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Project Summary

Determination of Hemoglobin Adducts Following Acrylamide Exposure

L. G. Costa and C. J. Calleman

The present project was undertaken to develop new methodologies for biological monitoring of exposure to the toxicant acrylamide (AA) in laboratory animals as well as humans. Methods were developed to measure the adducts of AA and its epoxide metabolite glycidamide (GA) to cysteine in rat hemoglobin (Hb) and to valine in human Hb by means of gas chromatography/mass spectrometry (GC/MS).

Studies in rats indicated that both AA and GA adducts are formed following acute or chronic exposure to AA, while only the GA adducts are formed after exposure to GA. Both adducts, in addition to acrylonitrile (AN) adducts, were measured in a group of Chinese workers exposed to AA during its synthesis and polymerization. Significant signs of neurotoxicity were also found in this population.

Additional studies in rats indicated that AA, but not GA, is the proximate neurotoxicant while GA may be responsible for the male reproductive and genotoxic/carcinogenic effects of AA.

These studies suggest that these novel biomarkers to assess exposure to AA are useful to assess potential health hazards (including possible cancer risks) due to exposure to AA in occupationally exposed workers as well as in the general population.

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This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Humans are potentially exposed to acrylamide (AA) in industrial processes, grouting operations, synthesis of chromatography gels and leakage of the monomer from polyacrylamide used in the purification of drinking water. According to a survey undertaken by NIOSH, some 10,000 workers in 27 occupations are potentially exposed to AA, including about one thousand persons involved in the synthesis of polyacrylamides and another thousand licensed to perform grouting operations. The number of laboratory workers exposed to AA in the preparation of chromatography gels has been estimated to be as high as 100,000-200,000.

Since the early '50s the neurotoxic effects, involving both the central and the peripheral nervous system, have been the primary health concern of human exposure to AA; close to 150 cases of human intoxication have been reported in the last 30 years. In more recent years, following the demonstration that AA induces tumors

in mice and rats, attention has been increasingly focused on the genotoxic and reproductive effects of the compound.

Despite the fact that AA has been shown to induce this wide range of adverse effects in mammalian species, few studies have investigated the role of metabolism in the toxicity of this compound, and although reports have appeared indicating that AA may undergo further metabolism, the structures of the intermediates generated have not been elucidated. From the point of view of quantitative risk assessment of human exposure this has represented a serious gap of knowledge, since the extrapolation of toxic effects in animals requires not only the identification of the agent(s) responsible for the induction of the effects, but, ideally, also information about the relationship between exposure dose and *in vivo* dose (defined as the time-integrated concentration of free electrophilic agent *in vivo*). In addition, since exposure to AA may occur through several different routes, the total amount absorbed by a person may be very difficult to assess.

The structural elucidation and quantitative determination of hernoglobin (Hb) adducts can be used to obtain this type of information. For example, in response to long-standing speculation about the possible involvement of a cytochrome P-450-generated agent in the toxicity of AA, we were recently able to demonstrate the formation *in vitro* and *in vivo* in rats of glycidamide (GA), an epoxide formed by oxidation of the olefinic bond of AA, through the identification of S-(2-carboxy-2-hydroxyethyl) cysteine in hydrolyzed Hb samples from AA-exposed rats.

It was the central aim of this project to develop and apply methods for simultaneously determining the adducts formed by AA and GA in Hb as a means of assessing occupational exposure to AA and to use these data in combination with dosimetric and metabolic studies in experimental animals undergoing toxicological tests to arrive at a risk estimation of human AA exposure that may serve as a basis for regulatory action.

In addition to the practical implications of the proposed methods for biomonitoring, AA appears to be a suitable compound for the theoretical development of a risk model based on *in vivo* dosimetry because of the wide range of toxic effects it is known to induce. Since both AA and GA are reactive electrophilic compounds, it was not clear whether the various toxic effects associated with AA exposure are induced by the parent compound, the epoxide, or the combined action of the two agents. Our finding that AA was metabolized to GA, thus generated a few hypothetical solutions to theoretical problems particular to the toxicology of AA.

Procedure

The specific aims of this project were, therefore

1. To develop a method for measuring the adducts of AA and GA to Hb in rats and to determine both adducts following administration of AA or GA.

2. To develop a method for measuring the adducts of AA and GA in humans, to measure adducts in workers occupationally exposed to AA, and to correlate adduct levels with neurotoxicity.

3. To evaluate and compare the neurotoxicity and reproductive toxicity of AA and GA in the rat.

Results and Discussion

1. Development of a GC/MS method for measuring Hb adduct of AA and GA.

A method was developed to determine Hb adducts formed by the neurotoxic agent AA and its mutagenic epoxide metabolite GA. The method was based on simultaneous measurements of the cysteine adducts formed by these two agents by means of gas chromatography/mass spectrometry (GC/MS) in hydrolyzed Hb samples. Rats were injected ip with AA or GA in doses ranging from 0 to 100 mg/kg body wt, and the Hb-adduct levels were determined. The Hb-binding index of AA to cysteine was found to be 6400 pmol (g Hb)$^{-1}$/μmol (kg body wt)$^{-1}$, higher than for any other substance studied so far in the rat, and 1820 pmol (g Hb)$^{-1}$/μmol (kg body wt)$^{-1}$ for GA. In rats injected with AA, formation of adducts of the parent compound was approximately linear with dose (0-100 mg/kg), whereas adducts of the epoxide metabolite GA generated a concave curve, presumably reflecting the Michaelis-Menten kinetics of its formation. On the basis of the rate constants for cysteine-adduct formation determined *in vitro*, the first-order rates of elimination of AA and GA from the blood compartment of rats were estimated to be 0.37 and 0.48 hr^{-1}, respectively, using a linear kinetic model. It was further estimated that the percentage of AA converted to GA in the rat decreased from 51% following administration of 5 mg/kg to 13% after a dose of 100 mg/kg. Subchronic treatment of rats with AA (10 mg/kg/day for 10 days or 3.3 mg/kg/day for 30 days) confirmed that the conversion rate of AA to GA, as determined from Hb-adduct formation, is higher at low-administered doses. These findings suggest that dose-rate effects may significantly affect risk estimates of this compound and that different low-dose extrapolation procedures should be employed for effects induced by the parent compound AA and those induced by the metabolite GA.

