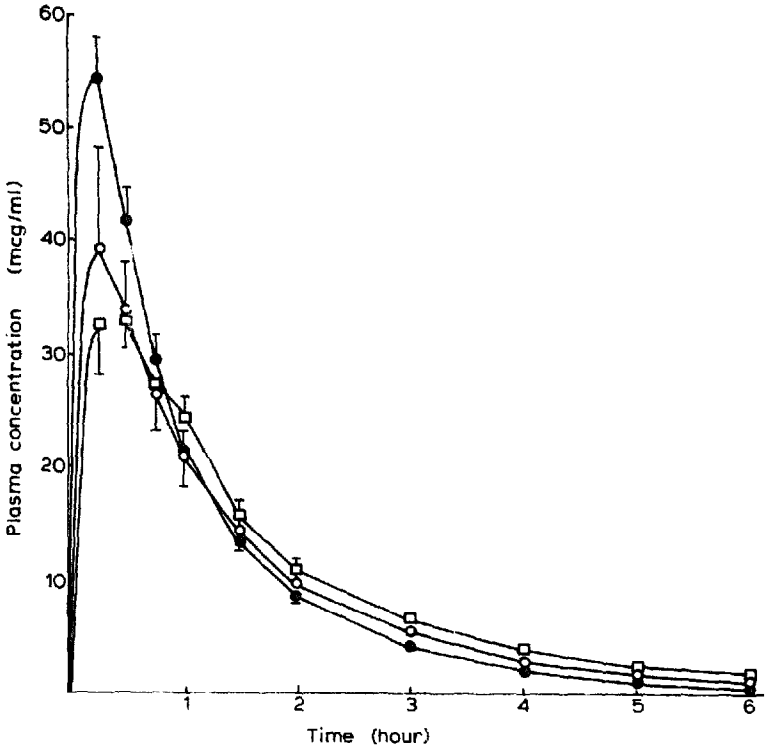

Fig. 1. Plasma concentration–time curves for 100 mg/kg oral dose of acetaminophen alone (●) and in combination with diphenhydramine, 10 mg/kg (○) and 20 mg/kg (□). Each point represents the average concentration of 6 rabbits.

TABLE 2

EFFECT OF DIPHENHYDRAMINE ON THE MAXIMUM PLASMA CONCENTRATION (C_{max}) AND THE TIME TAKEN TO REACH THE MAXIMUM PLASMA CONCENTRATION (T_{max}) OF ACETAMINOPHEN IN RABBITS

Rabbit	C_{max} (μg/ml)			T_{max} (h)		
	Control	Diphenhydramine		Control	Diphenhydramine	
		10 mg/kg	20 mg/kg		10 mg/kg	20 mg/kg
A	67.7	72.7	41.1	0.25	0.25	0.25
B	45.8	22.6	38.5	0.25	0.50	0.50
C	49.4	34.4	25.6	0.25	0.25	0.75
D	58.1	23.8	34.4	0.25	0.50	0.50
E	53.3	36.6	30.4	0.25	0.25	0.25
F	52.9	54.1	47.6	0.25	0.25	0.25
Mean ± S.E.	54.5 ± 3.1	40.7 ± 7.9 *	36.3 ± 3.2	0.25 ± 0.00	0.33 ± 0.05	0.42 ± 0.08
Statistcs	ANOVA, $P < 0.05$			ANOVA, NS		

* Significantly different from control, $P < 0.05$ (Duncan's multiple-range test).

prolongation of T_{max} may become obvious at a larger diphenhydramine dose. The distinction of T_{max} among treatment groups may also be observed by increasing the number of rabbit studies or upon more frequent sampling during the absorption phase.

Table 3 presents the area under the plasma concentration–time curve (AUC_0^∞) and the elimination half-life ($t_{1/2}$) of acetaminophen after oral administration of the drug alone or in combination with diphenhydramine. No significant differences were found in AUC_0^∞ and $t_{1/2}$ of acetaminophen among treatments. These results, together with the observation on T_{max}, indicate that diphenhydramine probably decreases the rate but not the extent of acetaminophen absorption. In a comparable study, McGillveray and Mattok (1972) found that the concomitant intake of food, which has been shown to delay gastric emptying (Kojima et al., 1971), affected the rate but not the extent of acetaminophen absorption.

In order to elucidate the mechanism of acetaminophen–diphenhydramine interaction, gastric emptying experiments were conducted in the presence of 10 and 20 mg/kg doses of diphenhydramine. As shown in Table 4, the weight percentage of phenol-red remaining in the rabbit stomach was significantly different among treatments. Diphenhydramine at 10 and 20 mg/kg doses significantly increased the weight percentage of phenol-red remaining in the rabbit stomach from 25.1 ± 3.8 to 32.1 ± 3.2 and 43.8 ± 3.8%, respectively ($P < 0.05$). Furthermore, the effect of diphenhydramine on gastric emptying was dose-related. The relative gastric emptying rates were 100% for the control, 78% at the 10 mg/kg diphenhydramine dose, and 57% at the 20 mg/kg diphenhydramine dose, respectively. Thus, the effects of diphenhydramine on gastric emptying, as referenced to the control group, were 22 and 43% for the 10 mg/kg and the 20 mg/kg doses, respectively. These data (Table 4) indicate direct proportion between the diphenhydramine dose and the inhibition of gastric emptying.

