

Arsenic-induced bladder cancer in an animal model[☆]

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Abstract

Dimethylarsinic acid (DMA^V) is carcinogenic to the rat urinary bladder, but not in mice. The carcinogenic mode of action involves cytotoxicity followed by regenerative cell proliferation. Dietary DMA^V does not produce urinary solids or significant alterations in urinary composition. The cytotoxicity is due to formation of a reactive metabolite, likely dimethylarsinous acid (DMA^{III}), concentrated and excreted in the urine. Urinary concentrations of DMA^{III} are dose-dependent, and the urinary concentrations are at cytotoxic levels based on *in vitro* studies. The no observed effect level (NOEL) in these rat dietary studies for detectable levels of DMA^{III}, cytotoxicity, and proliferation is 2 ppm, with marginal changes at 10 ppm. The tumorigenic dose is 100 ppm. Recent investigations have demonstrated that arsenicals administered to the rat result in binding to a specific cysteine in the hemoglobin alpha chain as DMA^{III}, regardless of the arsenical being administered. Monomethylarsonic acid (MMA^V) is not carcinogenic in rats or mice. In short term experiments (≤ 10 weeks), sodium arsenate in the drinking water induces significant cytotoxicity and regenerative proliferation. There is little evidence that the cytotoxicity produced following administration of arsenicals is caused by oxidative damage, as antioxidants show little inhibitory activity of the cytotoxicity of the various arsenicals either *in vitro* or *in vivo*. In summary, the mode of action for DMA^V-induced bladder carcinogenesis in the rat involves generation of a reactive metabolite (DMA^{III}) leading to cytotoxicity and regenerative proliferation, is a non-linear process, and likely involves a threshold. Extrapolation to human risk needs to take this into account along with the significant differences in toxicokinetics and toxicodynamics that occur between different species.

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Introduction

Inorganic arsenic is carcinogenic to humans, inducing carcinomas of the skin, urinary bladder, lung, and possibly other tissues (NRC, 1999). Exposure has been through the drinking water, occupational (mining) inhalation, diet, and pharmaceuticals. Although inorganic arsenic has long been known to be carcinogenic to humans, there is still little known regarding its mechanism of action, in part due to the general lack of animal models. In the past few years, animal models of arsenic-induced cancer have been developed, although all have

limitations, as would be expected for any animal model. However, these models have been useful in delineating some of the details that might be involved in the induction of cancer by arsenicals.

In rodents, inorganic arsenic has generally not been carcinogenic except in selected model systems involving co-administration with known carcinogenic agents (NRC, 1999; Cohen et al., 2006). However, Waalkes et al. (2003) have recently demonstrated that high doses of arsenite administered to mouse dams transplacentally induces cancer in the offspring in a variety of tissues. In contrast, administration to adult mice does not appear to produce tumors. Although the specific mechanisms involved in producing cancer in this model have not been delineated, in at least some of the tissues there appears to be a strong estrogenic effect (Waalkes et al., 2004). The relevance of these changes in the mouse transplacental model to human carcinogenesis is yet to be determined.

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Carcinogenicity of organic arsenicals

Organic arsenicals, primarily monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V), have long been considered detoxification products formed in mammals following exposure to inorganic arsenic (NRC, 1999). However, it has recently been demonstrated that the trivalent forms of these methylated arsenicals are highly toxic to a variety of tissues, and are now considered to be toxicologically active and possibly intermediates in the induction of cancer in humans and in animal models (Cohen et al., 2006).

