ACRYLONITRILE AND TISSUE GLUTATHIONE: DIFFERENTIAL EFFECT OF ACUTE AND CHRONIC INTERACTIONS[1]

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SUMMARY: Acrylonitrile (vinyl cyanide) is a reactive chemical extensively used in the synthesis of buna rubber and polymerized plastics (e.g., disposable bottles). A single dose of the chemical causes adrenal hemorrhage, while chronic ingesting results in adrenocortical hypofunction. Present acute experiments in rats show a rapid time- and dose-dependent decrease in reduced glutathione (GSH) in the liver, lung, kidney and adrenal. The diminution of cerebral GSH concentration is gradual and seems to correlate with the occurrence of mortality in acute experiments. Chronic ingestion of acrylonitrile in drinking water results in a dose dependent increase of hepatic GSH concentration, similar to that caused by chemical carcinogens.

Acrylonitrile (CH_2=CH-CN) is among the 50 most extensively synthesized chemicals in the U.S. Its annual production for 1974-76 was only 1/4 of that of vinyl chloride, but about ten times more than that of DDT (1). The compound, a liquid with substantial reactivity, is used in the production of synthetic buna rubber. In this reaction sequence, acrylonitrile is the most toxic ingredient (2). The chemical has also been used as a grain fumigant. Polymerised acrylonitrile is incorporated into numerous plastics and synthetic fibers (e.g., "Acrilan", "Dynel" and "Orlon". More recently, disposable plastic beverage bottles made of polyacrylonitrile have been marketed.

Despite these extensive applications of acrylonitrile, only scanty data has existed on its toxicologic and pharmacologic properties. Based on animal experiments, the human limit of exposure was set at 20 ppm (3,4). Subsequently, several cases of acrylonitrile intoxication resulting from exposure to fumes in

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factories of synthetic rubber and polymerization, as well as accidental, some fatal, acrylonitrile poisonings were described (2,5,6,7,8). These reports emphasized the involvement of the central nervous system (CNS) and gastrointestinal tract. Sakurai and Kusumoto (9) in an epidemiologic survey of acrylonitrile workers found an increased incidence of neurologic symptoms and functional liver changes. We (10) described that one dose of acrylonitrile in rats produces a rapidly progressing, fatal adrenal apoplexy which resembles the Waterhouse-Friderichsen syndrome in man. The cause of death is unknown but seems related to acute adrenocortical insufficiency, toxicity to the CNS (e.g., excitation and convulsion followed by paralysis which probably involves the respiratory center as well) and congestive lung edema. Pretreatment of rats with ACTH or phenobarbital, unlike that with steroidal inducers of hepatic mixed function oxidase (MFO) completely prevents the adrenal lesions and mortality induced by acrylonitrile (11). More recently, Gut et al. studied the role of phenobarbital and MFO on the metabolism of acrylonitrile (12).

Reduced glutathione (GSH) is an endogenous nucleophile which can react with electrophilic chemicals or electrophillic metabolites of less reactive chemicals. The latters are usually generated by the catalytic action of hepatic MFO and as reactive intermediates, frequently have short half lives, e.g., epoxides which readily interact with endogenous nucleophiles, like GSH (13,14). GSH also seems to act as an antioxidant in protecting the liver or lung from chemical injury (15). To gain insight into the mechanism of action of acrylonitrile, we investigated the effect of this chemical on tissue GSH concentrations in acute and chronic experiments.

METHODS AND MATERIALS

Female Sprague-Dawley derived Charles River (Wilmington, Mass.) rats with an initial body weight of 200g were maintained on Purina Lab Chow and tap water (with or without acrylonitrile) ad libitum. Each group consisted of 4-5 animals. Following the repetition of each experiment the results were pooled.

In the first acute experiments, rats were given as aqueous, 0.2% polysorbate 80 (Tween 80) suspension of acrylonitrile (Aldrich), 15 mg/100g i.v. once. This dose causes 100% incidence of adrenal apoplexy and 80-100% mortality in 60-90 min. Controls received only the 0.2% polysorbate suspension. Rats

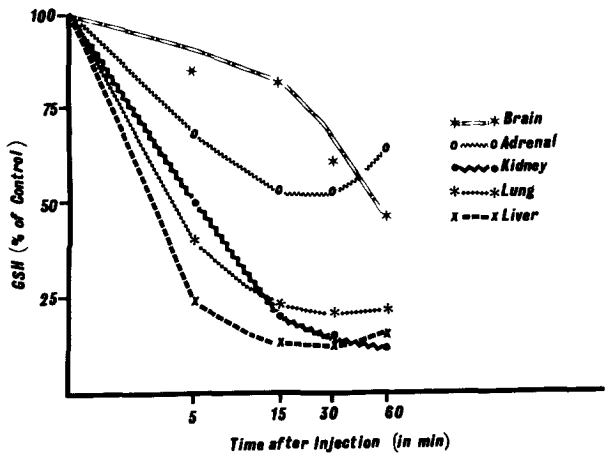

Fig. 1. Time-dependent depletion of glutathione (GSH) in liver, adrenal, brain, lung and kidney in rats after one injection of acrylonitrile.

were decapitated 5, 15, 30 and 60 min after treatment, organs were collected and kept on dry ice for subsequent determination of GSH. In the second acute experiments, animals were injected i.v. with 0, 1, 5 or 15 mg/100g of acrylonitrile and were sacrificed 30 min later.

