STUDIES ON THE MECHANISM OF CCl_4-INDUCED POLYRIBOSOMAL DAMAGE

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1. Introduction

Administration of CCl_4 to animals causes an increased lipoperoxidation in their livers [1–3] but antioxidants protect the liver against the triglyceride accumulation produced by this poison [4]. Protein synthesis is also impaired soon after giving CCl_4. Smuckler and Benditt [5] showed a decreased incorporation of amino acids into ribosomal proteins, both *in vitro* and *in vivo*, 1 hr after giving CCl_4 to rats; the polyribosomal patterns were also severely affected. The relationship between these changes in protein synthesis and lipoperoxidation is at present unknown. Alpers and coworkers [6] suggest that these phenomena may be independent and they did not find any protection from CCl_4-induced polysomal damage by prior treatment of the animals with antioxidants such as *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) or tocopherol. Investigations in this laboratory have, on the other hand, shown that glutathione diminishes the impaired *in vitro* amino acid incorporation into liver microsomal proteins in CCl_4-treated rats [13].

The work reported here shows that antioxidants diminish or prevent the changes in liver polyribosomal patterns induced by CCl_4 administration. CCl_4 has no effect *in vitro* and cycloheximide prevents the changes provoked by CCl_4 *in vivo*. We therefore suggest that pro-oxidant substances appearing as a consequence of CCl_4 poisoning may act at the level of initiation of translation.

2. Methods

Female Sprague-Dawley rats, each weighing 250–280 g, were used; the animals were starved for 15–18 hr before intoxication, but had free access to water. 250 μl CCl_4/100 g body weight, as 1:1 (v/v) mixture with mineral oil, were administered by stomach tube. The animals were then killed by decapitation at times ranging from 5 to 40 min.

Antioxidants were given by intraperitoneal injection: glutathione (GSH– 80 mg/100 g body wt), and propyl-gallate (30 mg/100 g body wt), were administered 30 min before intoxication; *N,N'*-dipehnyl-*p*-phenylenediamine (DPPD– 60 mg/100 g body wt, suspended in water with gum arabic 1% and Tween 0.5%), was administered in two injections, respectively 27 and 3 hr before intoxication.

Rat livers were homogenized, 20% (w/v) in a medium (TKM buffer) containing 0.15 M sucrose, 0.05 M tris-HCl buffer pH 7.8, 0.025 M KCl and 0.005 M $MgCl_2$, in a Potter-Elvehjem homogenizer fitted with a Teflon pestle. The postmitochondrial supernatant was prepared by centrifuging the homogenate at 15,000 *g* for 10 min, and was treated with 1% Na-deoxycholate. 0.2 ml of deoxycholate-treated postmitochondrial supernatant were layered over 5.5 ml exponential gradients of 0.5–1.5 M sucrose, obtained by a method similar to that described by Henderson [7], with a mixing chamber of 2.5 ml; the sucrose in gradients was in TKM buffer. The gradients were centrifuged at 204,000 *g* for 40 min in the SW 50 rotor of a Beckman-Spinco model HV ultracentrifuge and then monitored at 254 nm in an ISCO UV2 – Model D apparatus with a flow cell with a 0.2 cm light-path.

3. Results and discussion

The presence of severe changes in the size-distribution of liver polysomes from CCl_4-treated animals has been confirmed. The changes begin to appear as early as 15 min after giving the poison and mainly consist of a strong decrease in polysomes with a correspondent increase in the monomeric-dimeric ribosomes (fig. 1). By 40 min after poisoning, almost all polysomes are in the monomer-dimer peak. At 5 and 10 min after giving CCl_4, the patterns are still unaffected.

Previous administration of GSH, 30 min before giving CCl_4, produces a marked protection against such changes (fig. 2). This protection was present at all the times studied, but was never complete, in the conditions used. Very similar results were also obtained with propyl-gallate. No difference was seen in the ribosomal patterns of animals treated by either GSH or propyl-gallate only, compared with normal animals.