Administration of MMA^V (Arnold et al., 2003b) or DMA^V (Arnold et al., 2006) in the diet to mice in 2-year bioassays has been negative with respect to carcinogenicity. In rats, MMA^V has also been negative for carcinogenesis, although a low but statistically insignificant incidence of bladder hyperplasia was observed (Arnold et al., 2003b). In contrast, DMA^V administered at high doses in either the diet (Arnold et al., 2006) or drinking water (Wei et al., 2002) resulted in a statistically significantly increased incidence of urinary bladder carcinomas in 2-year bioassays. DMA^V in the drinking water has also been shown to enhance the incidence of urinary bladder cancer in male rats when administered after the known DNA reactive bladder carcinogen, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), in a 32-week standard two-stage bioassay (Wanibuchi et al., 1996). DMA^V has not been evaluated in long-term bioassays in other species, although dietary administration to male and female hamsters for 10 weeks did not produce any evidence of an effect on the urinary tract, including no evidence of hyperplasia of the urothelium (Cano et al., 2001).

In the 2-year bioassay involving DMA^V administration in the drinking water to male rats, Wei et al. (2002) showed a statistically significantly increased incidence of bladder tumors at 50 and 200 ppm, whereas no tumors or hyperplasia was observed at 12.5 ppm. In a 2-year bioassay involving dietary administration of DMA^V to male and female rats, the incidences of hyperplasia and urothelial tumors were increased as shown in Table 1 (Arnold et al., 2006). It is evident that there is a significantly greater response in females compared to males, with a statistically significantly increased incidence of bladder tumors occurring only in the females at 100 ppm, and a significant increase of hyperplasia at 40 ppm of the diet. There is no evidence of a response, either hyperplasia or tumors, at 2 and 10 ppm. We have used the results of this 2-year bioassay involving dietary administration to evaluate the mode action of DMA^V in the rat.

Mode of action of DMA^V rat bladder carcinogenesis

For bladder carcinogenesis in general, several modes of action have been identified (Cohen, 1998) which can help in formulating hypotheses regarding DMA^V carcinogenicity in the rat. The chemical carcinogens that have produced bladder cancer in all species, including rats, mice, dogs, or humans can be classified as either DNA reactive, such as aromatic amines, nitrosamines, or phosphoramidate mustards, or as non-DNA reactive. If the agent is non-DNA reactive, it induces bladder cancer by increasing urothelial cell proliferation. Increasing cell proliferation can occur either by increasing cell births or decreasing cell deaths. Increasing cell births can occur either by direct mitogenesis, usually involving alterations in hormones and/or growth factors, or it can be produced by toxicity and regenerative proliferation. Decreased cell deaths, which result in an increase in cell number, can be produced either by inhibiting apoptosis or inhibiting terminal differentiation since terminally differentiated cells do not proliferate. No bladder carcinogenic agents have been shown to act by inhibiting cell death. In contrast, increasing cell births has been demonstrated for all non-DNA reactive urothelial carcinogenic agents. Direct mitogenesis has only been demonstrated for one chemical, propoxur, by an unknown mechanism. Most non-DNA reactive agents that produce urothelial tumors in animal models or in humans do so by causing toxicity and regenerative proliferation, including hyperplasia. For urothelial carcinogenicity, toxicity can be produced by three possible means (Cohen, 1998): (1) production of urinary solids (precipitate, crystals, and/or calculi); (2) production of reactive metabolites; or (3) extreme alterations in urinary composition, such as extremes of volume, pH, or urinary dilution.

When considering possible modes of action of DMA^V for urothelial carcinogenesis in the rat, several possibilities can be postulated, including genotoxicity (not only DNA reactivity, but indirect effects, such as oxidative damage, inhibition of DNA repair or mitotic spindle interactions), direct mitogenesis, or cytotoxicity and regeneration (Cohen et al., 2006).

