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A PRELIMINARY STUDY ON THE ACUTE TOXICITY OF TARTRAZINE IN RABBIT AND GUINEA PIG

by
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要 約

食用色素 Tartrazine을 토끼의 경우 7.5~50mg/kg, 물모트의 경우 1,000~2,000mg/首를 經口 또는 腹膜注射, 靜脈注射로 投與하여 그 動物에 있어서의 色素의 毒性을 觀察調査하였다. 血淸은 化學分析하였고 各 臟器는 현미경的 觀察을 通하여 調査하였다.

그 結果 使用한 量의 色素로서 大部分의 臟器가 充血 또는 出血되었고 特히 肝臟機能障害를 認定할 수 있었다.

Tartrazine is one of the widely used dyes in foods, drugs, and cosmetics legally in most countries. It is also synonymously named as C.I. Food Yellow 4, FD and C Yellow No. 5, L-Gelb 2, or E.E.C. Serial No. E 102,[1] a trisodium

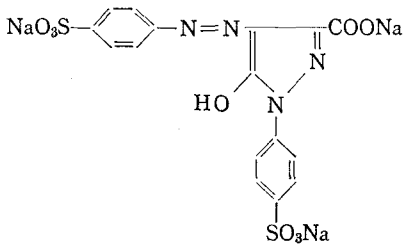

Fig. 1 Structure of the tartrazine

salt of 5-hydroxy-1-p-sulfophenyl-4-(p-sulfophenylazo) pyrazol-3-carboxylic acid (Fig. 1).

Because of the fact that the dye is commonly used in foods, interest in their pharmacology and toxicology has been aroused. Practically, the effect of its wide use on human health probably insignificant on the ground that it is poorly absorbed from the intestinal tract and may not be hazardous as long as the amount of its intake is kept under the level causing no significant toxicological effect, that is, 1% in the diet, equivalent to 750 mg/kg body weight for rats and 0-7.5 mg/kg body-weight for men.[1]

Nevertheless, the compound still has additional pharmacologic significance due to its participation in the excretory transport process of the liver and kidney as reported by serveral workers.[2,3] Although the demonstration of the fact that the compound was actively concentrated by slices of rabbit kidney cortex,[4] suggesting biliary excretion, the results only demostrate what liver and kidney are capable of achieving when appropriate quantities of dyes are made available for excretion as emphasized by the author.

In addition to this, the biochemical studies in rats and rabbits are fairly extensive. The long-term toxicity study on mice suggests even the formation of liver tumors,[5] notwithstanding various reports, including the study on rats,[6] showing that no dietary carcinogenic effect of the compound. The short term studies report generally no significant abnormalities;[1] however, they are confined to the amount acceptable to each animals they used.

In this respect, the author was tempted to study the acute toxicological effect of the dye in the present preliminary paper, using rabbits and guinea pigs, and significant abnormalities were found.

METHODS

Animals:

Rabbits weighing approximately 1.5kg. were divided into 6 groups regardless sex, each group being consisted of oral and parenteral subgroups. The water-suspended dye was intubatedto the oral groups daily in the early morning with the volumes equivalent to 7.5mg, 25 mg, and 50 mg/kg. body weight respectively, while the parenteral groups were fed via intravenous injection of the dye with the same series of amount as indicated above.

Guinea pigs, weighing approximately 260gm., were also divided into 3 groups, one being control and the other two receiving 1,000mg. and 2,000 mg. daily per capita by means of injection into the peritoneal cavity, each group being consisted of 5 animals.

Sample preparations:

The rabbits were fed *ad libitum* and sacrificed after 10 days of successive adminstration of the dye with a blow on the head; and cardiac puncture was made in order to obtain blood specimens and the serum was separated for analysis. In the guinea pig experiment the same procedure was employed, but the liver was homogenized additionally with the ice-cold 0.25*M* sucrose solution to obtain 20% (w/v) homogenate.

The internal organs, brain, lung, liver, kidney, and bone-marrow, were excised and the fresh tissues were subjected to fixation with formalin and stained with hematoxylin-eosin to study microscopically.

