Arsenic Possibly Influences Carcinogenesis by Affecting Arginine and Zinc Metabolism

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Received November 10, 1982; Accepted March 9, 1983

Abstract

The role of arsenic in carcinogenesis is controversial. There is no doubt arsenic can influence carcinogenesis under certain conditions. However, a review of the findings relating arsenic to cancer indicates that arsenic mainly affects carcinogenesis indirectly by influencing other metabolic systems (i.e., immune system) or nutrients (i.e., arginine, zinc) that may have a more direct role in the carcinogenic process. Depending upon the level of exposure, arsenic can either inhibit or activate interferon, an inhibitor of virus replication. Furthermore, arsenic can apparently inhibit some virus-induced tumorigenesis. However, once a tumor is initiated, arsenic enhances tumor growth, possibly by affecting the immune response. Recent experiments in our laboratory demonstrated that arsenic metabolically interacts with arginine and zinc, both of which apparently influence the immune response. Arsenic evidently has a role that strongly influences the metabolism of arginine, which is an immunostimulatory amino acid. Furthermore, the effect of arsenic on arginine metabolism is apparently modified by the zinc status of the animal. Because arsenic can apparently affect cancer development through several indirect or direct mechanisms, probably the only general conclusion that can be made about arsenic and cancer is that arsenic, depending upon dosage, route of administration, and chemical form, modifies the induction or development of some tumors.

Index Entries: Arsenic, role in carcinogenesis; carcinogenesis, role of arsenic in; arginine, effect of As on during carcinogenesis; zinc, effect of As on during carcinogenesis; arsenic, interaction with zinc and arginine; arginine, interaction with arsenic and zinc; zinc, interaction with arginine and arsenic; cancer, effects of arginine, As and Zn interactions; immune response, effects of arginine, As and Zn interactions.
Introduction

The role of arsenic in cancer prevention or causation is a topic that can evoke much emotion. Epidemiologic evidence has convinced many individuals that arsenic is a carcinogen. Others feel that, because attempts to experimentally induce cancer using arsenic have been generally unsuccessful, further information is needed to establish the role of arsenic in carcinogenesis. There are also a few individuals who, on the basis of limited experimental evidence, believe some arsenicals may possess anticarcinogenic properties. Close examination of numerous studies indicates to us that there apparently is some influence of arsenic on carcinogenesis. However, this influence is not that of a direct carcinogenic action, but that of indirectly influencing other metabolic systems (i.e., immune system) or nutrients (i.e., zinc) that have a more direct role in the carcinogenic process. This indirect action means that, depending upon the form, method of administration, and dosage, arsenic may have antagonistic, synergistic, or no action in carcinogenesis. In other words, arsenic is similar to many other nutrients, such as vitamin A, selenium, or zinc, that do not initiate carcinogenesis, but under certain conditions and with certain types of cancer, can positively or negatively modify later tumor development. One possible locus for modification by arsenic is that of host–immune systems. The findings that support this possibility will be reviewed here.

The Immune System, Carcinogenesis, and Arsenic

A review by Bull (1) indicated that the effect of nutrition on tumor immunity is quite complex because the immune system apparently exerts multiple and sometimes opposing effects on tumor growth. Thus, when immune function is involved, paradoxical effects of nutritional insults on tumor growth may be expected. For example, some nutritional deficiencies interfere with antibody synthesis. Logically, this should lead to less antibody-dependent cellular cytotoxicity and thus to accelerated tumor growth. However, the phenomenon of blocking antibody in tumor immunity complicates this logic. In some cancers, antigen–antibody complexes are formed that interfere with immune cytotoxic actions on tumor cells, thus giving an apparent enhancement of tumor growth. In this case, malnutrition-induced reduction in antibody synthesis would lead to slower tumor growth. It appears, therefore, that nutritional insults that affect the humoral and cellular immune system could possibly affect tumor development. However, the direction of that effect would be hard to predict.

Both organic and inorganic forms of arsenic apparently can, directly or indirectly, affect the immune system. Gainer (2) found that high concentrations of arsenicals inhibited both the synthesis and the action of interferon, whereas low concentrations of arsenicals increased the antiviral activity of low levels of interferon in cell cultures inoculated with vesicular stomatitis virus. Interferon, an inhibitor of virus replication, is one of the physiologically active lymphokines synthesized and released by sensitized lymphocytes that have become activated by contact with antigens. It was suggested that increased antiviral activity of interferon could pro-
vide a rationale for the beneficial "growth-promoting" effect of arsénical feed additives in animals through reduction in disease incidence or severity.