DPPD, given twice, respectively 27 and 3 hr before CCl_4, produces complete protection of the polysomal patterns (fig. 3). It has been observed that protection is much less when DPPD is given either in a single dose, 27 hr before CCl_4, or in two doses, respectively 48 and 24 hr before intoxication. These facts may explain the negative result reported by Alpers and coworkers [6]; they also used a different vehicle (corn oil) for DPPD. No substantial differences were seen between untreated animals and those treated by DPPD only, with the exception of a small decrease in the height of the monomer peak in the latter.

Smuckler and Benditt [5] have shown that ribosomal changes never occur when CCl_4 is added directly to the polysomal preparations *in vitro*; they conclude that CCl_4 only acts on polysomes *in vivo*, the effect being due to products of its metabolism. We therefore carried out experiments in which 2.5 μl of CCl_4 were placed in the side arms of Warburg flasks containing 5 ml of liver homogenate, prepared as described above. After stoppering, the system was incubated at 37°. In these conditions CCl_4 diffuses from the side arm into the main compartment of the flask, where it is metabolized, which stimulates lipid peroxidation, as it is shown by accumulation of malonyldialdehyde [8]. After 30 min, the homogenate was centrifuged and the polysomal patterns studied. No differences were observed in the size-distribution of ribosomes between homogenates incubated either in

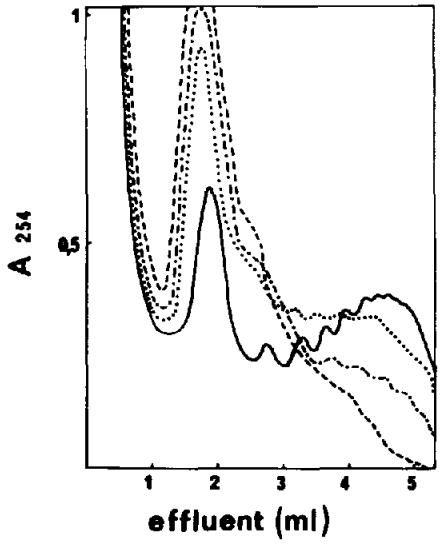

Fig. 1. Size-distribution of the ribosomes from the livers of control (——) and CCl_4-intoxicated rats. Time following CCl_4 administration: 15 min (.....), 30 min (-·-·-·-) and 40 min (- - - - -). The top of the gradients is to the left.

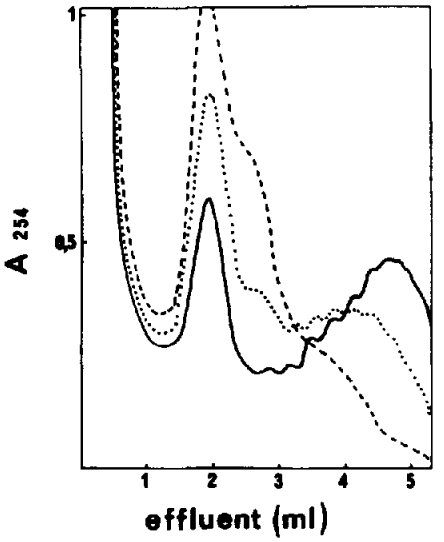

Fig. 2. Size-distribution of the ribosomes from the livers of control rats (——) and rats receiving CCl_4 alone (- - - - -) and CCl_4 plus GSH (.....). GSH was given 30 min before CCl_4. The rats were killed 40 min after administration of CCl_4. The top of the gradients is to the left.

