5 R01 OH00702-02
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Acrylonitrile Pharmacodynamics and Mutagenesis

7/1/77-6/30/79

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Problem:

Acrylonitrile (AN) exposure has been associated with excess cancer among exposed workers. In bacterial mutagenesis tests, it has been found weakly positive. Its molecular configuration is similar to vinyl chloride and trichloroethylene, carcinogenic in man or animals, and also mutagenic.

A concern with chlorinated ethylenes is lipid solubility and persistence in the body 3-30 days post-exposure. The proposed mechanism of action of mutagenic and carcinogenic activity is metabolic epoxide formation, also possible with AN. The pharmacodynamics of acrylonitrile were investigated to explore elimination routes and mechanisms of metabolism.

Materials and Methods:

The pharmacodynamics of acrylonitrile in the rat were investigated by study of kinetics in blood following doses of 5-60mg/kg. Blood samples were obtained at 5-10 minutes intervals from an indwelling jugular cannula. Acrylonitrile (AN) concentrations in blood were measured using a nitrogen sensitive detector with headspace gas chromatography. Levels of AN in urine and feces were obtained where possible, and levels in fat and liver were obtained at 3-18 hours after dosing in some experiments. To explore the potential molecular determinants of metabolism and blood binding, two AN analogues, crotononitrile (1-methyl,2-cyanoethylene) and methacrylonitrile (1-methyl, 1-cyanoethylene) were also studied.

Efforts to characterize possible metabolites were unsuccessful. A gas chromatograph peak corresponding to HCN was observed in many samples, but was not positively identified.

Pharmacokinetics:

Crotononitrile and methyacrylonitrile concentrations in blood followed a first-order kinetic elimination from blood with half-lives of 30 to 100 minutes, respectively (Fig. 1). Acrylonitrile showed biphasic kinetic elimination from blood with half-lives of ca. 10 and 50 minutes for the "fast" and "slow" rate constant (Fig. 2, Table 1). The "slow" rate was consistent with in vitro blood binding kinetics, which showed a dose-dependent half-life of 30-77 minutes. The "fast" reaction rate also appeared to be dose-dependent. Both fast and slow rates (14 and 53 minutes) were observed in liver tissue in vitro (Fig. 2). Compartmentalization of acrylonitrile in blood was 5:1 plasma to red blood cells. Methacrylonitrile and crotononitrile exhibited little, if any blood binding behavior. For crotononitrile, which was a mixture of the cis and trans isomers (35:65), the cis isomer showed a slightly shorter half-life (30 min.) than trans (35). Induction with phenobarbital did not change the pharmacokinetic curve. Repeated (2-5X) 5 or 10mg/kg doses showed almost superimposable curves, suggesting no accumulation, or change in metabolic rate. The relative reaction rates for the three homologs are consistent with their respective predicted Michael addition reaction rate or with epoxidation.

Compartmentalization of AN:

AN was measurable in fat, liver, and blood for 18 hours after a 37mg/kg dose. Fecal excretion was significant, with levels of 3-9ppm measurable within three hours after exposure. Urinary excretion continued for 24 hrs. post-exposure, with overnight (collected) urine levels of 0.03ppm after 5.3mg/kg dose and 0.1 for 81mg/kg. Levels were approximately 1/5 that in blood for the first three hours. The

highest urinary concentration observed (20ppm) followed an intravenous dose (20mg/kg), which also corresponded to the highest blood level of AN (400ppm). All other doses referred to herein were ip. Crotononitrile and methacrylonitrile were also measurable in urine (2-20ppm; 50-60mg/kg ip). Crotononitrile levels in urine closely paralled those in blood for both isomers. The 4-24 hr. collection had non-detectable levels.

The multicompartment distribution of AN derives from its dipolar nature. The total AN measured in feces, urine, and tissues, even at very short intervals after ip administration, represented only 3-30% of the administered dose, possibly due to occurrence during absorption of extensive binding such as that observed in blood. AN does not appear to bioaccumulate, due to rapid excretion, unchanged, in urine and feces and to rapid metabolism. Measurement of AN in urine or feces would thus be inadequate as a measure of exposure, except within the first ten hours after exposure, and then would be difficult to relate to dose. However, the technique for analyzing AN in urine is simple and sensitive (low parts per billion).

The pharmacodynamics of AN are complex, with at least 2 major metabolic pathways. The "fast" AN pathway was similar for liver in vitro and blood in vivo, which supports the idea that this pathway is a metabolic one, instead of a rapid redistribution phenomenon. The fact that it was apparently unaffected by phenobarbital pretreatment (enzyme induction) does not support the epoxidation pathway. The fast rate is consistent with Michael addition to AN or its analogs. This reaction would also readily account for cyanide formation:

$$CH_2{=}CH{-}CN \xrightarrow{R^-} R{-}CH_2CH_2CN \xrightarrow{OH^-} R{-}CH_2CH_2OH + CN^-$$

Attempts to simulate the AN reactions with glutathione or cysteine were unsuccessful. Since AN has been reported to react with several nucleic acids, it is possible that these reactions of AN represent addition of endogenous nucleophiles including macromolecules.

