Z. Ernährungswiss. **16**, 256–261 (1977)
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ISSN 0044–264 X

National Research Centre, Dokki, Cairo (Egypt)

Effect of AMP on acute carbon-tetrachloride hepatotoxicity

Tahani H. Mikhail, R. Awadallah,
and *E. A. Dessoukey*

With 2 tables

(Received August 1, 1977)

The liver is specifically attacked by a large number of chemical agents. Serum enzyme changes associated with chemical hepatotoxicity indicate liver-cell alteration.

Early reports on clinical enzymology suggested that increased serum-enzyme activity indicated cell disruption. However, it soon became apparent that frank cellular destruction was not the only cause of change in serum-enzyme levels. Increases have been associatad with the non-specific morphologic alteration characteristics of hypoxia, hypothermia, and shock (1). All these conditions are reversible and not necessarily associated with cellular dealth. Furthermore, there is evidence that in the fact of tissue damage cellular enzyme activity actually increases (2, 3), simultaneously with serum-enzyme increase.

It has been shown, however, that CCl_4 can increase the level of some serum enzymes and decrease that of others (3).

One of the earliest lesions observed in animal treated with CCl_4 is the rapid development of fatty liver (4).

A spontaneous process of recovery after initial liver damage by carbon tetrachloride has been generally observed if the animals are given stock diet. More specifically protection, as indicated by increased survival time, has been observed with methionine, choline, methyl purine etc., sulphur-containing amino acids, vitamin E, vitamin B_{12}, and folic acid (5).

Dianzani (6) several years ago suggested that the fatty liver induced by carbon tetrachloride might result from an ATP deficiency.

Recent work in our laboratory showed that Adenosine triphosphate can protect the animal from the toxic effect of CCl_4 (7).

In the present work, we studied the protective effect of adenosine monophosphate on CCl_4 hepatotoxicity. Liver fat, serum transaminases as well as serum-alkaline phosphatase, total serum proteins, and electrophoretic pattern were determined.

Material and methods

Adult albino rats of both sexes (Sprague-Dawley strain) weighing 150–200 g and maintained on stock diet were used. Food and water were allowed ad libitum.

The animals were divided into three groups. Control group, group treated with CCl_4 and group treated with AMP ½ hour before CCl_4. Each group consisted of 10 animals.

The animals were injected intraperitoneally with CCl_4 (1:1 v/v in mineral oil) 0.5 ml mixture per 100 g body wt. The control animals were similarly given corresponding amounts of mineral oil.

Where protection by AMP was studied, AMP was given intraperitoneally (30 mg/animal) 1/2 hour before the injection of carbon tetrachloride.

Twenty-four hours after administration of carbon tetrachloride, animals were killed by decapitation.

Blood was withdrawn by venepuncture after ether anaesthesia before the animals being sacrificed. Blood was secured for the determination of serum enzymes.

Specimens of liver were taken for fat determinations by the method of *Folch* (8). Transaminase activity glutamic oxalacetic and pyruvic transaminases were determined by the method of *Reitman* and *Frankel* (9). Alkaline phosphatase was determined according to *King* and *Armstrong* (10).

Total serum proteins were determined by the biuret method and electrophoretic separation of serum proteins was done according to *King* and *Wootton* (11).

Results and discussion

The protective action of AMP against carbon-tetrachloride injury is evident from the data in (table 1 and 2).

Prior treatment with AMP (30 mg/rat) afforded protection when administered 1/2 hr. before carbon tetrachloride as judged from the decrease in liver lipids and serum-alkaline phosphatase as well as the increase in A/G ratio. There was, however, a slight but significant decrease in serum GOT and GPT within the 24 hrs. period of study, but it remained still higher than that of the control.

