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Project Summary

Carcinogenic Effects of Arsenic Compounds in Drinking Water

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The primary objective of this research project was to determine whether there was a dose-response effect of arsenic (AsIII) as a promoter of DENA-initiated tumors in the kidneys of partially hepatectomized rats. Secondary objectives were to complete the study extending from the previous grant which was to determine whether arsenite (AsIII), arsenate (AsV) and dimethylarsinic acid (DMA) were initator carcinogens and/or promoters of DENA-initiated tumors in the rat kidney. In addition an analytical method for measuring the above three arsenic species simultaneously in the same biological sample was to be developed.

A dose-response was observed for AsIII promotion in partially hepatectomized DENA-initiated rats at concentrations of 40, 80 and 160 mg/L in the drinking water but it was not statistically significant.

AsIII and AsV did not have any initiator activity in intact male Wistar rats but significantly promoted DENA-initiated renal tumors in these animals. DMA had neither initiator nor promoter activity.

A continuous flameless HPIC-electrothermal atomic absorption spectrophotometric method was developed to detect and to quantitate simultaneously nanograms quantities of AsIII, AsV and DMA in biological fluids.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC 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

Arsenic has been associated with cancer in humans for a number of years based mainly on epidemiological studies (IARC, 1973; Yeh, 1973; Zaldivar, 1974; Bencko, 1976; NAS Committee, 1977; and Wildenberg, 1978). Experimental evidence in animal studies have basically been negative (Wildenberg, 1978) or the epidemiological evidence has been complicated by the presence of other chemicals or conditions which complicated the interpretation of the data (Pershagen, 1981). Thus, arsenic has not been unequivocally demonstrated to be a carcinogen; at best it may be a very weak carcinogen.

More recently, the contractor's laboratory has been involved in determining whether arsenic might be a promoter of carcinogenesis rather than acting as an initiator carcinogen. Thus, the project used an initiator-promotion experimental design in partially hepatectomized rats utilizing diethylnitrosamine as the initiator. Results showed that arsenite (AsIII) was a promoter of DENA-initiated renal tumors which were cortical in origin and associated with the renal tubular cells (Shirachi et al., 1983).

Associated with this effect was also a decrease in thymus weight, implicating a possible immunosuppressive effect as a contributory factor in the carcinogenesis process. In addition, these effects were observed in partially hepatectomized rats, a surgical procedure used to study hepatic carcinogenesis. Thus, it was possible that the partial hepatectomy might have been also a contributory factor in the promotion effect of AsIII, complicating the interpretation of the data obtained.

Therefore, the first objective of this study was to determine whether partial hepatectomy was a contributory factor by repeating the previous study in intact rats, using the same experimental design. If the results showed that partial hepatectomy was not contributory, then the second objective was to determine whether this promotion effect was a dose-response related phenomenon. This latter effect would be important in determining safe drinking water standards Since we have determined in preliminary studies that the various arsenic species are interconvertible in the liver (unpublished results), a final objective was to develop an analytical methodology for determining simultaneously the levels of the different arsenic species in biological samples. This would allow for blood and tissue level measurements to determine the effects of endogenous biotransformation of the different arsenics administered.

Materials, Methods and Kidneys

THE PROMOTION EFFECTS OF ARSENIC COMPOUNDS ON LIVERS FROM CONTROL AND DENA-INITIATED WISTAR RATS Cronically Treated with AsIII, AsV and DMA in the Drinking Water for 10, 15 and 24 Months.

A two stage initiation-promotion protocol was utilized for these experiments. Male Wistar rats 5 to 6 weeks old were injected with either 0.9% saline or DENA (Eastman Kodak) at a single i.p dose of 30 mg/kg and placed on regular Purina rat chow and deionized water *ad lib.* On day 7 the animals were placed in treatment groups and the promotion was instituted The control groups were continued on deionized water, the AsIII, AsV and DMA treatment groups pretreated with 0 9% saline were put on drinking water *ad lib* containing elemental arsenic in the form of sodium arsenite (AsIII), sodium arsenate (AsV) or dimethylarsinic acid (DMA) in deionized water equivalent to elemental arsenic at concentration of 160, 160 and mg/L respectively, and the AsIII, AsV and DMA treatment groups pretreated with DENA were put on drinking water *ad lib* containing sodium arsenite and dimethylarsinic acid in deionized water also equivalent to 160, 160 and 80 mg/mL respectively These animals were sacrificed at 10, 15 and 24 months

