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142. Akio Hoshi, Reiko Yanai, and Kazuo Kuretani: Effect of Terephthalic Acid upon Sulfadimethoxine Contents in Blood Plasma.

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The effect of terephthalic acid (TPA) upon sulfadimethoxine contents in blood plasma was studied.

The drug contents in blood plasma were increased by TPA feeding both in the rat and in the rabbit. The maximum contents of the drug in blood plasma were not so affected. However, 24 and 48 hr. after a single administration, they showed significant increases from that of the control.

The drug excretion amounts in the urine of the TPA group was lower than those of the control and the plasma half-life of the drug was elongated by TPA administration, about 1.5 times in the rat and 1.9 times in the rabbit.

Absorption of the drug through the gastrointestinal tract decreased with TPA administration and high contents of TPA might have a hindering effect on drug absorption from the small intestine.

The contests of N^4-acetate of the drug were increased by TPA administration.

This increasing effect of TPA upon sulfadimethoxine seems to be concerned with excretion from the kidney. As a result, it is supposed that the plasma half-life of the drug is elongated.

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The drug potentiating effect of terephthalic acid (TPA) has been studied by several groups of workers. The blood plasma contents of tetracycline-type antibiotics were increased by TPA feeding.[1~4] It has been shown by our group in recent years that TPA also has the effect of increasing the thiamine contents in blood plasma.[5]

The purpose of the work described below is to determine the effect of TPA upon sulfadimethoxine contents in blood plasma. The drug contents in blood plasma, liver and kidney increased and excretion in urine decreased, while at the same time the plasma half-life of the drug was elongated by TPA feeding.

Materials and Methods

Animals——The animals used were rats (female only) and rabbits. The rats used were Wistar King-A strain with a body weight of 200 g. The control group was fed only a special diet (a product of Central Laboratory for Experimental Animals (CLEA), Tokyo: CA-1). The TPA group was fed the diet supplemented by TPA at a ratio of 0.5% in the basal one for 7 days before the drug administration. The diet and water were offered *ad libitum*.

The same number of random-bred rabbits were used: male and female weighing 2 kg. The basal diet was RA-1 (a product of CLEA). The TPA diet was supplemented with TPA at a ratio of 0.5% in the basal one.

Materials——Sulfadimethoxine was J. P. grade. Terephthalic acid was 99.7% pure (Teijin Limited).

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Analytical Methods——Sulfadimethoxine was determined by the Bratton-Marshall method,[6] metabolites of which were determined with ethylacetate extraction.[7] A Bausch and Lomb spectronic 20 colorimeter was used for colorimetry.

Administration of the Drug——The sulfadimethoxine was suspended at a ratio of 2.5% in the 0.5% sodium carboxymethyl cellulose (CMC) solution by a glass homogenizer. This suspension was administered orally through a stomach tube in a dosage of 50 mg./kg. body weight.

The same rabbits were used for both the control and the TPA treatment groups. They were fed with the basal diet for 2 weeks from 1 week before the drug administration to the last day of the control experiment. Then the diet was changed to the TPA diet and the experiment was continued in the same manner.

Collection of Plasma——In the rat experiment, each group of 5 rats was used at 0, 4, 24 or 48 hr. after the drug administration and 4 ml. of each rat blood sample was drawn from the vena cava under light ether anesthesia. In the rabbit experiment, 2 ml. blood sample was drawn by heart puncture immediately before the drug administration for the control, and at intervals of 2, 4, 6, 8, 24, and 48 hr. after the drug administration for the test. They were centrifuged (3000 r.p.m. for 10 min.) to separate the blood plasma.

Collection of Liver and Kidney——The livers and kidneys of the rats were collected after bleeding from the carotid artery.

Collection of Urine——Urine was collected in the metabolism cage. Acetic acid (99%) had been put into the collecting flask, 1 ml. each for the rats and 5 ml. for the rabbits. The drug and metabolites were determined every 24 hr. for 4 days after the drug administration.

Determination of Drug Absorption from the Gastrointestinal Tract——The rat was not fed during the night prior to the experiments but water was allowed freely. The animals were anesthetized with subcutaneous sodium pentobarbital (75 mg./kg.) and the small intestine was tied at 10 cm. intervals. The upper and lower sides of these loops were not used, so about six loops were used.

