Hepatotoxicity of the condensation product of adrenaline with acetaldehyde

D. MOURA*, I. AZEVEDO, W. OSSWALD, *Laboratório de Farmacologia, Faculdade de Medicina, Porto, Portugal*

Non-enzymatic condensation of adrenaline with acetaldehyde was reported to occur in the bovine isolated adrenal gland perfused with physiological solution containing acetaldehyde (Cohen & Collins, 1970). Later, preparation of this condensation product was reported by Osswald, Polónia & Polónia (1975), who described the amorphous product formed in the reaction (MA 3) as well as its two constituents, MA 4 and MA 5. The chemical nature of these compounds was described by the same authors; they are tetrahydroisoquinoline derivatives, one of them (MA 4) having been identified as 1,2-dimethyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline. These compounds exert sympathomimetic actions, are taken up in nerve terminals and may function as false adrenergic transmitters (Greenberg & Cohen, 1973; Mytilineou, Cohen & Barrett, 1974; Osswald & others, 1975). These facts led us to study a possible influence of the condensation product of adrenaline and acetaldehyde (MA 3) on the structure of adrenergic nerve terminals in the rat. During this study, the abnormal appearance of the liver of animals injected by the intraperitoneal route prompted us to investigate this particular aspect of the toxicity of MA 3. We now report preliminary results obtained.

Male Wistar rats, 160–180 g, fed a standard rat chow (FF–G (M), E. Dixon and Sons, Ware, England) and given free access to tap water were used.

In a first series of experiments, 8 animals were injected intraperitoneally with MA 3 50 mg kg^{-1} (in a volume of 0·5 ml) and killed by decapitation 24 h later. In another series of experiments, 10 rats were given intraperitoneally 50 mg kg^{-1} of MA 3 on day 1 and on day 8, and decapitated on day 9. These doses had been shown, in the experiments referred to above, to be tolerated without mortality and to induce marked alterations of the adrenergic nerve terminals and were therefore chosen for the present study. Control animals were injected with the same volume of saline by the intraperitoneal route.

At necropsy the liver was removed and examined for any gross abnormality. Paraffin sections were prepared and stained with haematoxylin and eosin. Samples of liver were also removed and homogenized for determination of triglyceride content according to Van Handel, Zilversmit & Bowman (1957), after extraction with chloroform–methanol.

In the first series, both gross and microscopical morphology of livers from rats that had been injected with MA 3 were essentially similar to those of their untreated controls. However, liver triglyceride content in treated animals (5·24 ± 0·97 mg g^{-1}) increased by 42·7% above control values (3·67 ± 0·66; $P < 0.001$).

In the second series, abnormal areas were evident on inspection, in 40% of the treated rats. The areas involved were 1 to 5 mm in diameter, pale, soft and occupied several lobes. The histologic appearance was that of massive hepatic necrosis. Confluent zones of amorphous, eosinophilic and anuclear cellular debris involved the bulk of hepatocytes in contiguous lobules, leaving only a few irregular islands of preserved parenchyma around portal tracts. Inflammatory reactions and regenerative activity could be identified especially in the periphery of the necrotic areas, but often they were of only minor importance. In sections of less severely damaged areas, centrilobular necrosis could be seen.

In this series, the concentration of hepatic triglycerides (4·07 ± 1·06 mg g^{-1}) was not significantly different from the controls, i.e. the elevation produced by the first dose was no longer observed on day 9 of treatment.

The results reported show that the condensation product of adrenaline with acetaldehyde exhibits a marked hepatotoxic capacity in the rat. It is also apparent that the main toxic effect is the causation of necrosis, fat accumulation being rather discrete and of transient nature, as shown by the modifications of hepatic triglyceride content.

Since acetaldehyde is the major metabolic product of ethanol, the hypothesis has been advanced that its condensation products with catecholamines may be formed *in vivo* and play a role in acute and chronic alcoholism (Walsh, Davis & Yamanaka, 1970; Locke, Cohen & Dembiec, 1973). However, it is clear that, at the present stage, it is not possible to speculate on the possible involvement of the condensation product under study in the hepatic toxicity of ethanol in man.

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* Correspondence.

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Convulsant activity of thiomalate; a possible metabolite of aurothiomalate

P. V. TABERNER, *Department of Pharmacology, Medical School, University of Bristol, Bristol BS8 1TD, U.K.*

It has recently been shown that thiomalic acid is a potent competitive inhibitor of glutamate decarboxylase, GAD (Taberner, Pearce & Watkins, 1977). Since other GAD inhibitors, e.g. mercaptopropionic acid, (Lamar, 1970), 4-deoxypyridoxine (Horton & Meldrum, 1973), have been found to possess convulsant activity, and in view of the fact that thiomalic acid, in the form of sodium aurothiomalate (Myocrisin), is used widely in the treatment of arthritis and rheumatism (Gold Therapy in 1975), it was felt that thiomalic acid should be examined for possible convulsant activity.

Thiomalic acid (A.R., Koch-Light Laboratories Ltd.) was converted to the sodium salt by the stoichiometric addition of sodium hydroxide and a 100 mM stock solution prepared and adjusted to pH 7·4 with 50 mM sodium phosphate buffer. Adult LACG mice of either sex were used. Values for the minimum convulsive dose and ED50 of thiomalate were calculated by the methods described previously (Taberner, 1976), and the dose ranges given below represent the 95% confidence limits.

Following intraperitoneal injection, thiomalate was inactive at doses up to 2 mmol (340 mg) kg^{-1}. Higher doses were not tested. After the intracerebroventricular injection of thiomalate, using the method described by Brittain & Handley (1967), the minimum convulsive dose was 0·85 – 0·87 μmol (145 – 150 μg). At higher doses the mice went into running fits within 20–45 s of the injection. Occasional full tonic clonic seizures were also observed. The ED50 for running fits was 1·28 ± 0·10 μmol (220 ± 18 μg) kg^{-1}. The running fits lasted, on average, 8–12 min and the mice fully recovered by 30 min.

Running fits are also a characteristic feature of the effects of mercaptopropionate (Lamar, 1970) suggesting that they may well reflect a common mechanism of action of mercaptopropionate and thiomalate. Both compounds are equipotent as inhibitors of GAD *in vitro* (Taberner & others, 1977) yet the ED50 for mercaptopropionate following intraperitoneal injection in mice is reported to be 0·27 mmol kg^{-1} (Horton & Meldrum, 1973). The lack of activity of thiomalate following intraperitoneal injection therefore suggests that it does not readily cross the blood-brain barrier.

Gold therapy, whilst effective, is subject to an extremely high incidence of side-effects (Girdwood, 1974) although seizure activity has not been reported. The thiomalate moiety has no intrinsic therapeutic value and is only one of several soluble salts with which gold can be combined (Harvey, 1975). A recent report (Danpure, 1976) has indicated that sodium aurothiomalate can react *in vitro* with cysteine to yield free thiomalate. Whilst this reaction may present no risk to patients with a fully-functioning blood-brain barrier, in pathological conditions such as meningitis, when the blood-brain barrier is less effective, there is the possibility that thiomalate may enter the brain and its convulsant properties thus become apparent.

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