Livers were taken at necropsy processed and examined microscopicallv for basophilic foci, neoplastic modules and hepatocellular carcinomas Kidneys were also taken and examined microscopically for atypical hyperplasia, adenoma and adenocarcinomas

THE DOSE-RESPONSE EFFECT IN TUMORIGENESIS IN DENA-INITIATED MALE WISTAR RATS PROMOTED WITH AsIII OR AsV FOR 6, 9 AND 12 MONTHS IN THE DRINKING WATER

Materials and Methods

A two-stage initiation-promotion protocol utilizing partially hepatectomized rats was used for these experiments. Male Wistar rats weighing 70-100g were partially hepatectomized (PH) under ether anesthesia and allowed to recover. The animals were injected 18-24 hours later with a single i.p. dose of 0.9% saline or DENA (Eastman Kodak) 25 mg/kg and placed on regular Purina rat chow and deionized water *ad lib.* On day 7 the animals were randomly placed in treatment groups and the promotion protocol was instituted. The saline control and DENA control treatment groups were continued on deionized water; the AsIII treatment group pretreated with 0.9% saline was put on drinking water *ad lib* containing elemental arsenic in the form of sodium arsenite (AsIII) in deionized water equivalent to 160 mg/L; and the AsIII treatment group pretreated with DENA were put on drinking water *ad lib* containing sodium arsenite in deionized water equivalent to 40, 80 and 160 mg/L. In the nonhepatectomized rats the animals were injected with a single i.p dose of 0.9% saline or DENA 40 mg/kg. On day 7 the animals were randomly placed in treatment groups and the promotion protocol was instituted.

The saline control and DENA control treatment groups were continued on deionized water; the AsIII and AsV treatment groups pretreated with 0.9% saline were put on drinking water *ad lib* containing elemental arsenic in the form of sodium arsenite and sodium arsenate (AsV) in deionized water equivalent to 160 mg/L; and the AsIII and AsV treatment groups pretreated with DENA were put on drinking water *ad lib* containing sodium arsenite or sodium arsenate in deionized water equivalent to 80 and 160 mg/L. These animals were sacrificed at 6, 9, and 12 months

At necropsy, all animals were weighed and the kidneys removed, weighted and placed in 10% buffered formalin The tissues were then processed for paraffin embedding and prepared by routine histological techniques for H and E staining. The slides were examined microscopically for atypical hyperplasia, adenoma and adenocarcinoma in renal tissues.

SIMULTANEOUS DETERMINATION OF ARSENIC COMPOUNDS SPECIES BY HPLC-ELECTROTHERMAL ATOMIC ABSORPTION SPECTROSCOPY IN BIOLOGICAL FLUIDS

The analytical method consisted of a HPLC connected to a low capacity anion exchange column which separated the different arsenic species and converted by post-column modification by means of a hydride generator to their respective arsine gases. The arsine gas was separated from the liquid by an on-line gas-liquid separator through which a carrier gas passed to deliver the arsine gas into a quartz cell mounted on an atomic absorption spectrophotometer. The hyride generator system was driven by a proportioning pump and manifold.

MONITORING OF FOOD CONSUMPTION, BODY WEIGHT AND WATER CONSUMPTION DURING CHRONIC EXPOSURE TO ARSENIC IN THE DRINKING WATER

Body weight. Because of the number of animals involved, they were weighed as a group from each cage rather than individually. Except for a few exceptions the number of animals in each cage did not exceed five. Because the animals from each cage were either sacrificed or may have died, the changes were noted but the remaining animals were not moved to other cages. Therefore, all animals remained in the same cage during the entire study. Animals in each cage were weighed as a group once a week and the weight recorded.

Food consumption. As with the body weight monitoring, food consumption of animals in each cage was group monitored Usually, the food was weighed on the same day, depending upon the number of cages and animals. The total amount of food provided for each cage was weighed and recorded periodically.

Conclusions and Recommendations

Promotion Effects In Liver

The study concluded that (1) basophilic foci are probably precursor cells tc neoplastic nodules in the liver; however it is equivocal whether these cells are preneoplastic lesions, (2) it is probable that neoplastic nodules are precursor lesions to hepatocellular carcinomas however, the presence of these lesions does not indicate necessarily a pathogenesis to hepatocellular carcinoma; (3

formation of basophilic foci and neoplastic nodules are delayed in nonhepatectomized animals; (4) AsIII and DMA may be weak promoter carcinogens for DENA-initiated hepatocellular carcinomas in the rat liver; (5) one of the mechanisms by which AsIII may be acting as a promoter is suppression of the immune system; and (6) hepatocellular carcinoma appears to take a longer time to occur when compared with the appearance of renal adenoma or adenocarcinoma.

