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THE EFFECT OF SULPHAMETHOXYPYRIDAZINE ON LIVER AND PLASMA LEVELS OF VITAMIN A IN RATS

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1 Long-acting sulphonamides are highly bound to plasma proteins; the present study deals with the effects of this binding property upon a vitamin A compound also transported by plasma proteins.

2 Sulphamethoxypyridazine was administered in rats either orally or by intraperitoneal injection.

3 A significant fall in plasma vitamin A level and an increase in hepatic vitamin A concentration were observed.

4 These results suggest an interference by sulphamethoxypyridazine with the transport of vitamin A, either through competition between the drug and vitamin A for binding sites of plasma proteins, or through altered secretion of the vitamin from the liver.

Introduction

During the past 15 years there has been increasing awareness that many commonly prescribed drugs can interact with endogenous compounds for binding sites in cells and plasma proteins (Anton, 1973), such interaction possibly affecting the concentration of the circulating endogenous compounds. These studies have been carried out especially on hormone transport (Oppenheimer, 1968; 1973; Larsen, 1972), but few data have been reported about interactions with plasma-protein transported vitamins. It is also widely recognized that most, if not all drugs are bound to plasma proteins for transport, but the role of this phenomenon in determining their overall pharmacological properties has not yet been clearly defined. During the past decade, a type of long-acting sulphonamide with high affinity for plasma proteins has been described (Weinstein, 1965), one of these, sulphamethoxypyridazine (SMP) being bound to plasma albumin to the extent of 85%. Since vitamin A is also transported by plasma proteins forming a complex with Retinol Binding Protein (RBP) α-globulin (Goodman, 1973) it seemed of interest to study the interaction between vitamin A and sulphonamides.

This paper describes the effect of high doses of SMP on plasma and hepatic vitamin A levels in rats.

Methods

Adult Fischer rats of either sex (CDF Fischer 344) bred in this department were used throughout all the experiments. The animals were housed individually and maintained on a standard commercial rat chow (containing 24% protein) and tap water *ad libitum* in a room where lighting and temperature (23 ± 2°C) were controlled.

Experiment I: Plasma vitamin A levels after sulphamethoxypyridazine administration

Two separate groups of 10 animals were given a buffered saline solution (pH 8) of SMP (14 mg/100 g body weight) by stomach tube once daily; they were killed after 7 and 14 days respectively. The control group (10 animals) received an equal volume of saline (0.9% w/v NaCl solution). The body weight was recorded daily and the skin, eyes and oral mucosa were observed carefully. Routinely, animals were starved overnight before they were killed. Animals were exsanguinated and blood was collected. Plasma vitamin A levels were measured according to the method of Bessey, Lowry, Brock & López (1946).

Experiment II: Relationship between dose of sulphamethoxypyridazine administered and plasma vitamin A levels

(a) *Intragastric administration.* Thirty-five three-week old rats of similar weight (50 ± 3 g) were distributed randomly into 5 groups receiving SMP in the following daily doses by stomach tube for 20 days: 1.4; 2.8; 4.2 and 7.1 mg/100 g body weight, the control group receiving saline. The animals were

observed for overt signs of toxicity and body weights were recorded daily. The rats were killed by exsanguination 16 h after the last administration of SMP. SMP and vitamin A were determined in plasma by the method of Bratton & Marshall (1939), and Bessey *et al.* (1946) respectively; the liver was homogenized in distilled water at 0.1% and vitamin A was determined in the homogenates.

(b) *Intraperitoneal administration:* Fifty three-weeks old rats were distributed in five groups receiving SMP in buffered (pH 6.9) saline in the doses indicated above. All determinations were run in triplicate. In all cases the significance of differences between results

Table 1 Effect of sulphamethoxypyridazine (SMP) treatment on vitamin A plasma levels

Length of treatment with SMP	*Plasma vitamin A level (μg/100 ml)*
No treatment (controls)	35.3 ± 5.02 (10)
7 days	ND (10)
14 days	ND (10)

ND = not detectable.
Animals were treated as described in the Methods section under Experiment I. The values represent the means ± s.e. mean with the number of observations in parentheses.