Drug solutions were prepared at the final contents of 20 mg./ml. of sulfadimethoxine in 0.01*N* phosphate buffer (pH 6.5) containing 0.25% CMC and 0, 0.01, 0.1, 1.0 and 10.0 mg./ml. each of TPA. These solutions were made up isotonic with NaCl and adjusted at pH 6.5 but in the case of 10.0 mg./ml. of TPA, it was slightly hypertonic.

The drug solution (0.5 ml. each) was injected into the intestinal loops of two controls and four TPA treatments. Two hours after the administration, the animals were killed and the intestinal loops were collected. These were cut and washed with isotonic saline. The drug contents in the wash and the intestinal wall were determined.

Results

Drug Contents in Blood Plasma of the Rat——Sulfadimethoxine contents in blood plasma were determined after the drug administration of a single oral dose of 50 mg./kg. of body weight.

The total drug contents in the blood plasma at 4, 24 and 48 hr. were 21.8, 13.4 and 6.8 mg./dl. in the control 20.6, 16.6 and 11.8 mg./dl. in the TPA group respectively.

Table I. Blood Plasma Contents of Sulfadimethoxine in the Rat after a Single Oral Dose of 50 mg./kg.

Time (hr.)		Drug contents (mg./dl.)[c]		
		4	24	48
Control	Total[b]	21.8±0.8	13.4±1.1	6.8±0.3
	Free[b]	20.4±1.3	12.9±1.4	5.9±0.2
TPA[a]	Total	20.6±2.6	16.6±0.6[e]	11.8±0.9[e]
	Free	18.8±2.2	15.2±0.8[d]	10.2±0.9[e]

a) They were fed TPA diet for 7 days.
b) Total and free were determined by Bratton-Marshall method.
c) Mean value ± SD. of each 5 rats.
d) Significantly greater than control ($P<0.05$).
e) Significantly greater than control ($P<0.001$).

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The drug contents of the TPA group were significantly greater than in the control at 24 and 48 hr. and it seemed to be somewhat lowered at 4 hr. but there were no significant differences. Free drug contents in the TPA group were higher than the control at 24 and 48 hr. These results are almost the same as in the case of the total drug (Table I).

The drug has a plasma half-life of about 25 hr. in the control and it was elongated to about 48 hr. by TPA feeding.

Plasma drug contents were increased and the plasma half-life was elongated by TPA feeding.

Drug Contents in Liver and Kidney——The drug contents in the livers and kidneys of the rats were determined. Changes of the drug contents in these organs were analogous to that of the blood plasma. It increased significantly at 48 hr. in the liver; 0.8 mg./100 g. in the TPA group and 0.4 mg./100 g. in the control, and at 24 and 48 hr. in the kidney; 2.7 and 1.4 mg./100 g. in the TPA group and 2.0 and 1.1 mg./100 g. in the control (Table II).

It was shown that the increase of the drug contents in the organs (as well as in the blood plasma) the TPA group was higher than in those of the control.

Table II. Sulfadimethoxine Contents in Liver and Kidney after a Single Oral Dose of 50 mg./kg. in the Rat

		Drug contents (mg./100 g.)[c]					
Time (hr.)		Liver			Kidney		
		4	24	48	4	24	48
Control	Total[b]	3.0±0.4	1.3±0.3	0.4±0.1	4.5±0.4	2.0±0.3	1.1±0.1
	Free[b]	3.0±0.3	1.3±0.3	0.1±0.1	4.5±0.4	1.9±0.3	1.0±0.1
TPA[a]	Total	2.8±0.9	1.4±0.2	0.8±0.2[e]	3.8±0.8	2.7±0.2[d]	1.4±0.1[d]
	Free	2.6±0.9	1.4±0.1	0.5±0.2[e]	3.6±0.9	2.6±0.1[d]	1.3±0.1[d]

a) They were fed TPA diet for 7 days.
b) Total and free were determined by Bratton-Marshall method.
c) Mean value ± SD. of each 5 rats.
d) Significantly greater than control ($P<0.01$).
e) Significantly greater than control ($P<0.001$).