There are reports that show that arsenic can reduce the occurrence of some virus-related cancers. Schrauzer et al. (3) studied the effect of oral arsenite on carcinogenesis in a strain of C3H mice that develop mammary adenocarcinoma with close to 100% incidence. The oncogenic agent is thought to be viral and transmitted vertically during breast feeding of the young by infected dams. Schrauzer et al. (3) found that 10 ppm of arsenite in the drinking water depressed tumor incidence in the C3H mice. In a subsequent study in which the controls exhibited a relatively low incidence of spontaneous tumors, 2 ppm of arsenite in the drinking water did not significantly depress tumor incidence (4). However, in both studies, arsenite enhanced tumor growth rates and raised the incidence of multiple tumors. Similar observations were made by Kerkvliet and Kollet (5) who studied tumors induced by a tissue culture cell line (MSB)-derived from Moloney sarcoma virus (MSV)-induced tumors in B6 mice. They found that MSB tumor development was delayed and tumor incidence and mortality was reduced by arsenite orally administered to C57BL/6 mice inoculated with MSB tumor cells. On the other hand, arsenic apparently depressed cell-mediated cytotoxicity and appeared to enhance tumor growth. However, the latter finding apparently was not statistically significant. No doubt there are several explanations for the actions of arsenic on these virus-related cancers. In light of the findings by Gainer (2) an appealing explanation is that arsenic inhibited tumor initiation by enhancing the action of interferon, but once a tumor was initiated, arsenic affected the immune response in some manner to enhance tumor growth.

The experiments that showed that arsenic possibly affects the immune response to viruses used luxuriant amounts of the element. The use of luxuriant, or toxic, amounts of a substance to evoke a response in a physiological mechanism does not necessarily mean that a response would occur in the mechanism in the absence of that substance. However, it does indicate a possible site for study in deficiency-type investigations. The objective of the rest of this presentation will be to present findings that suggest that arsenic deprivation can affect the immune response, and thus carcinogenesis, via interactions with arginine and zinc.

Arginine and the Immune Response

The finding that orally administered arginine affects the immune response in healthy or stressed animals and humans is relatively new. In 1980, Barbul et al. (6) reported that rats stressed with femoral fractures exhibited impaired thymic function as assessed by thymic size, numbers of thymic lymphocytes, and ability of thymic lymphocytes to respond to mitogenic stimulation. They found that a 1% dietary arginine supplementation effectively alleviated thymolysis and thymic lymphocyte dysfunction that appeared post-trauma in the rats. Furthermore, supplemental dietary arginine significantly elevated thymic weight, cellularity, and T-cell blastogenic responsiveness in uninjured rats. Barbul et al. (6) stated that the supplemental arginine apparently affected thymic lymphocytes directly, both by
increasing their numbers and by heightening their reactivity to mitogens. In a subsequent study, Barbul et al. (7) found that a daily dietary supplement of 30 g of arginine · HCl for 7 d fed to humans significantly elevated stimulation indices of peripheral blood lymphocytes following concanavalin A and phytohemagglutinin stimulation.

Barbul and coworkers also examined the effects on the immune response of other amino acids related to arginine in the urea cycle. They found that ornithine exhibited the thymotrophic effect of arginine (8), but that citrulline, which can replace arginine for growth requirement in rats, was not thymotrophic (9).

Other studies showed that the thymotrophic effects of arginine supplementation correlated with a strong antitumor effect of MSV viral tumors and on the C3HBA tumors (7, 10). Dietary arginine supplementation prolonged tumor induction, reduced tumor incidence, and tumor size, and in the case of the lethal C3HBA tumor, significantly lengthened survival time. A recent study indicated that ornithine, like arginine, inhibited C3HBA tumor growth (11).

Zinc and the Immune Response

There is no question that zinc is an element of importance to the immune system and host-defense mechanisms. There are numerous reviews available on that subject and thus only a few statements in a review by Nauss and Newberne (12) will be repeated here. Zinc deficiency has a profound effect on the anatomy of the lymphoid tissues of experimental animals. Zinc-deficient animals exhibit hypoplasia of the thymus, spleen, lymph nodes, Peyers patches, and other intestinal lymphoid tissues. Dysfunction of the immune system components in both zinc-deficient animals and humans has been reported, including a depression in T-helper lymphocyte functions, diminished activity of natural killer cells, decreased T-lymphocyte killer activities, and depressed cytotoxic activity of natural killer cells. Mitogen stimulation of lymphocytes in zinc deficiency apparently is depressed. However, this latter finding may depend upon the severity and/or duration of zinc deficiency (13).