The general impression obtained from review of literature concerning CCl_4-induced hepatotoxicity suggests that one should find a reciprocal relationship between serum enzymes and liver activity. An increase in cellular and serum enzyme could represent a homeostatic rather than retrograde response. There is a considerable depletion of liver glycogen early in the course of CCl_4 intoxication (12). The gluconeogenetic action of GOT and GPT (13) could represent a compensatory response by providing new supplies of glucose precursors (14). Also the return of liver activity to normal levels despite significant serum-enzyme elevation was found by *Dinman* et al. (15).

The serum-alkaline phosphatase was significantly increased after CCl_4 and it exhibited a significant reduction under the influence of AMP treatment (table 1).

Increased serum-alkaline phosphatase in case of CCl_4 may result from leakage out of damaged liver cells.

Adenosine monophosphate can form complexes with ions as copper and magnesium (16). The chelation of metal ions by adenosine monophosphate could affect enzyme systems requiring metal ions as activators.

Alkaline phosphatase is an enzyme system of this type in which zinc and magnesium ions, under suitable conditions, have been shown to be activators (17).

Also the relationship of fat to alkaline phosphatase was studied by *Sukumaran* et al. (18). They found that alkaline phosphatase in rats and man was lowered on fasting and increased by ingestion fat.

Table 1. Serum glutamic oxalacetic and pyruvic transaminases and alkaline phosphatase.

	GOT I.U./l	GPT I.U./l	Alk. phosphatase King-Armstr. units
Control Group (10)	69.3 ± 13.0	27.8 ± 7.9	16.8 ± 2.9
CCl_4 (6)	107.4 ± 12.9	152.3 ± 17.7	38.9 ± 4.2
Cont. vs. CCl_4	<.05	<.05	<.05
CCl_4 + AMP (10) P	91.2 ± 1.0	86.0 ± 16.9	19.9 ± 5.7
CCl_4 vs. CCl_4 + AMP	<.05	<.05	<.05
Cont. vs. CCl_4 + AMP	<.05	<.05	>.05

Figures between paranthese indicate number of animals.

The decreased alkaline-phosphatase level under the influence of AMP may also be due to its effect on fat metabolism. From table 2 it is clear that under the influence of AMP is a significant decrease in the fat content of the liver.

It was found that cyclic adenosine 3,5 monophosphate at 5×10^{-5} M decreased the incorporation of (2-^{14}C) acetate into fatty acid in rat-liver slices and decreased cholesterol synthesis by more than 80 % [*Bricker* and *Levy* (19)]. The data indicate that cyclic adenosine 3,5 monophosphate may be involved in regulating acetyl-CoA incorporation into de novo fatty acid and cholesterol in a specific manner in mammalian liver.

Harvill (20) reported that intramuscular administration of adenosine-5-monophosphate (AMP) to man significantly lowered the blood serum cholesterol levels. *Milch* et al. (21) found that in cholesterol-fed cockerels, serum-lipid analysis indicated that AMP administration was associated with a pronounced fall in total cholesterol and some trend toward lower values in serum-β-globulin concentration.

It was further observed that AMP administration was associated with significant decreases in the fat and cholesterol concentrations of atherosclerosis hen aortae (21).

In recent years a large amount of data has been accumulated showing that cyclic AMP is the critical intracellular mediator of the actions of many hormones on their target tissues (22).

It has been previously suggested that the influences of glucagon and insulin on cyclic AMP may in turn regulate aspects of lipogenesis (23, 24).

The fundamental metabolic deviation causing fatty degeneration has not been well understood. The intense fatty degeneration of liver and its depletion of pyridine nucleotide has been suggested to result from a lowered concentration of adenosine triphosphate as a result of impaired mitochondrial integrity (25). The depletion of RNA phospholipids, proteins etc. appears to reflect in a general manner the impaired production of energy-rich phosphate esters in damaged mitochondria. It has been ob-

Table 2. See text.

