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Changes of serum lipids in vitamin K3 (menadione) treated rats

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SUMMARY. The effect of high doses of vitamin K3 (10 to 50 mg/kg/day of menadione, administered intramuscularly) on the serum content of total lipids, cholesterol, phospholipids and triglycerides in male Wistar rats was evaluated. This experimental group was compared with another group that received intramuscular injections of 10 to 50 mg/kg/day of sodium bisulphite. Hypervitaminosis K3 was diagnosed by jaundice (due mainly to unconjugated bilirrubin) and by anemia which was particularly evident at doses of 40 and 50 mg/kg/day of menadione. These doses of menadione increased serum content of total lipids, phospholipids, and triglycerides but decreased cholesterol. The results show that overdoses of vitamin K3 alter lipid metabolism. The influence o participation of liver damage, fastening, as well as various endocrine and hematological changes are considered responsible for the alterations in serum lipids.
Keywords: menadione, hypervitaminosis K3, serum lipids, cholesterol, triglycerides, phospholipids.

RESUMEN. Modificaciones de los lípidos séricos en ratas tratadas con vitamina K3 (menadiona). En el presente artículo se evaluó el efecto de las dosis elevadas de vitamina K3 (10 a 50 mg/kg/día) administradas intramuscularmente, durante 7 días, sobre los niveles séricos de lípidos totales, colesterol, fosfolípidos y triglicéridos en ratas blancas Wistar (Grupos Tratados) y se comparó con lo que sucede en ratas tratadas con dosis equivalentes de bisulfito de sodio (10 a 50 mg/kg/día) por igual lapso de tiempo (Grupos Controles). La hipervitaminosis K3 se confirmó por la presencia de ictericia (a predominio de la bilirrubina no conjugada) y por la anemia, muy evidentes con las dosis de 40 y 50 mg/kg/día de menadiona. Estas dosis incrementaron significativamente ($p<0.05$) el contenido sérico de lípidos totales, de fosfolípidos y de triglicéridos, pero disminuyeron el colesterol. Estos resultados demuestran que las dosis elevadas de vitamina K3 alteran el metabolismo lipídico. El daño hepático, el ayuno y diversos cambios hematológicos y endocrinos se consideran los responsables de las alteraciones detectadas en los lípidos séricos.
Palabras claves: lípidos séricos, colesterol, fosfolípidos, triglicéridos, menadiona, hipervitaminosis K3.

INTRODUCTION

Experimental studies on hypervitaminosis K3 are scarse. The symptoms of hypervitaminosis K3 are not well described; in contrast, the symptoms of hypervitaminosis A and D have been extensively analyzed (1). The toxic effects of overdoses of vitamin K include: slow body growth (1), hemolytic anemia and hepatoesplenomegalia (2,3), jaundice due to unconjugated bilirrubin (4), albuminuria, porfirinuria and eventual death due to respiratory or cardiac failure (5). Previous studies have shown changes in the serum enzyme profile (6,7), as well as in tissue electrolytes in rats treated with menadione (7). Moreover, cytotoxic concentrations of menadione rapidly change intracellular thiol and Ca homeostasis (8,9). These changes are associated with alterations in the surface structure of the hepatocytes which may be an early indication of cytotoxicity (10-12). However, there are no reports on the effect of hypervitaminosis K3 on the serum content of lipids. This article reports our findings on the effect of high doses of vitamin K3 administered intramuscularly for seven days on the serum content of total lipids, total cholesterol, phospholipids and triglycerides in male rats.

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MATERIALS AND METHODS

Two hundred albino male Wistar rats weighing between 180 and 200 g were included in this study. They were kept in individual metabolic cages. The animals had free access to water and food during the experimental period. The formula for the basal diet was similar to the one recommended by the American Institute of Nutrition (13). After 7 days of adaptation the animals were randomly divided in 10 groups of 20 rats each. Groups one to five received equivolumetric intramuscular injections of 10, 20, 30, 40 and 50 mg/kg/day respectively of vitamin K (Sigma, menadione sodium bisulfite) for seven days (Experimental Groups). Groups six to ten received equivolumetric injections of 10, 20, 30, 40 and 50 mg/kg/day os sodium bisulfite intramuscularly for seven days (Control Groups). Sodium bisulfite was used in the control groups because menadione sodium bisulfite is dissociated into vitamin K and bisulfite in the liver of rats.