It is generally concluded by most investigators involved in arsenic research that arsenicals do not form DNA reactive

Table 1
Effects of dietary administration of DMA^V on the bladder epithelium of F344 rats in a 2-year bioassay

| Histopathology | DMA ^V (ppm) | | | | |
|---|------------------------|----|----|----|-----|
| | 0 | 2 | 10 | 40 | 100 |
| <i>Males</i> | | | | | |
| <i>n</i> | 60 | 59 | 60 | 58 | 59 |
| Simple hyperplasia | 0 | 0 | 0 | 4 | 35 |
| Papillary/nodular hyperplasia | 0 | 0 | 0 | 2 | 5 |
| Simple hyperplasia + papillary/nodular hyperplasia ^a | 0 | 0 | 0 | 6 | 40 |
| Papilloma ^b | 0 | 0 | 1 | 1 | 0 |
| Carcinoma ^c | 0 | 1 | 0 | 0 | 2 |
| Papilloma + carcinoma ^c | 0 | 1 | 1 | 1 | 2 |
| <i>Females</i> | | | | | |
| <i>n</i> | 60 | 59 | 60 | 59 | 60 |
| Simple hyperplasia | 0 | 0 | 0 | 28 | 40 |
| Papillary/nodular hyperplasia | 0 | 1 | 0 | 1 | 8 |
| Simple hyperplasia + papillary/nodular hyperplasia ^a | 0 | 1 | 0 | 29 | 48 |
| Papilloma ^b | 0 | 0 | 0 | 0 | 4 |
| Carcinoma ^d | 0 | 0 | 0 | 0 | 6 |
| Papilloma + carcinoma ^d | 0 | 0 | 0 | 0 | 10 |

^a Statistically significant positive trend ($p < 0.001$).

^b Number of lesions too small for statistical analysis. Statistically significant positive trend ($p < 0.01$) when data for both sexes were combined.

^c Number of lesions too small for statistical analysis. Statistically significant positive trend ($p < 0.001$) when data were combined with female data.

^d Statistically significant positive trend ($p < 0.001$).

intermediates (Cohen et al., 2006). However, indirect genotoxic effects have been postulated, especially focusing on oxidative DNA damage or DNA repair inhibition. Although there is some evidence for each of these mechanisms, their relationship to bladder carcinogenesis remains unclear.

We have evaluated the possibility of direct mitogenesis or cytotoxicity and regenerative proliferation using a relatively short-term model involving administration for 10 weeks or less. The potential of DMA^V to produce urothelial cytotoxicity and/or increased proliferation was investigated using the same doses used in the dietary bioassay of DMA^V in rats exposed to 0, 2, 10, 40, and 100 ppm. We also compared female to male rats, the effect of altering urinary pH (Arnold et al., 1999), and the effects in hamsters.

Female rats produced a greater response than the males (Arnold et al., 1999), similar to what was observed in the 2-year bioassay (Arnold et al., 2006). Cell proliferation was evaluated by a combination of light microscopic histology, scanning electron microscopy (SEM), and bromodeoxyuridine (BrdU) labeling index following a 1-h pulse administered by intraperitoneal injection. Utilizing these assays, there was an increase of hyperplasia at 40 and 100 ppm in the female rat, with a statistically significant increase in the labeling index at 40 and 100 ppm. There was also evidence of cytotoxicity at these two doses. The dose of 10 ppm in the diet resulted in a non-statistically significant change both by SEM and BrdU labeling index. The dose of 2 ppm was clearly a no observed effect level (NOEL).

There were minor changes in urine composition, including a slight increase in urinary pH and alterations in urinary calcium (Arnold et al., 1999), and although there was some evidence of calcification in the kidney tubules at the higher doses of DMA^V in the diet, this effect has not been reproducible in subsequent experiments or under other circumstances. There was no evidence of formation of urinary solids by light or scanning electron microscopy. This experiment provided strong evidence that there was an increase in cell proliferation along with cytotoxicity, and strongly supported the hypothesis that the increased cell proliferation and cytotoxicity were due to a reactive intermediate present in the urine rather than due to formation of urinary solids or extreme alterations of urinary composition. Both cytotoxicity and increased cell proliferation were present in this 10-week experiment. Which comes first becomes critical: is the sequence of events increased mitogenic proliferation accompanied by cytotoxicity and necrosis, or is the sequence cytotoxicity followed by a regenerative proliferative response?