	Liver total lipid g%	Serum total protein g/100 ml	Albumin %	Alpha 1 %	Alpha 2 %	Beta globulin %	Gamma globulin %	Total globulin %	A/G ratio
Control	4.67 ± .82 (10)	7.04 ± 1.56 (10)	42.5 ± 2.2 (5)	14.1 ± 2.2 (5)	10.4 ± 1.7 (5)	17.2 ± 1.3 (5)	15.2 ± 1.7 (5)	57.1 ± 2.2 (5)	0.73 ± .08 (5)
CCl_4	10.99 ± 1.42 (10)	5.95 ± .92 (6)	31.6 ± 1.7 (5)	19.7 ± 0.9 (5)	16.2 ± 1.7 (5)	17.6 ± 2.5 (5)	14.6 ± 5.5 (5)	68.4 ± 1.8 (5)	0.46 ± .03 (5)
P	<.05	>.05	<.05	<.05	<.05	>.05	>.05	<.05	<.05
CCl_4 + AMP	7.80 ± 0.73 (10)	6.35 ± .72 (10)	37.2 ± 3.4 (5)	17.12 ± 1.17 (5)	11.54 ± 1.95 (5)	15.4 ± 2.6 (5)	18.7 ± 2.4 (5)	62.8 ± 3.4 (5)	0.59 ± .08 (5)
P	<.05	>.05	<.05	<.05	<.05	>.05	>.05	<.05	<.05
CCl_4 vs. CCl_4 + AMP									

Figures between parantheses indicate number of animals.

served that in mitochondria from steatotic livers there is a decreased ability to retain nucleotides and other cofactors of oxidative phosphorylation (26).

However, *Recknagel* and *Anthony* (27) emphasized that the accumulation of fat in the liver occurs before mitochondrial injury and that the initial effect of CCl_4 on the liver occurs at about 3 hrs. *Recknagel* and *Lombardi* (28) made the critical observation that changes in endoplasmic reticulum function were evident clear before those seen in mitochondria. They found evidence of damage to the endoplasmic reticulum 2 to 3 hrs. after CCl_4 poisoning coinciding in time with the highest CCl_4 concentration in the liver and with the beginning of fat accumulation. They considered that the endoplasmic reticulum had a role in lipid secretion and postulated that in CCl_4 poisoning the fatty liver resulted from an accumulation of the triglycerides normally secreted by the liver as lipoproteins. This may be the result of either an inhibition in the synthesis of the protein moiety of lipoproteins or damage to the secretory mechanism. *Rees* et al. (29) found that within 2 hrs. of CCl_4 poisoning an inhibition to both of these processes takes place.

Smuckler and co-workers (30, 31) showed that the early morphologic changes seen in the endoplasmic reticulum could be related to a biochemical defect: failure to incorporate glycine into liver protein. *Robinson* and *Seakins* (32) have also shown decreased synthesis of serum liproproteins and liver proteins two hours after CCl_4.

In the present investigation in animals treated with CCl_4 it is certain that the total protein showed a tendency to decrease accompanied by a decrease in A/G ratio.

Administration of AMP has no effect on serum total protein. However, AMP resulted in a significant increase in the A/G ratio.

It is concluded therefore that acute carbon-tetrachloride hepatotoxicity produced fatty liver associated with elevation in serum enzymes and a decrease in A/G ratio.

Prior treatment with AMP ameliorated liver function without complete prevention of fatty liver.

Summary

The effect of carbon-tetrachloride poisoning and the protection caused by AMP were studied.

A single dose of CCl_4 has resulted in a rapid development of a fatty liver, a considerable increase in serum enzymes, glutamic oxalacetic and pyruvic transaminases as well as serum-alkaline phosphatase. Total serum protein showed a tendency to decrease accompanied by a decrease in A/G ratio.

Administration of adenosine-5-monophosphate prevented the increase in serum-alkaline phosphatase and increased the A/G ratio. There was, however, a slight but significant decrease in serum GOT and GPT within the 24-hrs. period of study, but it remained still higher than that of the control.

AMP lowered liver fat without complete protection against the development of fatty liver.