The rats were weighed and clinically checked daily. Twenty four hours after the last injection and after a fasting period of 8 hours, the rats were anesthetised with ether and a blood sample was extracted with a hematocrit tube from the orbital sinus through the external corner of the eye. The blood was allowed to coagulate spontaneously, centrifuged and serum samples were obtained to determine total lipids (14), cholesterol (15), phospholipids (16) and triglycerides (17).

Basic statistics including average and standard deviations were calculated. The differences between the blood lipid values in experimental and control groups were assessed by two **Anova** factor followed by Duncan's related test when granted by the Anova Test (18).

RESULTS

The animals treated with 10, 20 and 30 mg/kg/day of menadione did not show clinical symptons except two of them that had jaundice. In contrast, all the rats treated with 40 and 50 mg/kg/day of menadione showed remarkable jaundice. The autopsy revealed that internal organs also showed jaundice. In addition, these rats had low counts of red blood cells and low hemoglobin, hematocrit and mean corpuscular hemoglobin (4, 19); however, both conjugated and unconjugated bilirrubin were increased (19). Table 1 shows the results of the lipid statistical analysis. Again, the animals treated with 40 and 50 mg/kg/day of menadione had increased total lipids, phospholipids and triglycerides but decreased the cholesterol level.

TABLE 1
EFFECT OF VITAMIN K_3 AND SODIUM BISULFITE ON SERUM CONCENTRATION OF LIPIDS

COMPOUNDS	DOSES1	VITAMIN K_3	BISULFITE	p^2
Total Lipids	10	266±15	273±12	NS
	20	301±13	313±13	NS
	30	317±15	316±13	NS
	40	577±14	320–13	<0.005
	50	592±13 (*)	320±16	<0.005
Triglycerides	10	52±2	62±2	NS
	20	63±3	68±6	NS
	30	131±9	69±3	<0.05
	40	150±12	71±4	<0.05
	50	195±15	72±6	<0.05
Phospholipids	10	62±4	68±6	NS
	20	74±3	68±4	NS
	30	78±7	69±6	NS
	40	99±12	74±8	<0.05
	50	150±13 (*)	79±6 (*)	<0.05
Total Cholesterol	10	92±3	72±3	<0.05
	20	63±2	74±3	<0.05
	30	57±2	76±2	<0.05
	40	54±8	711±3	<0.05
	50	45±5 (*)	78±2	<0.05

The results are expressed in mg/dL (means± 2 S.D))1 Doses in mg/kg/day. 2 Statistical comparison between vitamin K and bisulfite (*) Analysis of variance of means of respective groups, $p<0.05$ statistically significant. NS is not significant.

DISCUSSION

The term lipids applies to all fatty compounds present in the blood. The lipid compound most often quantitated in clinical chemistry are total lipids, phospholipids, total fatty acids, cholesterol (free and sterified), neutral fats, triglycerides and lipoprotein (14). Except for the cholesterol each contains some fatty acids in its molecular structure. The normal value of total lipids, phospholipids, cholesterol and triglycerides found in the control groups (sodium bisulfite treated-animals) are similar to those reported in previous studies (20,21).

Acute hypervitaminosis K_3 increased total lipids, phospholipids and triglycerides but decreased cholesterol, particularly at the doses of 40 and 50 mg/kg/day. With these doses of menadione there is an important change in certain lipids that we do not determine in this study, among which are the lipoprotein fractions, total and free fatty acids, lecitine and other fatty material present in minor quantities (14) that could

be affected by the vitamin and which will be object of further studies. The decrease of total lipids and phospholipids in the group receiving 10 to 30 mg/kg/day of menadione are not considered significant since the values found are within the range of those reported normal for rats (20,21). The effect of high doses of menadione on the serum lipids could be a consequence of a direct action of vitamin K on the synthesis of these compounds. Eskelson et al (22) found that vitamin K_3 at 10^{-4} M inhibits cholesterol synthesis in vitro.