It is recommended that further study of the AsIII and DMA potential promoter effects for hepatocellular carcinoma be made in partially hepatectomized rats. However, since diethylnitrosamine (DENA) appears to be a good initiator for the inorganic arsenics, AsIII and AsV, in renal tumorigenesis, we recommend that several different initiators other than DENA be tested because the animals will more than likely die from the kidney lesions before the liver effects can be observed with DENA as the initiator.

Promotion Effects In Kidney

The data from this study suggest that (1) there is no direct correlation between formation of atypical hyperplasia and the appearance of renal tubular adenomas and/or adenocarcinomas; (2) atypical hyperplasia is a general response to toxic substances and occurs to the same degree in response to all three arsenic treatments studied; (3) AsIII and AsV are promoters for DENA-initiated renal tumors; (4) partial hepatectomy is not a prerequisite for DENA-induced renal tumorigenesis promoted by the inorganic arsenic compounds; (5) inorganic arsenics are not weak carcinogens under the experimental conditions of this study; and (6) a possible mechanism for the promotion effects of AsIII and AsV might be at least partially due to immunosuppression. Thus, the results from this study support earlier findings in partially hepatectomized rats that AsIII is a promoter of DENA-initiated renal tumors. Now AsV can also be said to have the same effect.

Since the doses used for both inorganic arsenics were the same, 160 mg/L in the drinking water, and they both promoted renal tumorigenesis initiated by DENA, it is important to determine whether these arsenic species were exerting their promotion effects via a common mechanism due to biotransformation or via different mechanisms. Since the chemistry of the two inorganic arsenics are very different but can be interconverted by biotransformation; and since the promotion effect occurred at the same dose, a common mechanism may have been involved. A systematic study of the metabolism of the various arsenic species should be done both in the liver and in the kidney to determine the metabolic fate of each specie after it is absorbed from the G.I. tract. This should indicate to which arsenic specie the kidney would be exposed, irrespective of which arsenic specie was ingested via the drinking water. This effort should provide some insight into the arsenic species responsible for the promotion effect. In addition, a followup study should be made to determine whether the immune response is implicated in some way and whether this might explain at least partially the occurrence of the tumorigenesis process in the renal tubules.

THE DOSE-RESPONSE TUMORIGENIC PROMOTION EFFECTS OF AsIII AND AsV

Some experimental evidence in this study indicates that AsIII and AsV might increase kidney growth to some extent, and also that the dose-response curve for renal tumorigenesis promoted by AsIII is very steep and not easily determined.

Further studies conducted on dose-response effects of AsIII and AsV in terms of promotion of renal tumorigenesis, should include' (1) use of the partial hepatectomy model; (2) an increased initiation dose to 30 mg/kg; and (3) observation of tumorigenesis at an earlier time period (between 3-6 months).

SIMULTANEOUS DETERMINATION OF ARSENIC SPECIES BY HPLC-ELECTRO-THERMAL ATOMIC ABSORPTION SPECTROSCOPY IN BIOLOGICAL FLUIDS

This method will be able to detect some of the arsenic species down to 5-10 ng and others down to 20-25 ng. Thus, the method will be useful in measuring nanogram quantities from blood, urine and tissue samples. It has the advantage over most of the other anlaytical methods in that it is sensitive and is a continuous flow system. This analytical method should be further developed for studying arsenic metabolism and measuring the various arsenic species in biological fluids.

MONITORING OF CUMULATIVE BODY WEIGHT, WATER AND FOOD CONSUMPTION

When AsIII and AsV are put into drinking water, they have an inhibitory effect on the cumulative body weight gained at the doses used in this study. This was a primary effect of AsIII and AsV because it occurred in the presence or absence of DENA-initiation. This decrease in cumulative body weight gain probably was due to the inhibitory effect these compounds has on water consumption which was significantly decreased at these doses. AsIII and AsV also inhibited food consumption during the initial rapid growth stage, and probably decreased weight gain. With respect to the tumorigenesis, it is not clear whether any of these inhibitory effects played a role. The decrease in food consumption could have had an adverse effect on the nutritional requirements of these animals, thereby contributing to the promotion of DENA-initiated renal tumors.

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The complete report, entitled "Carcinogenic Effects of Arsenic Compounds in Drinking Water," (Order No. PB 87-232 542/AS; Cost: $13.95, subject to change) will be available only from:

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