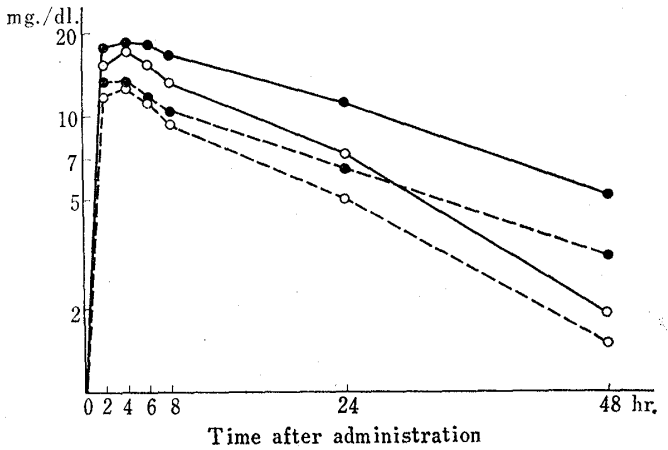

Fig. 1. Sulfadimethoxine Contents in Blood Plasma after a Single Oral Dose of 50 mg./kg. in the Rabbit

—●— TPA group (Total)
—○— Control group (Total)
·····●····· TPA group (Free)
·····○····· Control group (Free)

Drug Contents in Blood Plasma in the Rabbit——In order to eliminate the effect of individual variation on the drug distribution, the same rabbits were used for the control as for the TPA treatment.

Drug contents in blood plasma were determined after a single oral dose administration of 50 mg./kg. The drug contents in the blood plasma increased more in the TPA group than in the control in total and free amounts. The total amounts of the drug in the blood plasma at 2, 4, 6, 8, 24, and 48 hr. were 15.3, 17.4, 15.4, 13.1, 7.3, and 1.9 mg./dl. in the control and 17.8, 18.7, 18.2, 16.7, 11.0, and 5.2 mg./dl.

in the TPA group respectively. The maximum contents were observed at four hours in both groups with only slight difference between the two groups. The TPA group had shown significantly high contents at every period except 4 hr. (Table III).

The ratio of N^4-acetate to the total drug was greater in the rabbit than the rat, and furthermore, this ratio was increased by TPA feeding. It was 31% in the control and 41% in the TPA group at 24 hr. (Table IV).

The drug has a plasma half-life of about 15 hours in the control and about 22 hours in the TPA group. The plasma half-life was also elongated (as in the rat) by TPA feeding. The drug eliminating rates of the total and free amounts were almost the same in each group, as shown in two parallel lines in Fig. 1.

Table III. Sulfadimethoxine Contents in Blood Plasma after a Single Oral Dose of 50 mg./kg. in the Rabbit

Time (hr.)		Drug contents (mg./dl.)[a]					
		2	4	6	8	24	48
Control	Total	15.3±2.0	17.4±2.0	15.4±1.6	13.1±2.5	7.3±1.6	1.9±0.6
	Free	11.8±1.5	12.7±3.6	11.1±3.0	9.3±1.8	5.0±1.4	1.5±0.6
TPA	Total	17.8±2.0[b]	18.7±1.7	18.2±1.6[c]	16.7±2.2[c]	11.0±1.7[d]	5.2±1.3[d]
	Free	13.2±2.3	13.1±2.1	11.7±2.5	10.3±1.9	6.5±1.6[b]	3.1±1.1[d]

a) Mean value ± SD. of each 10 rabbits.
b) Significantly greater than control ($P<0.05$).
c) Significantly greater than control ($P<0.01$).
d) Significantly greater than control ($P<0.001$).

Table IV. Proportion of Sulfadimethoxine acetylated in Blood Plasma after a Drug Administration

Time (hr.)	Sulfadimethoxine acetylated (%)[c]					
	2	4	6	8	24	48
Control	23	27	28	29	31	21
TPA[a]	26	30	36	38	41	40
Control	34	30	35	40	39	40
TPA[b]	23	42	49	58	65	44

a) They were fed TPA diet for 7 days and were administered the drug orally.
b) They were administered the mixture of sulfadimethoxine and TPA.
c) Mean value of each 10 rabbits.

Drug Excretion in Urine of the Rat——The contents of the excreted drug and their metabolites were determined. Intact drug was excreted at 21% of the administered drug in the control and 14.1% in the TPA group for 4 days. N^4-acetates were excreted at 22.9% and 23%, glucuronides at 4.9% and 3.6% in the control and TPA group respectively. Total excretion amounts were 48.8% in the control and 40.5% in TPA group for 4 days (Table V).

The total amounts of drug excretion in TPA group decreased due to the decrease of intact drug excretion. On the other hand N^4-acetate was not changed.

Drug Excretion in Urine of the Rabbit——The total amounts of the excreted drug for 4 days were 82.6% in the control and 70.6% in the TPA group. In the TPA group, there was less excretion in the 1st and 2nd days and more excretion in the third and fourth days than in the control group and finally there was a significant difference in the total amounts of the two groups (Table I).