Whether zinc nutriture affects carcinogenesis by affecting the immune system is not known. However, some findings suggest that this may be true. For example Ciapparelli et al. (14) found that supplemental zinc in the drinking water retarded tumor growth induced by dimethylbenzanthracene implants in the submandibular gland of rats. Increasing supplemental zinc progressively decreased the amount of squamous epithelium, whereas an inflammatory response became more marked. Ciapparelli et al. (14) suggested that these findings were indicative of a zinc-modified immune response to the developing carcinoma. In apparent contrast, dietary zinc deficiency also may be beneficial against carcinogenesis. Zinc deficiency depressed tumor growth rate and subsequent survival of rats injected with Walker 256 carcinosarcoma (15), or of mice injected with leukemic cells (16). The logical explanation of these findings is that, because zinc is required for DNA synthesis and subsequent cell division, the depressed tumor growth is the result of lower cell turnover. However, another possible explanation can be that zinc defi-
ciency altered the immune response in such a way that the antagonistic, rather than the synergistic, effect on tumor growth was enhanced.

Interactions Among Arsenic, Arginine, and Zinc

Although the exact mechanisms for how zinc (or the lack of zinc) or arginine retards tumor development, or functions in the immune system, are unknown, there is no doubt that these nutrients can have marked effects. Thus, any nutrient that affects the metabolism of arginine and zinc may indirectly affect the immune response and thereby some carcinogenic processes. In our laboratory, findings have been obtained that show that arsenic interacts with arginine and zinc.

In 1978, Nielsen and Shuler (17) reported that arsenic might be an essential nutrient for growing chicks. In four experiments, 1-d-old cockerel chicks were fed a diet based on skim milk powder and acid-washed ground corn that was supplemented with arginine (20 g/kg). The basal diet contained about 20 ng of As/g in experiments 1 and 2, 35 ng/g in experiment 3, and 45 ng/g (low-arsenic skim milk became unavailable) in experiment 4. Controls were fed a supplemental 1.0 μg of arsenic/g. Although not described in the report, personal involvement of one of us with these experiments enables us to mention that these chicks were raised under environmental conditions that could have stressed the chick towards the end of the experiments. The chicks were housed in plastic cages inside rigid plastic isolators (18). As the chicks became older, proper cleanliness and humidity became difficult to maintain in the cages. Also, the experiments had to be terminated when chicks were age 28 d because the cages became too crowded. These environmental conditions are mentioned because they may explain, along with the high level of dietary arginine, why findings described by Nielsen and Shuler (17) did not agree well with our subsequent findings. Nielsen and Shuler (17) found that, in the first three experiments, the arsenic-deprived chicks weighed significantly less than controls at 28 d. They suggested that the growth difference might have been more marked if the experiments had been prolonged, because arsenic deprivation did not affect growth until days 17 to 20 of the experiments. This was the time the cages started to become crowded and hard to keep clean. In experiment 4, chicks fed the basal diet containing 45 ng As/g grew as well as controls. Two additional findings in these experiments were that the arsenic-deprived chicks exhibited elevated levels of zinc in the liver and possibly depressed white cell counts (19). These latter two findings, which have not been confirmed in subsequent studies, indicate that these chicks may have been under a stress that demanded a marked involvement of the immune system. It is known that during infection and other inflammatory stresses in rats, the level of zinc in liver is elevated (20).

After the initial experiments, the unavailability of low-arsenic skim milk powder and concern about the environmental conditions prompted us to develop a new diet and an improved environment for the chicks. In 1979, we successfully formulated a new diet which routinely contained less than 15 ng As/g. This diet was composed of high-protein casein (16%), acid-washed ground corn (68%), corn oil (7.5%), and supplemental vitamins, minerals, and amino acids to meet the nutri-
tional requirements of chicks (21). Environmental conditions were vastly improved by modifying the isolator so that small cages did not have to be used (22).

Initial experiments using the new diet containing normal levels of arginine, and an improved environment, were disappointing (unpublished observations) because the findings did not agree well with those of Nielsen and Shuler (17, 19). Thus, we decided to study arsenic deprivation in chicks stressed with zinc deficiency and high dietary levels of arginine. Among the experiments done was a three-way, two-by-two-by-two arranged experiment with supplemented arsenic, arginine and zinc as the variables (Table 1). The most interesting findings from this experiment were those that showed that dietary arsenic markedly influenced arginine metabolism (Table 1). In this experiment, arsenic deprivation apparently elevated kidney arginase activity and plasma uric acid in chicks fed a normal level of arginine. When dietary arginine was increased to 34 mg/g by a supplement of 20 mg/g, kidney arginase activity and plasma urea were substantially elevated. However, the elevation of these two parameters was markedly influenced by dietary arsenic and zinc. Zinc deficiency alleviated the elevation in plasma urea and kidney arginase.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma uric acid, mg/100 mL</th>
<th>Plasma urea, mg/100 mL</th>
<th>Kidney arginase, units$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>As, µg/g</td>
<td>Arg, mg/g</td>
<td>Zn, µg/g</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0–5</td>
<td>6.99</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0–5</td>
<td>5.83</td>
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<tr>
<td>0</td>
<td>0</td>
<td>50</td>
<td>6.66</td>
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<tr>
<td>2</td>
<td>0</td>
<td>50</td>
<td>4.71</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>0–5</td>
<td>7.64</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0–5</td>
<td>8.24</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>50</td>
<td>6.00</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>50</td>
<td>5.24</td>
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Analysis of Variance, $P$ Values