In chronic experiments, rats had access for 21 days to drinking water containing 0, 0.002% (20 ppm) 0.01% (100 ppm) or 0.05% (500 ppm) of acrylonitrile. These doses were previously shown to cause adrenocortical hypofunction (16). Additional groups of animals received acrylonitrile as a bolus twice daily by gavage with a rubber stomach tube. The bolus dose was calculated on the basis of measured water intake and the corresponding acrylonitrile concentration. The animals were killed and the organs were collected as described above.

Nonprotein sulfhydryl concentration expressed as reduced GSH was assayed with Ellman's reagent, according to the procedure of Jaeger et al. (17).

RESULTS AND DISCUSSION

Results presented in Fig. 1. demonstrate that an adrenocorticolytic dose of acrylonitrile drastically depleted GSH stores in the liver, lung and kidney within 5 min after i.v. injection. A low level plateau was reached within 15 min in these organs and the results did not change at 30 and 60 min. It is important to stress that despite the 80-90% decrease in hepatic, renal and pulmonary concentration of GSH no specific tissue damage was detected in these organs by either gross or light microscopic examination. The decline in cerebral GSH concentration was gradual and probably reached the lowest level when the rats started to die. The decrease in GSH level in the adrenal was between that found in the brain and the

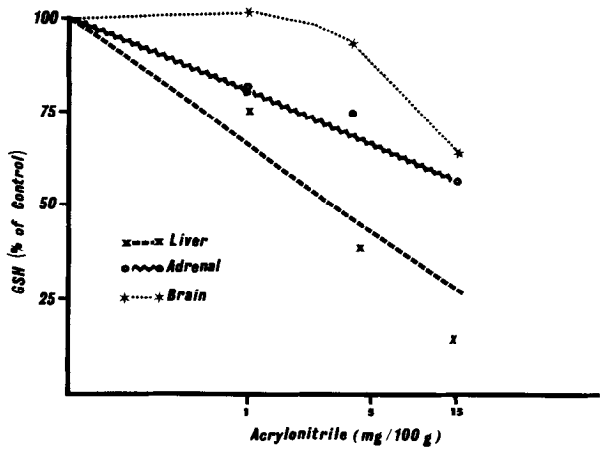

Fig. 2. Dose-dependent depletion of glutathione (GSH) in liver, adrenal and brain in rats 30 min after one injection of acrylonitrile.

value in the liver, lung and kidney. The slight elevation in the GSH concentration in the adrenals at 60 min is poorly understood, but it might be correlated with either a rebound of GSH (19), or severe congestion and/or early hemorrhage in the adrenals, since erythrocytes are known to contain high concentrations of GSH (14).

The dose-response data (Fig. 2) indicate a gradual and steady decrease in GSH concentrations in the liver and adrenal. On the other hand, while a minimal change occurs at 1-5 mg of acrylonitrile, a sharp drop in cerebral GSH concentrations was observed between 5-15 mg of acrylonitrile, at the dose range which induced mortality. Thus, these experiments suggest that a precipitous decrease in GSH level in the brain correlated with mortality in acute acrylonitrile poisoning. These results are also in agreement with those reported by Dinu (20) who found low tissue levels of protein and nonprotein sulfhydryl groups after a $^{LD}100$ of acrylonitrile (although no dose- or time-response studies were performed).

In chronic experiments only changes in the amount of liver GSH were prominent. Table 1 shows a dose-dependent increase in GSH concentration. This increase was always greater if the dose of acrylonitrile was given as a bolus

Table I.

Effect of chronic acrylonitrile ingestion on hepatic glutathione concentration in rats

	Glutathione (µg/g liver)
Control	1431.14 ± 24.08
Acrylonitrile 0.002%	
-drinking water	1506.00 ± 85.29
-bolus	1535.84 ± 14.44*
Acrylonitrile 0.01%	
-drinking water	1492.01 ± 75.59
-bolus	1621.45 ± 26.14**
Acrylonitrile 0.05%	
-drinking water	1666.00 ± 68.16**
-bolus	1782.02 ± 84.29**

Mean ± standard error of mean. Student's t-test.
* = $p < 0.05$; ** = $p < 0.005$, as compared to control

rather than in small amounts in drinking water during the 21 day period.

The changes in GSH after acrylonitrile suggest that acrylonitrile or its reactive, probably epoxy, derivative interacts with the endogenous nucleophile. Epoxides are frequently formed from unsaturated (e.g., vinyl) compounds and then undergo an enzymatic interaction with reduced GSH (13,14). The importance of the double bond in acrylonitrile and the possible formation of an epoxide are also underlined by additional preliminary experiments which showed that an LD_{100} dose of propionitrile (CH_3-CH-CN) in rats did not markedly alter GSH concentrations in the liver, brain and adrenal. The dose dependent increase in hepatic GSH

concentration found in the chronic experiments might represent a rebound phenomenon observed after the administration of other chemicals as well (e.g. trichloroethylene, vinyl chloride) (18,19) and might also represent a precancerous alteration. Elevations in the amount of liver GSH have been described as "early" lesions caused by chemical carcinogens (21). It remains to be seen whether these changes correlate with the "proliferative lesions" in the stomach and brain (22), and the recently reported (23) increase in mortality due to cancer in factory workers who were exposed to acrylonitrile.

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