Changes in drug absorption associated with delayed or accelerated gastric emptying have been reported for a number of drugs in man (Consolo et al., 1970; Manninen et al.,

TABLE 3

EFFECT OF DIPHENHYDRAMINE ON THE AREA UNDER PLASMA CONCENTRATION – TIME CURVE (AUC_0^∞) AND THE ELIMINATION HALF-LIFE ($t_{1/2}$) OF ACETAMINOPHEN IN RABBITS

Rabbit	AUC_0^∞ (μg · h/ml)			$t_{1/2}$ (h)		
	Control	Diphenhydramine		Control	Diphenhydramine	
		10 mg/kg	20 mg/kg		10 mg/kg	20 mg/kg
A	72.6	88.7	74.6	1.05	0.98	1.12
B	63.5	34.9	61.5	1.72	1.40	1.16
C	58.7	47.5	60.6	1.05	0.97	1.04
D	63.2	50.2	60.6	1.67	1.67	1.91
E	55.3	65.3	51.5	1.19	1.60	1.27
F	59.4	62.7	66.3	1.02	1.18	1.05
Mean ± S.E.	62.1 ± 2.4	58.2 ± 7.6	62.5 ± 3.1	1.28 ± 0.13	1.30 ± 0.12	1.26 ± 0.13
Statistics	ANOVA, NS			ANOVA, NS		

TABLE 4

EFFECT OF DIPHENHYDRAMINE ON THE WEIGHT PERCENTAGE OF PHENOL-RED REMAINING IN THE RABBIT STOMACH 0.5 h AFTER INJECTION, (PR%)

Rabbit	Phenol-red remaining (PR%)		
	Control	Diphenhydramine	
		10 mg/kg	20 mg/kg
G	18.8	23.3	36.2
H	41.2	38.7	48.9
I	22.2	31.6	42.4
J	21.0	36.4	48.2
K	30.7	40.4	56.5
L	16.5	22.0	30.8
Mean ± S.E.	25.1 ± 3.8	32.1 ± 3.2 *	43.8 ± 3.8 *
Relative gastric emptying rate	100.0	78.2	57.3
Inhibition of gastric emptying	0	21.8	42.7
Statistics	ANOVA, $P < 0.005$		

* Significantly different from control, $P < 0.05$ (Duncan's multiple-range test).

1973; Nimmo et al., 1975; and Algeri et al., 1976). Propantheline delays gastric emptying and markedly decreases the rate of acetaminophen absorption, while metoclopramide, which accelerates gastric emptying, markedly increases the rate of acetaminophen absorption (Nimmo et al., 1973). Atropine, a typical anticholinergic drug, decreases the rate of lidocaine absorption by delaying the gastric emptying (Adjepon-Yamoah et al., 1974). In a recent report, Imamura et al. (1980) have shown that chlorpromazine and atropine, which have been shown to delay gastric emptying, decrease the rate of acetaminophen absorption in rabbits. Heading et al. (1973) have demonstrated a highly significant correlation between the rate of gastric emptying and rate of acetaminophen absorption.

Recently, Hindmarsh et al. (1978) have reported that diphenhydramine markedly inhibits the oxidative metabolism of methaqualone. Diphenhydramine is chemically similar to SKF 525-A which is a typical inhibitor of oxidative drug metabolism. Consequently, diphenhydramine may affect the oxidative metabolism of acetaminophen. However, since major metabolites of acetaminophen are well known to be formed by direct glucuronide and sulfate conjugation at the 4-hydroxy position (Koch-Weser, 1976), diphenhydramine would have little effect, if any, on the metabolism of acetaminophen. In addition, since tissue distribution occurred rapidly for acetaminophen, the observation of similar half-lives for acetaminophen in the control and the treatment groups ruled out the possibility of metabolism inhibition by diphenhydramine.

In summary, evidence has been presented in this study that diphenhydramine delays gastric emptying in rabbits. The overall effects of diphenhydramine administration on C_{max}, T_{max}, AUC_0^{∞} and $t_{1/2}$ of acetaminophen lead us to conclude that diphenhydramine affects the rate of acetaminophen absorption in rabbits by delaying the gastric emptying.

Although the suitability of rabbit model for drug absorption study remains conditional (Chiou et al., 1969; Crouthamel et al., 1975; Maeda et al., 1975), we have been able to obtain reproducible data under the specified fasting conditions and clearly demonstrated the gastric emptying effect of diphenhydramine. The clinical significance of acetaminophen–diphenhydramine interaction awaits further investigation in man.

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