Chemical analyses of the serum and 20% liver tissue homogenate were performed with regard to the total protein, total and esterfied cholesterol, alkaline phosphatase, and transaminases. Conventional routine methods were employed: total protein by the biuret method,[7] cholesterol by the Shoenheimer and Sperry method,[8] alkaline phosphatase by the Gutman's method,[9] and the transaminase by the Reitman & Frankel's method.[10]

Table I. Serum analysis of the tartrazine administered rabbits

		7.5mg/kg for 10 days		25mg/kg for 10 days		50mg/kg for 10 days	
	Control	Per Os.	intravenous	Per Os.	intravenous	Per Os.	intravenous
Cholesterol, total mg%	90	95	50	90	55	90	65
// ester mg%	50	55	30	50	10	25	15
GOT unit	95	96	45	67	60	90	53
GPT unit	85	92	38	88	51	83	57
Protein, total g%	7.1	7.3	7.1	7.1	6.9	7.3	7.3
albumin g%	4.9	4.9	4.9	4.9	4.9	4.9	4.9

Table II Serum and liver analysis of the tartrazine administered guinea pig

	Serum			20% Liver Homogenate		
	Control	1,000mg for 5 days	2,000mg for 1 day	Control	1,000mg for 5 days	2,000mg for 1 day
Cholesterol, total mg%	123	147	183	106	106	138
// ester mg%	76	118	120	65	77	105
Alkaline phosphatase	12.4	11.8	19.5	3.6	11.9	16.8
GOT unit	60	630	650	165	920	965
GPT unit	44	74	120	80	480	500

RESULTS AND DISCUSSION

Chemical analysis of the blood and tissue:

As tabulated in Table I, cholesterol showed no significant variation in the oral group studied; however, it was dropped markedly as the doses of administration increased both in parenteral groups of the rabbits. No marked changes were also noted in the oral group with regard to the transaminases, while they decreased in the parenteral group. The total protein content has not changed, but rather remained constant, ranging from 6.9 to 7.3 gm%.

From Table II, it is quite apparent in guinea-pigs that, with the more massive amount of the dye cholesterol in the serum, either total or esterified, showed an impression of hypercholesterolemia, both in the serum and the liver tissue homogenate. The enzymes, alkaline phosphatase and transaminases, were in any of the case shown to be higher in their activities.

The results indicate that severe liver damage could be resulted by massive amount of tartrazine, 1,000-2,000mg per animal, by the injection into peritoneal cavity in guinea pig, though not so marked in its effect by oral or parenteral (intravenous) administration of it in the rabbit with the moderate amount, 7.5-50mg/kg body weight daily. The damage of liver function is so well relected in the data for the 20% liver tissue homogenate in Table II.

Even with the minute amount of the tartrazine, 0.4% of the food ration, liver aldolase activity was reported recently to drop significantly in the rat, supporting the present result to the effect that the dye could play a role as a hepatotoxic agent.

With the amount of the dye ranging from 7,5 ~50mg/kg in the rabbits, the dye seemed to have brought dysfunction or at least decreased liver function; while, with massive amount of it in the guinea pigs, it is quite clear that active liver damage was in progress.

Microscopic observations:

In the organs of the rabbit, pathologic findings were not particular to be of note except only an eosinophilic infiltration in the liver of all groups, and slight hydropic degeneration of liver cells and kidney tubular cells in the group, received daily 50mg/kg body weight for 10 days.

However, in the guinea pig experiments, the situation was a little more aggravated. Opening the body cavity, there was observed yellowish clear pleural and ascitic fluid accumulated, approximately 15 ml. in pleural cavity and 20 ml. in abdominal cavity in the 50mg/kg administration group. All the internal organs, pleura, pericardium and peritoneum were deeply yellowish, with some petechia in pericardium.

The surface of lung showed normal appearance, but on cut section, there were some congestion and edema. When microscopically studied, extensive hemorrhage and marked congestion were observed, which resulted in complete obliteration of alveolar spaces focally. The alveolar septa were thickened due to hemorrhage and capillary congestion.