An experiment to demonstrate the time course of events utilizing light microscopy, SEM, and BrdU labeling index was performed in female rats treated with 100 ppm DMA^V in the diet for 6 and 24 h, and 3, 7, and 14 days (Cohen et al., 2001). Cytotoxicity of the superficial cells of the urothelium was evident by SEM as early as 6 h (earlier time points have not been evaluated), whereas proliferation as determined by BrdU labeling index was not increased over control until after 3 days of administration and did not become statistically significantly increased until 7 days.

Based on these studies, the mode of action was clearly defined as cytotoxicity with subsequent regenerative proliferation resulting in hyperplasia and ultimately tumors. Urinary solids and extreme alterations in urine composition were excluded as possible mechanisms, leaving the likelihood that the mode of action involved a reactive metabolite present in the urine. The pentavalent methylated arsenicals are stable chemicals and have little cytotoxic potential except at extremely high concentrations. Thus, it was likely that a reactive metabolite was formed from DMA^V that was concentrated in the urine and was cytotoxic to the urothelium.

The metabolism of arsenic involves repeated reduction of the pentavalent form to the trivalent form followed by oxidative methylation (Cohen et al., 2006). Beginning with inorganic arsenate, this is reduced to arsenite which is then methylated to MMA^V. This is reduced to monomethylarsonous acid (MMA^{III}) which is methylated to DMA^V, which is reduced to dimethylarsinous acid (DMA^{III}), which can be methylated to trimethylarsine oxide (TMAO). In mammalian systems this is essentially a unidirectional metabolic pathway, since the arsenic–carbon bond is thermodynamically extremely strong. Demethylation does not appear to occur in mammals, only in various unusual bacterial systems (Cullen and Reimer, 1989).

Cytotoxicity of DMA^{III}

Given this background concerning metabolism, we postulated that the reactive metabolite present in the urine following DMA^V administration would be DMA^{III}. If a trivalent arsenical is involved with the cytotoxicity produced by DMA^V administration, it should be possible to inhibit it by co-administering 2,3-dimercaptopropane-1-sulfonate (DMPS), which interacts with trivalent arsenicals, inhibiting their reactivity (Cohen et al., 2002). Utilizing an abbreviated period of 2 weeks of administration as a model system, since both cytotoxicity and increased proliferation are already present when DMA^V is administered for this period of time, the effect of DMPS on DMA^V-induced urothelial cytotoxicity and cell proliferation was evaluated. DMPS was administered in the diet at a level of 5600 ppm and DMA^V at a level of 100 ppm to female rats. DMA^V produced cytotoxicity as detected by SEM, and the BrdU labeling index was statistically significantly increased to 0.63 ± 0.10 compared to the controls (0.16 ± 0.02). In the rats co-administered DMA^V with DMPS, the cytotoxicity was inhibited and the BrdU labeling index (0.09 ± 0.01) remained at control levels. This provided evidence that a trivalent arsenical was involved in the cytotoxicity and regenerative hyperplasia induced by DMA^V administration to rats.

An initial attempt at measuring urinary metabolites following administration of DMA^V without or with DMPS failed to demonstrate the presence of DMA^{III} or other trivalent arsenicals above control levels in fresh voided urine specimens. In aqueous solutions, MMA^{III} and DMA^{III} are unstable compounds. By taking a variety of precautionary measures (immediate preservation in liquid N₂ and evaluation within 24 h) to prevent the re-oxidation of these trivalent methylated species, adequate amounts of the trivalents could be preserved

to detect DMA^{III} in the urine (Cohen et al., 2002). Since some re-oxidation of DMA^{III} to DMA^V likely occurs within this time span, the levels of DMA^{III} detected likely underpredicts actual amounts.