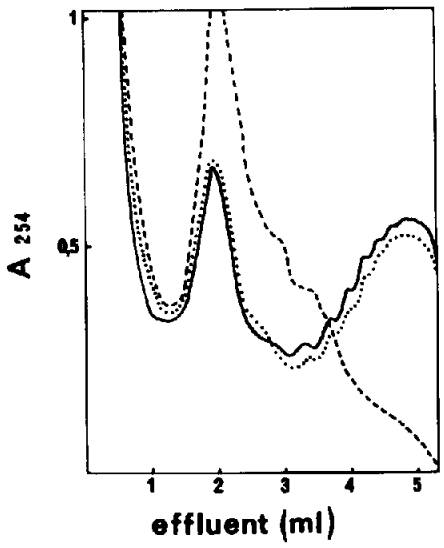

Fig. 3. Size-distribution of the ribosomes from the livers of control rats (——) and rats receiving CCl_4 alone (- - - -) and CCl_4 plus DPPD (.....). DPPD was given in two injections, 27 and 3 hr before CCl_4. The rats were killed 40 min after administration of CCl_4. The top of the gradients is to the left.

the absence or in the presence of CCl_4. The production of malonyl-dialdehyde was, however, much higher in the last case. These results show that *in vitro* metabolism of CCl_4, as well as lipoperoxidation, are not immediately related to the ribosomal changes observed after *in vivo* intoxication. The appearance of the latter must depend upon something happening within the intact cell.

About the nature of the damage produced by CCl_4, it seems improbable that CCl_4 acts by provoking the hydrolysis of polysomal messenger RNA; in fact Weksel and Gelboin [9] have shown that liver ribosomes from CCl_4-treated rats appear to be free from mRNA, or at least they behave similarly to ribosomes devoid of mRNA activity. The fact that the observed changes occur rather soon after poisoning, suggests that a decreased formation of mRNA cannot be responsible for the disappearance of the polyribosomes.

The possibility has to be considered, therefore, that CCl_4 acts by preventing the binding of the ribosomes to mRNA after a normal polysomal cycle; in other words, ribosomes set free from the polysomal complex at the end of mRNA translation would be unable to form new polysomes. This mechanism agrees with the time necessary for the appearance of changes in polysomal patterns after giving CCl_4. To investigate this possibility we studied the effect of giving cycloheximide before CCl_4. It is known that cycloheximide inhibits protein synthesis by blocking the translation of ribosomes along the mRNA [10, 11], probably due to inhibition of transferase II [12]. As is shown in fig. 4, cycloheximide (100 μg/100 g body wt, 10 min before CCl_4) prevents the changes in the polysomal patterns caused by CCl_4. Cycloheximide has no antioxidant activity; malonyl-dialdehyde production *in vitro* in liver homogenates was not influenced by the addition of 0.1–1 μg/ml cycloheximide. These facts show that the action of CCl_4 on polysomes does not occur when ribosomes are bound to mRNA, and it seems then possible that pro-oxidant substances, appearing as a consequence of CCl_4 poisoning, act at the level of chain initiation. The theoretical possibility exists, however, that "polysomal freezing" caused by cycloheximide can protect these structures from some other type of damage.

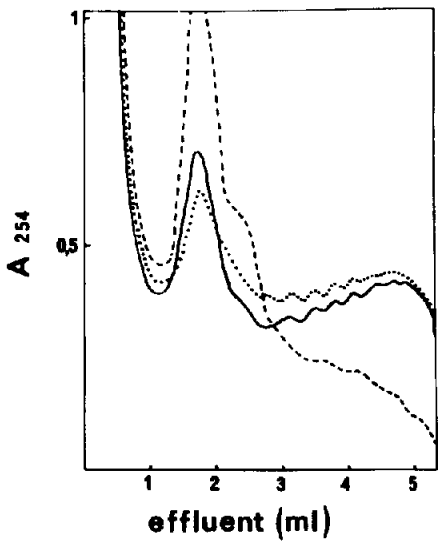

Fig. 4. Size-distribution of the ribosomes from the livers of control rats (——) and rats receiving CCl_4 alone (- - - -) and CCl_4 and cycloheximide (.....). Cycloheximide was given 10 min before CCl_4. The rats were killed 40 min after administration of CCl_4. The top of the gradients is to the left.

Acknowledgement

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