ANTIDOTAL EFFECT OF AMINOACETONITRILE AGAINST THE BIOCHEMICAL INJURY OF THE MICROSOMAL AMINO-ACID INCORPORATING SYSTEM INDUCED IN VIVO BY CARBON TETRACHLORIDE OR DIMETHYLNITROSAMINE

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Received January 15, 1965

Aminoacetonitrile (AAN) is known for its potent lathyrogenic activity i.e. the peculiar capability of inducing in susceptible animals specific lesions of the connective tissue (see German, 1960). While attempting to devise an appropriate system for studying in vitro the biochemical disturbances involved in the lathyritic syndrome, it was noted that AAN in suitably low amounts prolonged the survival and increased the overall protein yield of chick embryo fibroblast cultures (Bornstein, Halbreich and Mager, unpublished results). It occurred to us that this observation may be pertinent to the interesting discovery made by Fiume (1962 a, 1962 b) on the ability of AAN to prevent the appearance of the histopathological signs of liver damage in rats treated with a variety of poisons, such as carbon tetrachloride, dimethylnitrosamine (DMNA), chloroform or bromobenzene. Of particular relevance to the problem concerned appeared to be the recent announcement by the same author that pretreatment with AAN obviated the inhibition of ^{14}C-glycine incorporation into the liver proteins observed in rats treated with DMNA (Fiume, 1964).

Prompted by the above considerations, the present study was initiated with the aim of exploring in subcellular preparations the protective effect of AAN against the

deterioration of the protein synthesizing system induced by CCl_4 or DMNA (see Smuckler and Benditt, 1963; Arrhenius and Hultin, 1962).

MATERIALS AND METHODS

Each experimental group comprised 4 male rats weighing 90-110 g. One tenth of CCl_4 mixed with 0.1 ml paraffin oil or 20 mg of DMNA dissolved in 0.2 ml saline were given intraperitoneally 2 to 5 hours, respectively, before the animals were killed. Two tenths of a neutralized 10% solution of aminoacetonitrile hydrogen sulfate (recryst. from aqueous ethanol) in saline were injected subcutaneously 12-20 hours prior to the administration of the liver poisons. Suitable controls were run in parallel. All the animals were fasted overnight and at the times specified were killed by cervical dislocation. The livers were rapidly excised, rinsed with ice-cold 0.25M sucrose solution and those belonging to the same group were pooled by mincing together and homogenized with 4 volumes of cold 0.25M sucrose - 0.05M Tris buffer (pH 7.5) per each gram of tissue. The homogenates were centrifuged at 30,000xg for 15 min and the resultant supernatants were spun again at 105,000xg for 60 min. The pellets were carefully rinsed with 0.25M sucrose solution and resuspended in the same medium. The assays were run in duplicate. The standard reaction mixture contained in 1 ml final volume the following components: 50μmoles Tris buffer (pH 7.5), 100μmoles KCl, 7.5μmoles $MgSO_4$, 0.1 μmole GTP, 1μmole ATP, 5μmoles sodium phosphoenolpyruvate, 20μg pyruvate kinase (EC 2.7.1.40) 0.2 μmoles of either 1-^{14}C - DL-leucine or 3-^{14}C-DL-phenylalanine (spec. act. approx. 2.5μC/μmole), 0.2 ml microsome suspension containing 2.5 mg protein and 0.2 ml soluble fraction (105.000xg supernatant) containing 2 mg protein. Following incubation at 35^{o}C for 30 min, the reaction was terminated by adding trichloroacetic acid to a final concentration of 5% and the proteins were isolated by a slightly modified procedure of Siekevitz (1952). The radioactivity was determined by the conventional techniques in a gas-flow counter.

TABLE I

Prevention by AAN of the decline of amino acid incorporation into protein in cell-free rat liver preparations following treatment in vivo with CCl_4 or DMNA

	Experiment No.					
	1	2	3	4	5	6
	Incorporation activity per cent of control					
Treatment	1-^{14}C-Leucine			3-^{14}C-Phenylalanine		
AAN	111	98	103	115	114	101
CCl_4	47	51	48	74	60	55
AAN plus CCl_4	98	88	99	92	96	95
DMNA	-	-	52	50	-	-
AAN plus DMNA	-	-	117	89	-	-

Abbreviations: AAN; aminoacetonitrile; DMNA, dimethylnitrosamine.

RESULTS

The experimental data assembled in Table 1 show that the marked decline in the amino acid incorporating activity exhibited by cell-free liver preparations from rats treated with DMNA or CCl_4 could be prevented or considerably attenuated by a single dose of AAN injected 12-20 hours prior to the administration of the liver poisons. No protective response resulted, however, when the interval between the two injections was shortened to 2 hours or when AAN and the liver poisons were given simultaneously. Furthermore, addition of AAN to the reaction mixture (up to 0.002M) failed to reverse the decay of amino acid incorporation, following the treatment in vivo with CCl_4 or DMNA.