References

1. *Henley, K. S., E. Schmidt, F. W. Schmidt,* Enzymes in serum, their use in Diagnosis (Springfield, Ill. 1966). – 2. *Schmidt, E., F. W. Schmidt, E. Wildhert,* Klin. Wschr. 36, 280 (1958). – 3. *Cornish, H. H., W. D. Block,* Arch. Environm.

Health **1**, 96 (1960). – 4. *Farber, E., M. V. Simpso, H. Tarver,* J. Biol. Chem. **182**, 91 (1950). – 5. *Kasbekar, D. K., W. H. Lavate, D. V. Rege, A. Sreenivasan,* Biochem. **72**, 384 (1959). – 6. *Dianzani, M.,* U. Biochem. J. **65**, 116 (1957). – 7. *Tahani, H. Mikhail* (Private communication). – 8. *Folch, J., M. Lees, G. H. Sloane,* J. Biol. Chem. **226**, 497 (1957). – 9. *Reitman, S., S. Frankel,* Amer. Clin. Path. **28**, 56 (1957). – 10. *King, E. J., A. R. Armstrong,* Canad. Med. Assoc. J. **31**, 376 (1934). – 11. *King, E. J., I. D. P. Wootton,* Micro-analysis in Medical Biochemistry, 4th ed. (London 1964). – 12. *Leduc, E. H., J. W. Wilson,* Arch. Path. 65, 147 (1958). – 13. *Rosen, F., N. R. Roberts, C. A. Nichol,* J. Biol. Chem. **234**, 476 (1959). – 14. *Dinman, B. D.,* Arch. Environm. Health **11**, 265 (1965). – 15. *Dinman, B. D., I. A. Bernstein,* Arch. Environm. Health **16**, 777 (1968). – 16. *Claude Liebeco,* Bulletin de la Société de chimie biologique, Extrait du Tome XL III no. 2–3, 331 (1961). – 17. *Cohen, A. E., L. D. Scheel, J. F. Kopp, R. G. Keenan,* Amer. Ind. Hyg. Assoc. G. **20**, 303 (1959). – 18. *Sukumaran, M., L. Walter Bloom,* Proc. Soc. Exptl. Biol. Med. **84**, 631 (1953). – 19. *Bricker, L. A., G. S. Levey,* J. Biol. Chem. **247**, 4914 (1972). – 20. *Harvill, E. K.,* quoted from Amer. J. physiol. **170**, 346 (1952). – 21. *Milch, L. J., R. F. Redmond, W. W. Calhoun, H. I. Chinn,* Amer. J. physiol. **170**, 346 (1952). – 22. *Sutherland, E. W., G. A. Robison, R. W. Butcher,* Circulation **37**, 279 (1968). – 23. *Berthet, J.,* Proc. Int. Congr. Biochem. **17**, 107 (1960). – 24. *Steiner, G., V. K. Murtty,* Diabetes 20, Suppl. **1**, 379 (1971). – 25. *Dianzani, M. U.,* Biochim. biophys. Acta **17**, 391 (1955). – 26. *Kasbekar, D. K., A. Sreenivasan,* Nature (Lond.) **187**, 989 (1956). – 27. *Recknagel, R. O., D. D. Anthony,* J. Biol. Chem. **234**, 1052 (1959). – 28. *Recknagel, R. O., B. Lombardi,* J. Biol. Chem. **236**, 564 (1961). – 29. *Rees, K. R., V. L. Shotlander,* Proc. Roy. Soc. Biol. **157**, 517 (1963). – 30. *Smuckler, E. A., O. A. Iseri, E. P. Benditt,* Biochem. Biophys. Res. Commun. **5**, 270 (1961). – 31. *Smuckler, E. A., E. P. Bendilt,* Science **140**, 308 (1963). – 32. *Robinson, D. S., A. Seakins,* Biochem. J. **82**, 9 (1962).

Authors' address:

Tahani H. Mikhail, R. Awadallah, and *E. A. El-Dessoukey,*
National Research Centre, Dokki, Cairo (Egypt)