In these analyses, the concentration of DMA^{III} that was detected ranged from approximately 1 to 5 μ M, whereas DMA^{III} was below the level of detection in the control animals (Cohen et al., 2002). However, DMA^{III} was detectable in the urine of rats co-administered DMA^V and DMPS, although at lower levels than those found in rats treated only with DMA^V. However, it remains uncertain whether DMA^{III} was actually present *in situ* or whether it was generated by separation from the DMPS during the processing of the specimen.

The control rats had levels of arsenate, arsenite, and MMA^{III} in the urine of less than 0.1 μ M, and DMA and TMAO were present at levels of 0.2 and 0.3 μ M, respectively (Cohen et al., 2002). In contrast, rats administered DMA^V as 100 ppm of the diet had markedly increased urinary concentrations of DMA^V and TMAO, approximately 60 to 70 μ M or higher. When co-administered with DMPS, the amount of DMA^V increased to greater than 500 μ M whereas TMAO was reduced to below 5 μ M. In addition, two unknown metabolites were detected in the urine. The overall distribution of metabolites was similar in rats administered DMA^V in the drinking water, although precautions were not taken to preserve DMA^{III} and it was not detected. Thus, the concentration of arsenicals in the urine following DMA^V administration includes concentrations of 1–5 μ M DMA^{III} and concentrations of DMA^V and TMAO of 60 μ M or higher.

Utilizing *in vitro* systems of immortalized but not transformed urothelial cells from rats (MYP3) or humans (1T1), cytotoxicity of the various arsenicals was evaluated (Cohen et al., 2002). The IC₅₀ for arsenite was approximately 1 μ M and for arsenate was approximately 5 μ M. For MMA^V, DMA^V, and TMAO, the cytotoxic concentrations were at mM levels rather than μ M concentrations. In contrast, MMA^{III} and DMA^{III} were highly cytotoxic to these cells, producing cytotoxicity at concentrations below 1 μ M. For DMA^{III}, nearly complete cytotoxicity occurred between 0.2 and 0.5 μ M, depending on the specific assay.

Although quantitative extrapolations between *in vitro* and *in vivo* systems must be done with caution, since the *in vitro* cell system does not have a fully differentiated urothelium and metabolic differences may occur, these *in vitro* studies at least demonstrated the approximate concentrations that are likely to be cytotoxic in an *in vivo* system (Cohen et al., 2002). These results clearly showed, as has been shown in other cell culture systems involving other epithelial systems, that the pentavalent methylated arsenicals are cytotoxic only at extremely high concentrations whereas the trivalent methylated arsenicals are highly cytotoxic, with cytotoxicity occurring at concentrations at or below 1 μ M, and generally at concentrations lower than concentrations necessary to produce cytotoxicity with arsenite or arsenate.

Based on the quantitative measures of the various metabolites in the urine of rats administered 100 ppm DMA^V in the diet, the levels of DMA^{III} produced in the urine are well above the

concentration anticipated to be necessary to produce cytotoxicity of the urothelium, whereas the levels of DMA^V and TMAO are well below the concentrations necessary to produce cytotoxicity. These results must be interpreted with some caution since the two unknown metabolites that were also found in the urine following DMA^V administration have not yet been identified.

DMA^{III} is produced in the urine in a dose responsive manner following administration of DMA^V in the diet (Arnold et al., 2003a; Cohen et al., 2006). Based on measures of DMA^{III} in the urine, concentrations of DMA^{III} that are likely to be cytotoxic are attained when DMA^V is administered in the diet at levels of 40 and 100 ppm, and is present at marginally cytotoxic urinary concentrations when DMA^V is administered at 10 ppm of the diet. However, at levels of 2 ppm of the diet, there are no detectable levels of DMA^{III} in the urine (Arnold et al., 2003a; Cohen et al., 2006). The urinary concentrations of DMA^{III} correlate well with the observed cytotoxicity and urothelial proliferation observed in short-term assays and with the long-term bioassay tumor results. They strongly support a NOEL of 2 ppm of the diet with marginal activity at 10 ppm of the diet, which is consistent with the previously described dose-response for urothelial hyperplasia.