The changes in serum lipids here detected could be clarified, partially at least, by the liver damage (7,10-12) produced by high doses of vitamin K3. It is well known that serum cholesterol decrease in severe liver damage (23,24), whereas triglycerides and phospholipids increase in the case of swelling or inflammatory processes of liver (25). The level of total serum cholesterol also falls rather consistently in severe forms of anemia, specially in hemolytic anemia (23-25). Previous studies conducted in our Department (19) have shown that hypervitaminosis K_3 is responsible for severe hemolytic anemia.

Alternatively the changes in lipids might be indirectly caused by two mechanisms: 1) Hypoxia, due to low hemoglobin and to hemolytic anemia (19). Hypoxia constitutes a stimulus for fatty acids movilization from their storage (26) overwhelming the oxidative capacity of the liver. This oversaturation would divert these lipids toward alternate routes such as resynthesis of hepatic lipids, increased synthesis of triglycerides and phospholipids as well. These compounds might pass to the blood. Hypoxia is also a powerful stimulus for the secretion of adrenocorticotrophic hormone (ACTH) (27) which in turn stimulates the adrenal glands to release glucocorticoids. The glucocorticoids increase blood lipids due to their lipolytic action (28). In addition, Feketé and co-worker (29,30) demonstrated in vitro in the rat that coadministration of menadione and corticoids, such as prednisolone or cortisol, yielded an increase in the activities of these glucocortiocids. The in vivo transformation of cortisol into physiologically inactive derivatives is thought to involve ring A reduction as the rate limiting step (28). The findings of Kupfer and Peets (31) have demonstrated that menadione inhibits the ring A reduction. This mechanism involves an interference at the cofactor level. Such an action by menadione could potentiate the cortisol activity in vivo. 2) The high doses of vitamin K3 decrease the appetite and cause a fasting state. In such situation insulin release decreases and glucagon secretion increases (32). These two endocrine phenomena lead to lipolysis (32). Moreover, fasting increases sympathethic activity (33), a powerful lipolytic mechanism (34). According to Chiodi and Bass(35) the increase of triglycerides observed in hypoxia and fasting situations could be explained by: a) abnormal release of triglycerides from the liver which surpasses the uptake capacity of the adipose tissue and b) decrease of the storage capacity of the adipose tissue. Menadione might also changes the activity of lipoprotein lipase, the main catabolic enzyme of the circulating triglycerides(36).

The phospholipid increment seems to be due, at least partially, to the changes of activity of alkaline and acid phosphatases already described in experimental acute overdose of vitamin K3 (2,6).

In conclusion, the present study shows that high doses of vitamin K3 changes the level of blood lipids. Therefore, the hypervitaminosis K3 produces serious cellular metabolic changes.

Although this work is basically experimental, and does not admit or allow extrapolation to humans, we consider the statements of Smith and Custer (37) quite appropriate regarding the toxicity of vitamin K «.Among the more patent abuse in present-day therapy is the indiscriminate administration of vitamin K and its analogues. Free use of these substances is made on the assumption that they are harmless, and the physician generally believes that he is doing something of real value for his patient by giving him vitamin K during a hemorrhagic episode, be it bleeding from a tonsillar fossa, a prostatic bed or a duodenal ulcer. Rarely is such hemorrhage due to a defective coagulation mechanism, and the drug is generally given without prior evaluation of the prothrombin activity of the patients´blood.

In the face of predetermined hypoprothrombinemia, it is likewise common practice to give vitamin K in greater than adequate doses and to continue the treatment well beyond the time required to correct a true deficiency. Hypoprothorombinemia due to a seriously diseased liver cannot be corrected by vitamin K therapy, and many patients with this disorder may actually be harmed by frantic administration of menadiol sodium diphosphate, phytonadione or menadione, the only benefit being realized by the manufacturer...»

One should not be very censurios of physicians using vitamin K, however. The medical literature is virtually devoid of data relating to toxic effects of vitamin K. The textbooks of pharmacology that one consults made mention of the possibility of such effects, limited to the comment that hypoprothrombinemia, kernicterus and hemolytic anemia could be induced by giving excessive amounts of the vitamin K (37).

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