The heart revealed no remarkable changes, except slight degree of muscular degeneration in myocardium. The hemorrhagic tendency was also noted in the liver in its portal spaces and venous congestion in the tissue noted as well. The same observation was also hold to be true in adrenal gland, where marked hemorrhage in medulla, but focal in cortex. Slight degree of hemorrhage in the sinuses of spleen and focal hemorrhage in cerebral cortex were observed. Kidney showed capillary congestion in cortex and hydropic degeneration of tubular epithelium, and in bone mar-

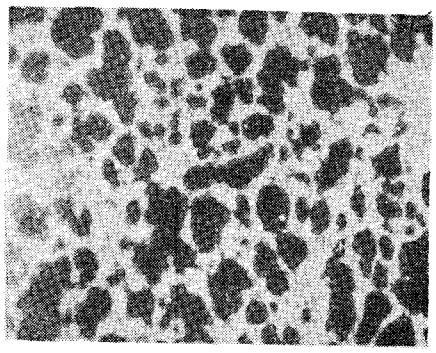

Fig. 2 Lung

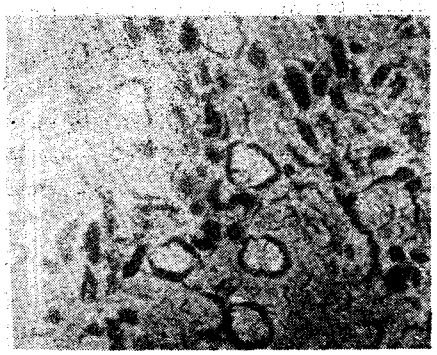

Fig. 3 Kidney

row marked increase of eosinophils was noted.

Fig. 2 and 3 are the most typical preparation of the lung and kidney in the dye-administered guinea pigs.

SUMMARY

To the rabbit and guinea pig, tartrazine was administered, either parenterally through venous or peritoneal injection or orally, with the ammount of 7.5-50 mg/kg body weight in case of rabbit and 1,000-2,000mg per animal in case of guinea pig, in order to observe acute toxic effect of the dye in the animals.

Blood serum was analyzed chemically and all the internal organs were microscopically observed for this purpose.

The results point to the fact that severe hemorrhagic and congestive findings in most of the organs can be induced by the amount of the dye utilized, and particularly liver damage could be resulted.

REFERENCES

(1) F.A.O. Utilition Meetings Report Series No. 38B, WHO-Food-Ed-66.25. p.88

(2) Scheline, R.R. and Longberg, B.: The absoro absorption, metabolism and excretion of the sulfonated azodye, Acid Yellow, by rats: Acta Pharmacl. Toxicol. 23, 1 (1965)

(3) Priestly, B,G. and O'Reilly, W.J.: Protein binding and the excretion of some azo dyes in rat bile: J. Pharm., 18, 41(1966)

(4) Despopoulos, A.: Renal and hepatic transport of food dyes: J. Pharm. and Exper. Therap., 163, 222(1968)

(5) Waterman, N. and Lignac, G.O.E.: Acta physiol. pharmacol. neerl., 7,35(1958), In WHO p.88

(6) Willhelm, R. and Ivy, A.C. A preliminary study concerning the possility of dietary carcinogenesis: Gastroenterology, 23, 1(1953)

(7) Parvin, R., Pande, S.V., & Venkitasubramanian, T.A.; On the colorimetric biuret method of protein determination; Anal. Biochem., **21**, 219(1965)

(8) Sperry, W.M. & Schoenheimer, R.; A comparison of serum heparinized plasma and oxalated plasma in regard to cholesterol content; J. Biol. Chem., **110**, 655(**1935**)

(9) Gutman, A.B.; Serum alkaline phosphatase activity in diseases of the skeletrol and hepatobiliary system; Amer. J. Med, **27**, 875(**1959**)

(10) Reitman, S. & Frankel, S.; A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic trans-aminases; Amer. J. Clin. Pathol., **28**, 56(1957)