Table 2 Effect of different doses of sulphamethoxypyridazine (SMP) administered by stomach tube on vitamin plasma level

Groups	*No. of animals*	*Age of animal in days*[1]	*SMP (μg/100 ml)*		*Vitamin A (μg/100 ml)*
Control	7	42			34.57 ± 4.04
SMP treated					
1.4 mg/100 g body weight	7	42	[2]FS	= 0.045	24.59 ± 5.22*
			[3]TS	= 0.062	
2.8 mg/100 g body weight	7	42	FS	= 0.057	25.53 ± 4.60**
			TS	= 0.085	
4.2 mg/100 g body weight	7	42	FS	= 0.071	18.35 ± 4.64**
			TS	= 0.114	
7.1 mg/100 g body weight	7	42	FS	= 0.423	1.27 ± 1.20

[1] Age at the time animals were killed; [2] FS free SMP; [3] TS total SMP.
Animals were treated as described in the Methods section under Experiment IIa. The values represent the means ± s.d. of 7 observations.
* $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$, when compared with corresponding control values.

Table 3 Effect of different doses of sulphamethoxypyridazine (SMP) administered intraperitoneally on SMP in plasma and on hepatic vitamin A levels

			Plasma			*Liver*
Groups	*No. of animals*	*Age of animals in days*[1]	*Sulphonamide (μg/100 ml)*		*Vitamin A (μg/100 ml)*	*Vitamin A (μg/g)*
Control	10	42			33.76 ± 6.19	104.79 ± 25.87
SMP treated						
1.4 mg/100 g body weight	10	42	[2]FS	= 0.043	23.91 ± 4.48*	80.06 ± 22.56
			[3]TS	= 0.062		
2.8 mg/100 g body weight	10	42	FS	= 0.047	14.52 ± 6.01**	193.55 ± 23.41
			TS	= 0.095		
4.2 mg/100 g body weight	10	42	FS	= 0.069	14.14 ± 2.12**	164.84 ± 16.00**
			TS	= 0.185		
7.1 mg/100 g body weight	10	42	FS	= 0.100	4.84 ± 2.93**	200.48 ± 16.29**
			TS	= 0.127		

[1] Age at the time animals were killed; [2] FS free SMP; [3] TS total SMP.
Animals were treated as described in the Methods section under Experiment IIb. The values represent the mean ± s.d. of 10 observations.
* $P < 0.01$; ** $P < 0.001$, when compared with corresponding control values.

was analysed by the use of Student's test for independent means (Snedecor, 1956).

Results

Effect of sulphamethoxypyridazine on plasma vitamin A levels

Table 1 shows that the intragastric administration of high doses of SMP caused a drop in plasma vitamin A circulating levels which was already evident after 7 days treatment. Clinically the animals showed skin and mucosal lesions even at the fifth day of treatment: 7 of 10 animals receiving SMP for 14 days showed xerophthalmia, nasal chaps, red conjuntiva with scabs in the eyelids.

Dose-effect relationship

The stomach tube administration of SMP over 20 days caused a retardation of body weight gain, which correlated well with the dose. Similar findings were obtained with intraperitoneal administration of SMP over the same period of time.

Table 2 shows the effect of different doses of SMP on plasma vitamin A levels. A relationship was observed between the dose of SMP administered and the plasma vitamin A level, so that the higher the dose, the greater the drop in plasma vitamin A concentration.

When the sulphonamide was administered intraperitoneally, a drop in plasma vitamin A levels was also observed and, at the same time, a significant increase in hepatic vitamin A concentration was found (Table 3).

Discussion

Our results have demonstrated that prolonged administration of high doses of SMP either by stomach tube or by intraperitoneal injection causes a fall in vitamin A plasma levels which is already noticeable (results not shown) on the fifth day of administration. These low levels of vitamin A in plasma are accompanied by the typical skin and mucosal lesions of avitaminosis A.

Treated animals also show an increase in hepatic vitamin A level. It is known that under physiological conditions vitamin A is stored in liver and transported by the plasma (Garbers, Gillman & Peisach, 1960) after its binding to a complex formed by the prealbumin and α-globulin (Goodman, 1973). SMP is also transported by the plasma largely bound to plasma proteins (Anton, 1973), mainly to albumin and when its blood concentration is very high, also to globulins. It is therefore possible to explain the present results on the basis of a competition between vitamin A and SMP for binding sites of plasma proteins, although it is also possible that SMP interferes in some other way with the secretion of vitamin A from the liver into the blood.

The doses of SMP administered to the rat in these experiments are high as compared with those used therapeutically in man. Nevertheless, the present results raise the possibility that patients given the drug for a long time may develop alterations in vitamin A metabolism.

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