Oxidative damage

A postulated mechanism of toxicity for arsenicals is oxidative damage (NRC, 1999; Cohen et al., 2006). The role of antioxidants as preventive measures for the cytotoxicity of DMA^V was evaluated utilizing *in vitro* and *in vivo* models. Utilizing the MYP3 cell line for *in vitro* analysis, the potential inhibitory activity of a variety of antioxidants was evaluated, including melatonin, N-acetylcysteine (NAC), vitamin C, Tiron, and Trolox (Wei et al., 2005). The results are summarized in Table 2. No inhibitory activity was seen with either Tiron (superoxide anion scavenger) or Trolox (vitamin E analog) and only minimal activity with melatonin when arsenite was used as the arsenical. Melatonin had no activity against any of the other arsenicals. N-acetylcysteine inhibited cytotoxicity of some of the methylated arsenicals, but not MMA^V or the inorganic arsenicals. The only antioxidant that inhibited cytotoxicity

Table 2

Effects of antioxidants on the *in vitro* cytotoxicity of various arsenicals on rat urothelial MYP3 cells^{a, b}

| | Melatonin | NAC | Vitamin C | Tiron | Trolox |
|--------------------|-----------|-----|-----------|-------|--------|
| Arsenate | – | – | + | – | – |
| Arsenite | +/- | – | ++ | – | – |
| MMA ^V | – | – | – | – | – |
| MMA ^{III} | – | +++ | ++ | – | – |
| DMA ^V | – | ++ | – | – | – |
| DMA ^{III} | – | +++ | ++ | – | – |
| TMAO | – | + | – | – | – |

^a Maximum nontoxic effect dose of antioxidants: Melatonin, 0.2 mM; NAC, 1 mM; Vitamin C, 0.8 mM; Tiron, 0.2 mM; Trolox, 0.1 mM.

^b Increases in the survivability of cells treated with an arsenical plus antioxidant compared to that of cells treated with the arsenical alone: <20%, +/-; 20%–40%, +; approximately 50%, ++; 100%, +++.

induced by trivalent arsenicals was vitamin C (ascorbic acid). Vitamin C also inhibited, to some extent, the cytotoxicity induced by arsenate.

Trolox and Tiron cannot be evaluated in an *in vivo* system involving urothelial reactivity since they are not excreted at significant levels in the urine. Melatonin, N-acetylcysteine, and vitamin C were evaluated in the *in vivo* model of dietary DMA^V administration to female rats for a period of up to 10 weeks (Wei et al., 2005). Minimal inhibitory activity was observed when 100 ppm DMA^V was co-administered with 1000 ppm melatonin in the diet, but some inhibitory activity, although not complete, was produced when vitamin C was co-administered in the diet at a level of 10,000 ppm. No inhibitory activity was observed when 0.4 mg/kg/day N-acetylcysteine was co-administered in the drinking water. Based on these *in vitro* and *in vivo* investigations, it is evident that oxidative damage plays a minimal role in the cytotoxicity and regenerative hyperplasia produced following the administration of DMA^V to the rat.

The evidence for oxidative damage playing a role in the urothelial carcinogenicity of arsenicals is based on studies mostly *in vitro* and involving concentrations of the arsenicals well above the concentrations necessary to produce cytotoxicity (Cohen et al., 2006). Also, in the *in vivo* setting it is difficult to separate oxidative damage as the cause of the cytotoxicity or as the result of cytotoxicity, since cell death will lead to oxidative damage regardless of the mechanism by which the cell death is produced.

Binding to hemoglobin

Extrapolating the results from rats to humans poses a significant challenge, not only because of differences in susceptibility of the urothelium, but also because of the known significant differences in toxicokinetics of arsenicals in rats compared to humans (Aposhian, 1997). Rats sequester arsenicals in red blood cells (RBCs) by binding to hemoglobin, whereas this does not occur in other species, including humans. Furthermore, TMAO is a major metabolite of arsenicals in the rat, is produced to a lesser extent in mice and other species, and is produced to a limited extent in humans, if at all (Cohen et al., 2006).