Mutagenic studies with acrylonitrile were planned as part of the study. In particular, it was planned to evaluate the epoxide of acrylonitrile. Budgetary cuts from the original proposal prevented extensive mutagenic studies, and attempts to synthesize the epoxide via a published route were unsuccessful. The synthetic products were thought to be of insufficient purity to permit useful testing.

Publications: None. Contemplated: publication of pharmacokinetic findings.

Table 1

Pharmacokinetic data for Acrylonitrile in the rat

	Rate constant (ppm/min x-1 ≈ mole/l/min)	half-life (min)	dose mg/kg
Acrylonitrile-blood binding	0.009-0.02 (0.016±0.006)	30-77	4-160
"Fast" *in vivo* acrylonitrile in blood	0.065-0.11	5-12	5-81
In vitro acrylonitrile in liver, fast	0.050	14	37
slow	0.013	53	
Crotonitrile in blood	0.022	31	49
Methacrylonitrile in blood	0.007	100	62

Doses were administered ip in corn oil. *In vivo* kinetics were studied by analysis of the nitrile in serial blood samples obtained from an indwelling jugular cannula. *In vitro* kinetics were followed by analysis of blood or liver, obtained from a rat sacrificed immediately following treatment; the blood or liver was allowed to stand at room temperature; aliquots were taken for analysis at 10-30 min. intervals.

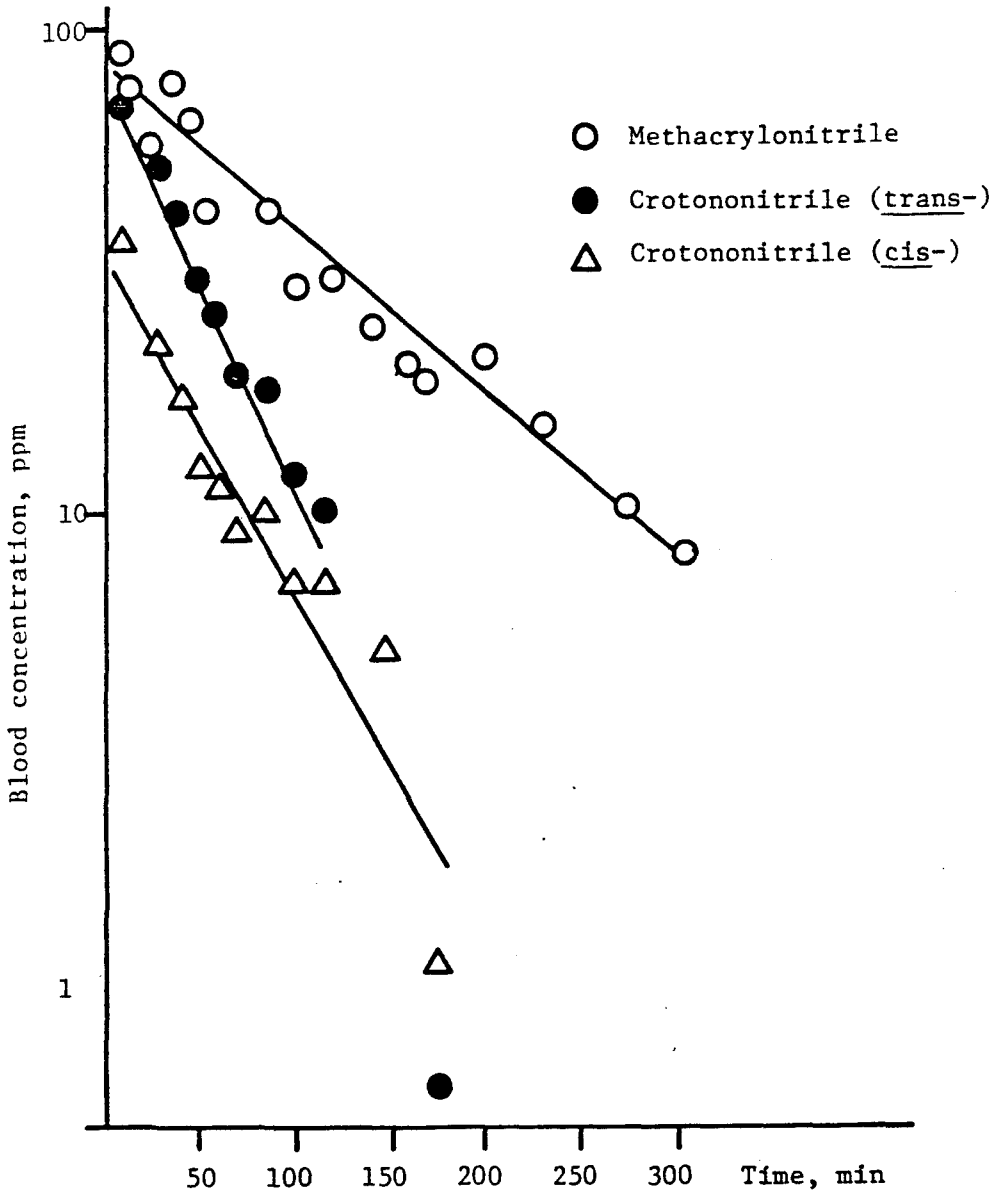

Fig. 1. In vivo kinetics of methacrylonitrile (1-methyl, 1-cyanoethylene) and crotononitrile (a mixture of cis- & trans-1-methyl, 2-cyanoethylene).