Table V. Excretion of Sulfadimethoxine and Its Metabolited in Urine after a Single Oral Dose of 50 mg./kg. in the Rat

Period (day)		Excretion of the drug (%)[a]			
		Intact drug	N^4-Acetate	Glucuronides	Total
Control	0~1	13.0±3.7	14.8±2.0	2.8±1.0	
	1~2	4.3±0.5	4.5±0.6	1.0±0.0	
	2~3	2.5±0.6	2.3±1.0	0.8±0.5	
	3~4	1.5±0.6	1.3±0.5	0.3±0.5	
	Total	21.3±3.9	22.9±2.1	4.9±1.0	48.8±4.2
TPA	0~1	8.8±2.6	12.5±4.0	2.3±0.5	
	1~2	3.0±0.8	5.0±0.8	1.0±0.0	
	2~3	1.8±1.0	3.5±1.3	0.3±0.5	
	3~4	0.5±0.6	2.0±0.8	0	
	Total	14.1±4.2[c]	23.0±4.3	3.6±1.0[b]	40.5±6.1[b]

a) Mean value ± SD. of each 5 rats.
b) Significantly less than control ($P<0.05$).
c) Significantly less than control ($P<0.01$).

Table VI. Excretion of Sulfadimethoxine in Urine after a Single Oral Dose of 50 mg./kg. in the Rabbit

Time (day)	Excretion of the drug (%)[b]				
	1	2	3	4	Total[c]
Control	51.7±21.3	23.7±17.0	6.6±8.6	0.6±2.2	82.6±6.8
TPA[a]	38.4±22.3	20.2±12.7	7.8±3.8	4.3±4.3	70.6±14.6[d]

a) They were fed TPA diet.
b) Mean value ± SD. of each 10 rabbits.
c) Integral excretion for 4 days.
d) Significantly less than control ($P<0.05$).

Effect of Single Oral Administration of TPA——In an effort to determine the direct effect of TPA upon the drug contents in the blood plasma and the excretion in urine, a mixture of sulfadimethoxine and TPA was administered through a stomach tube.

The mixture of the two drugs was prepared at a ratio of 2.5% sulfadimethoxine and 10% TPA in a 0.5% CMC solution. For the control, 2.5% sulfadimethoxine in a 0.5% CMC solution was used.

The total plasma drug contents of the TPA group were lower than the control group at 2 hr. and higher at 6, 8 and 24 hr. after the administration. No significant difference from the control was shown at 4 and 48 hr. The free drug contents of the TPA group were lower than the control group at 2, 4, and 6 hr. There were no significant differences at 8, 24, and 48 hr. N^4-Acetate of the drug in the blood plasma was raised by TPA administration, this ratio was 39% in the control and 65% in the TPA group at 24 hr. (Table IV). The plasma half-life of the drug was not changed (Table VII).

There were no significant differences in excretion of the drug in urine between two groups (Table VIII).

High contents of TPA might have some inhibiting effect on the drug absorption through the gastrointestinal tract. The increase of the drug contents in the plasma might be lowered by this inhibition in the early stage of the experiment.

Table Ⅶ. Sulfadimethoxine Contents in Blood Plasma after a Single Oral Dose of 50 mg./kg. of Sulfadimethoxine and 200 mg./kg. of Terephthalic Acid Mixture in the Rabbit

Time (hr.)		Drug contents (mg./dl.)[c]					
		2	4	6	8	24	48
Control	Total[b]	15.4±2.0	16.2±1.0	15.0±1.3	11.7±1.1	5.7±1.9	1.5±0.7
	Free[b]	10.2±1.3	11.3±1.3	9.8±1.6	7.0±1.7	3.5±1.3	0.9±0.6
TPA[a]	Total	10.3±3.6[g]	15.5±2.5	16.0±0.6[d]	14.5±1.1[e]	7.8±1.7[d]	1.6±0.6
	Free	7.9±2.9[f]	8.9±2.0[g]	8.2±1.6[f]	6.1±2.0	2.7±1.4	0.9±0.7

a) They were administered the mixture of the drug and TPA.
b) Total and free were determined by Bratton-Marshall method.
c) Mean ± SD. of each 10 rabbits.
d) Significantly greater than control ($P<0.05$).
e) Significantly greater than control ($P<0.001$).
f) Significantly less than control ($P<0.05$).
g) Significantly less than control ($P<0.01$).