2. Development of methods for AA and GA Hb adducts in humans.

Hb-adduct determinations were used to monitor occupational exposure to AA and acrylonitrile (AN). Forty-one workers in a factory in the People's Republic of China who were involved in the synthesis of AA by catalytic hydration of AN and the manufacturing of polyacrylamides were studied. Ten nonexposed workers in the same city served as controls. AA and AN exposures were monitored using the modified Edman degradation procedure for the determination of their respective Hb adducts to N-terminal valine. The adduct levels in the exposed workers were 0.3-34 nmol/g Hb for AA and 0.02-66 nmol/g Hb for AN, as determined by GC/MS. The formation of GA, the epoxide metabolite of AA, in humans was demonstrated by GC/MS analysis of its Hb-adduct N-terminal valine following acid hydrolysis, ion-exchange chromatography, and derivatization. The GA adduct was detected in samples from exposed persons with levels of 1.6-32 nmol/g Hb. There was a linear relationship between the AA- and GA-adduct levels (r=0.96) and the ratio of the *in vivo* doses of GA and AA was 3:10. These results suggest that AA is metabolized to GA in humans, as had previously been shown in the rat. The high AA-adduct levels in the exposed workers, as compared to those expected from air concentrations, indicate that dermal exposure may contribute significantly to the total uptake of AA. Observations at the factory are consistent with this indication. The average daily *in vivo* doses of AA and GA in the highest exposed Chinese workers were comparable to the *in vivo* doses in rats injected with 3 mg/kg AA. Since a regimen of 2 mg/kg/day is known to cause a significant increase of tumors in rats, preventive measures may be necessary for humans exposed to high levels of AA in industrial settings.

The workers underwent a complete medical and neurological examination and provided blood and urine for the determination of several biomarkers of exposure. Among the exposed workers, signs and

symptoms indicating peripheral neuropathy were found with statistically significant increased frequencies as compared to a group of controls from the same city. Based on signs and symptoms and quantifiable indicators of peripheral nervous dysfunction, such as increased vibration detection thresholds, neuropathic signs and electroneuromyography measurements, a Neurotoxicity Index (NIn) specific for AA-induced peripheral neuropathy was designed. The NIn, which adequately predicted the clinical diagnosis of peripheral neuropathy, was significantly correlated with the levels of mercapturic acids (products of AA metabolism) in 24-hr urine, Hb adducts of AA, accumulated *in vivo* dose of AA, employment time, and vibration sensitivity. The NIn was correlated also with Hb adducts of AN, which was explained primarily by a correlation between AA and AN exposure in this workshop. It was however not significantly correlated with momentary measures of exposure such as concentrations of AA in the air or in the plasma of exposed workers.

This study is the first in which adduct monitoring has been applied to the same group of individuals where adverse health effects have been observed. The results seem to indicate that Hb adducts are useful as predictors of AA-induced peripheral neuropathy and that measurements of vibration thresholds are useful for identifying early neurotoxic effects in workplaces with hazardous exposures to AA.

3. Comparative neurotoxicity of AA and GA.

In rats treated with 0-100 mg/kg of AA, significant dose-rate effects were observed on adduct formation by both AA and GA. The high rate of formation of the metabolite, especially at low doses where approximately 60% of AA was converted to GA *in vivo*, prompted us to investigate its potential role in the induction of neurotoxic and reproductive effects attributed to AA exposure. In initial neurotoxicological experiments, the effects of the parent compound (8-14 days, 25 and 50 mg/kg/day) and the metabolite (8-14 days, 50 and 100 mg/kg/day) were compared. While at the higher dose both compounds affected the rats' performance on the rotarod, only AA had a significant effect in the hindlimb splay test, which is considered a more sensitive indicator of peripheral neuropathy. On the other hand, a stronger effect was seen for GA than for AA on the male reproductive system, especially on sperm cell viability. These preliminary results suggest that while the parent compound appears to be primarily responsible for the induction of peripheral neuropathy other toxic effects associated with AA exposure, such as reproductive toxicity, may be attributed to GA.

Conclusions

A GC/MS method was developed for measuring AA and GA adducts to cysteine residues of Hb from the rat. Results of a human study in which AA and GA were measured as adducted to the N terminal valine of Hb, suggest that AA is metabolized to GA in humans as has been shown in the rat. Dermal exposure is thought to be the major route of exposure in the factory workers who were monitored. Indicators of peripheral nervous dysfunction were found to be correlated significantly with Hb adducts of AA. Further experiments with rats indicate that AA is the neurotoxicant, while GA may be the reproductive- and geno-toxin.

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L. G. Costa and C. J. Calleman are with the University of Washington, Seattle, WA 98195.

***Charles H. Nauman** is the EPA Project Officer (see below).*

The complete report, entitled "Determination of Hemoglobin Adducts Following Acrylamide Exposure," (Order No. PB94-144235; Cost: $19.50; subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:

Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
Las Vegas, NV 89193-3478

United States
Environmental Protection Agency
Center for Environmental Research Information
Cincinnati, OH 45268

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