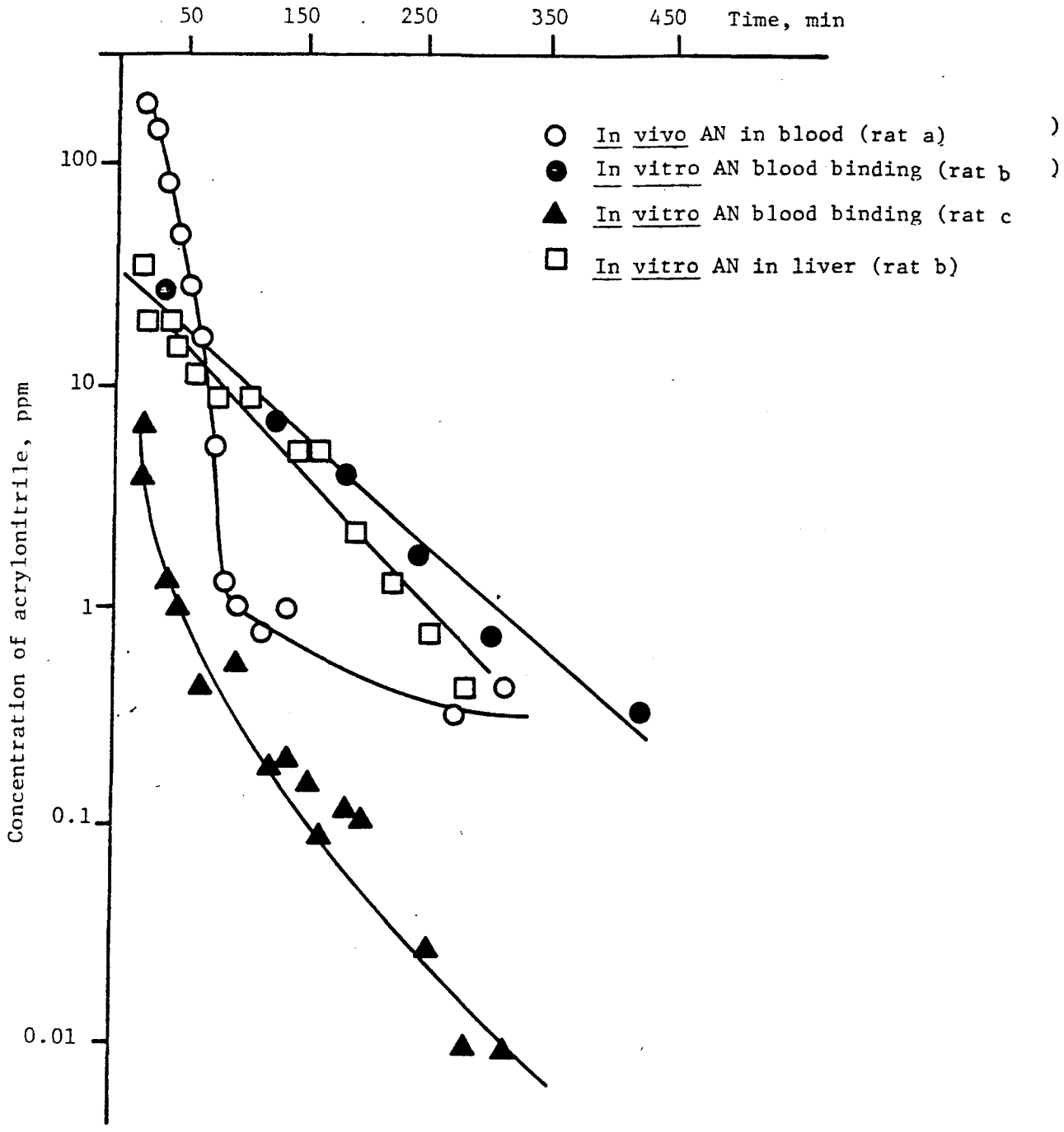

Fig. 2. Comparison of in vivo and in vitro acrylonitrile (AN) kinetics in blood of the rat. Blood and liver in vitro study (rat b) was performed simultaneously. All three experiments followed a 36-37 mg/kg dose (ip in corn oil). Similar results were obtained in other experiments at several dose levels (see Table 1).