By examining various combinations of the particulate and soluble fractions derived from the postmitochondrial supernatants of liver homogenates from controls and treated animals, the behavior of the reconstituted system was found in all instances to be

essentially determined by the origin of the microsomes, regardless of the source of the soluble fraction employed (Table 2). Thus, both the intracellular target of the toxic injury and the site of the protective effect of AAN appeared to be located in the microsomal particles.

The early signs of the deleterious action of the liver poisons on the microsomal system have been shown in a recent study to be attended by a striking enhancement of the polyuridylic acid (poly U)-directed incorporation of phenylalanine (Mager,

TABLE II

Incorporation of 1-^{14}C-leucine into protein (counts/min/mg protein) in various combinations of microsomes and soluble fractions from control and treated rats

	Soluble fractions					
Microsomes	Control	AAN	CCl_4	DMNA	AAN plus CCl_4	AAN plus DMNA
Control	374	-	328	401	410	444
AAN	-	384	311	347	-	-
CCl_4	201	175	180	193	-	-
DMNA	186	198	-	193	-	-
AAN plus CCl_4	376	-	-	-	370	-
AAN plus DMNA	350	-	-	-	-	437

Bornstein and Halbreich, 1965). The elevated sensitivity of the system to poly U was interpreted as reflecting an increased availability of the ribosomal combining sites, uncovered by the destruction of the ribosome-bound messenger RNA. It appeared of interest, therefore, to test the effect of AAN on this parameter. The experiment depicted in Fig. 1 clearly indicates that pretreatment with AAN resulted in a significant reduction in the degree of augmentation of the response to poly U elicited by CCl_4 or DMNA.

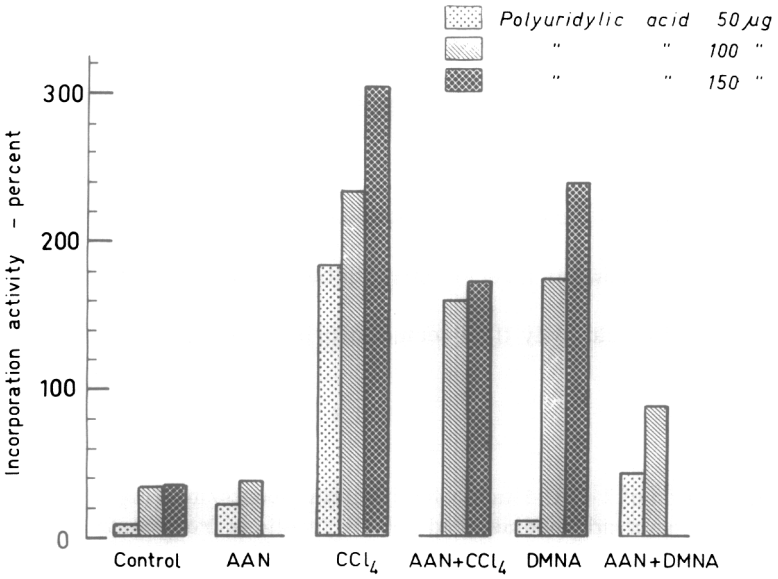

Fig. 1.

Enhancement of the poly U-directed polymerization of phenylalanine induced by CCl_4 or DMNA and its partial reversal by pretreatment in vivo with AAN. The poly U-dependent activity is expressed as the increment in the incorporated radioactivity observed in the presence of poly U in per cent of the intrinsic incorporation activity (without poly U added).

DISCUSSION

It may be concluded from the present results that the protective influence of AAN bears on the major parameters featuring the injury inflicted upon the microsomal system by CCl_4 and DMNA. The temporal aspects of the AAN effect suggest that the antidotal activity of this substance is linked to some relatively slow metabolic process, the nature of which remains to be elucidated.

The observation reported by Fiume and Laschi (1963) that AAN does not prevent the early lesions of the endoplasmic reticulum due to CCl_4-poisoning, seems to argue against the possibility that this compound acts by interfering with the enzyme systems involved in the transformation of the potentially toxic substance into its reactive molecular form (see Smuckler and Benditt, 1963; Hultin, Arrhenius, Löw and Magee, 1960).

The above finding, if confirmed by more quatitative data, would imply a direct "stabilizing" effect of AAN on the ribosomal particle.

Work currently in progress is designed to explore the relationship of the phenomena described in this report to the lathyrogenic activity of AAN. It is also intended to examine the possible role of AAN in counteracting the carcinogenic properties inherent in the hepatotoxic agents studied.

Supported by a grant No. AM 06123 of the National Institutes of Health.

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