Table Ⅷ. Excretion of Sulfadimethoxine in Urine after a Single Oral Dose of 50 mg./kg. of Sulfadimethoxine and 200 mg./kg. of Terephthalic Acid Mixture in the Rabbit

Time (day)	Excretion of the drug (%)[b]				
	1	2	3	4	Total[c]
Control	46.5±16.9	25.8±13.7	7.6±10.2	0.7±1.2	80.6±3.9
TPA[a]	38.6±19.4	29.8±18.4	8.3±8.1	0.3±0.8	77.0±9.7[d]

a) They were administered the mixture of the drug and TPA.
b) Mean value ± SD. of each 10 rabbits.
c) Integral excretion for 4 days.
d) Not significantly different from control ($P>0.05$).

Drug Absorption through the Small Intestine——The direct effect of TPA upon the drug absorption from the intestinal loop was studied to *in situ*, to determine whether high contents of TPA will affect drug absorption through the gastrointestinal tract or not.

The drug absorption was 100, 79.5, 91.8, 79.5, and 85.0% in the contents of 0, 0.01, 0.1, 1.0, and 10.0 mg./ml. of TPA respectively, and the tendency to inhibit drug absorption was slightly recognized at each content of TPA, but there were no significant differences among them (Table Ⅸ). The drug contents of intestinal wall samples were 25.0 mg./100 g. of wet tissue in all cases.

Table Ⅸ. Absorption of Sulfadimethoxine from the Intestinal Loop (*in situ*) in the Rat

TPA contents (mg./ml.)	Drug absorption (%)[a]				
	0	0.01	0.1	1.0	10.0
	100.0±18.1	79.5±30.7	91.8±22.8	79.5±24.7	85.0±25.0

a) Mean value ± SD. of each 10 rats.

Discussion

Previous investigations of the potentiating effect of TPA have dealt almost exclusively with tetracycline-type antibiotics (Oxytetracycline and Chlortetracycline).

Furthermore, such interesting effects of TPA as the growth acceleration in the fowl[8] and in the mouse[9] and the increasing of the thiamine contents in blood plasma of the rat and fowl[5] have been studied.

In the present investigation, TPA feeding effected the increase of sulfadimethoxine contents in the blood plasma of the rat and the rabbit. At the same time excretion of the drug decreased and the plasma half-life of the drug was elongated. So, one of the effects of TPA upon increasing the drug contents in blood plasma seems to be lowering drug excretion in urine.

Antibiotics contents in plasma increased with TPA feeding at a ratio of 1.5 times in chlortetracycline[3] and 1.7 times in oxytetracycline at four hours[10] and 1.7 times in thiamine at four hours.[5] On the other hand, the increasing rate of sulfadimethoxine contents in the blood plasma of the control and the TPA groups was almost the same in the early stage. The maximum content was reached at four hours in both groups.

The increase by TPA feeding of sulfadimethoxine contents in the blood plasma was observed in the rat and also in the rabbit. Though the drug contents in the plasmas of these two species were different, the increase of the drug contents was significant at 24 and 48 hours after the administration in both cases. Furthermore, the plasma half-lives of sulfadimethoxine were also elongated. In the case of thiamine, the plasma half-life of thiamine was not changed by TPA feeding.[5]

There was a difference in the excretion rate of the drug in these two species but the drug excretion was decreased by TPA feeding in both cases.

In the rabbits administered the mixture of the drug and TPA, the effect of TPA upon the drug contents in the blood plasma was somewhat different from that of the effect of TPA feeding. The contents of TPA in the gastrointestinal tract were higher than that of the TPA feeding group. As a result, the high contents of TPA might have a negative effect on the drug absorption through the intestinal wall. The changes of TPA contents in plasma are supposed as an another cause of this difference. In TPA feeding, the TPA content in plasma is constant, on the other hand, in single oral administration of TPA, it is higher in a short period than the former, however, it is gradually lowered into lower concentration than in the TPA feeding.[11] From these results, it was suggested that the drug was absorbed slowly and its contents of blood plasma was lowered. The increasing effect of TPA might be continued for a limited period in the single oral administration of TPA.

In view of the above facts, we conclude that the increasing effect of TPA upon the sulfadimethoxine contents in blood plasma is a different mechanism from that of TPA upon antibiotics and thiamine.

The contents of free drug in the blood plasma decreased with TPA administration especially with a single administration, in other words, N^4-acetate increased with TPA administration.

The increasing effect of TPA upon sulfadimethoxine seems to be concerned mainly with excretion from the kidney and not with absorption through the gastrointestinal tract. High contents of TPA tend to inhibit drug absorption from the intestine.

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