The basis for the sequestration in red blood cells in the rat was examined by investigating the binding of the various arsenicals to rat hemoglobin. Lu et al. (2004) demonstrated that the various trivalent arsenicals could bind to rat hemoglobin *in vitro*, with DMA^{III} binding to a greater extent compared to MMA^{III} and only limited binding with arsenite. In contrast, binding of these arsenicals occurred to a much lesser extent to human hemoglobin. The binding of DMA^{III} to rat hemoglobin was demonstrated following the administration of DMA^V to rats, occurred in a dose-responsive manner, and appeared to saturate at dietary levels of 40 ppm and above. The amount of unbound arsenical in the plasma continued to increase with dose above 40 ppm. Binding of arsenicals to RBCs was also demonstrated *in vivo* following administration of either arsenate or MMA^V. Quite surprisingly, however, was the observation that the form of arsenic bound to the hemoglobin was DMA^{III},

regardless of the form of arsenic administered to the rat (Lu et al., submitted for publication). Thus, it would appear that in the rat, sequestration of arsenic in RBCs was due to binding to hemoglobin, and that the form of arsenic that is bound to the hemoglobin is DMA^{III}, regardless of the form of arsenic administered in the diet or drinking water.

Possible role of interaction with protein sulfhydryl groups

In addition to providing an explanation for the difference in toxicokinetics for arsenicals in the rat compared to humans, this binding to hemoglobin via one of the sulfhydryl groups on the hemoglobin provides some evidence for another possible mechanism that could explain the cytotoxicity of arsenicals in mammalian systems. Arsenicals are well known to bind to sulfhydryl groups, including those on proteins, as demonstrated for hemoglobin and previously demonstrated for metallothionein (Jiang et al., 2002; Lu et al., 2004). A possible mechanism for producing cytotoxicity in urothelial cells and other cell types could be an interaction of a trivalent arsenical, such as DMA^{III}, with a critical cellular protein in the target cell. In the urothelium, the major proteins at the luminal surface are a group known as the uroplakins (Kong et al., 2004). These proteins have sulfhydryl groups, and could be the target for the DMA^{III} present in the urine.

Conclusion

Regardless of the actual mechanism involved, the data are accumulating to demonstrate that DMA^V carcinogenicity toward the rat urothelium is a non-linear process, and likely involves a threshold phenomenon related to the cytotoxicity. The key events involved in the urothelial carcinogenicity in rats induced by DMA^V administration are: (1) reduction of DMA^V to the reactive trivalent DMA^{III}; (2) concentration in the urine; (3) urothelial cytotoxicity; (4) regenerative cell proliferation (hyperplasia); and (5) tumor induction. Our working hypothesis is summarized in Fig. 1.

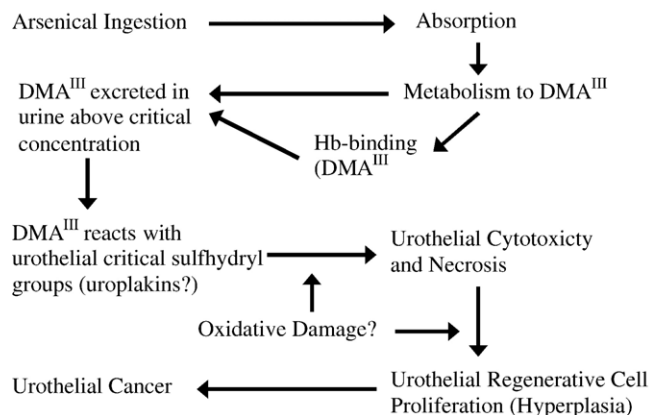


Fig. 1. Hypothesized mode of action for arsenical-induced urinary bladder carcinogenesis.

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