<table>
<thead>
<tr>
<th>Effect</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic effect</td>
<td>0.001</td>
</tr>
<tr>
<td>Arginine effect</td>
<td>0.001</td>
</tr>
<tr>
<td>Zinc effect</td>
<td>NS</td>
</tr>
<tr>
<td>As × Zn</td>
<td>0.03</td>
</tr>
<tr>
<td>As × Arg</td>
<td>0.0007</td>
</tr>
<tr>
<td>Zn × Arg</td>
<td>NS</td>
</tr>
<tr>
<td>As × Zn × Arg</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

$^*$Chicks were killed at age 35 d.

$^1$Amounts of arsenic (sodium arsenate), arginine, and zinc (zinc acetate) supplemented to basal diet containing approximately 15 mg As, 5 µg Zn, and 14 mg Arg/g.

$^2$Initially, no supplemental zinc was given to the zinc-deficient groups. After age 12 d, a supplement of 5 µg of zinc (zinc acetate) was given.

$^3$Units were µg urea formed/min/mg protein (×100).
TABLE 2
Effect of Dietary Arsenic on the Level of Certain Amino Acids in Plasma of Chicksa

<table>
<thead>
<tr>
<th>Dietary arsenicb</th>
<th>Plasma, nmol/mL</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/g</td>
<td>Arginine</td>
<td>Cystine</td>
<td>Glutamine</td>
<td>Glycine</td>
<td>Histidine</td>
</tr>
<tr>
<td>0</td>
<td>94 ± 22</td>
<td>212 ± 28</td>
<td>1336 ± 225</td>
<td>879 ± 66</td>
<td>225 ± 49</td>
</tr>
<tr>
<td>2</td>
<td>125 ± 25</td>
<td>171 ± 14</td>
<td>1019 ± 244</td>
<td>724 ± 95</td>
<td>194 ± 46</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.07</td>
<td>&lt; 0.02</td>
<td>&lt; 0.06</td>
<td>&lt; 0.02</td>
<td>&lt; 0.07</td>
</tr>
</tbody>
</table>

aChicks were killed at age 28 d.
bAmount of arsenic (sodium arsenate) supplemented to basal diet containing approximately 15 ng As, 17 µg Zn, and 15 mg Arg/g.

The enzyme arginase is composed of four subunits, to each of which is bound one atom of manganese and is specific for L-arginine. The exact point at which arsenic affects arginine metabolism is not known. Most likely, it is not through a direct role in the reaction catalyzed by arginase because arsenic deprivation elevated or depressed kidney arginase activity with the direction determined by the zinc and arginine status of the chick.

Regardless of the mechanisms involved, evidence has been obtained that shows that dietary arsenic influences the effects of high dietary arginine and low dietary zinc. As described vide supra, both of these dietary treatments alter some types of tumor development, possibly through altering the immune response.
Concluding Remarks

At present, little is understood regarding the mechanism of action of physiological levels of arsenic at the cellular and molecular levels. Thus, much work still must be done to ascertain the importance of arsenic for arginine and zinc metabolism, and whether arsenic directly or indirectly affects the immune response. The tantalizing findings to date suggest that such research be done because it probably will eliminate some of the confusion regarding the relation between arsenic and cancer. The findings to date indicate to us that arsenic, in the forms usually ingested, is not carcinogenic. However, nontoxic levels of dietary arsenic probably can affect the development of certain tumors, especially those involving viral induction, by affecting the metabolism of other nutrients, or by directly affecting the immune system. Furthermore, because altered nutritional status, or immune function, can either positively or negatively affect tumor development, future findings probably will show that general statements about arsenic causing or preventing cancer cannot be made. Most likely, the only general conclusion will be that arsenic, depending upon dosage, route of administration, and chemical form, modifies the induction or development of some tumors.

References