Effects of Diet on Urinary Bladder Carcinogenesis and Cancer Prevention$^{1,2}$

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ABSTRACT Urine plays a major role in bladder carcinogenesis, acting as a transport mechanism for carcinogens, containing several growth factors stimulating cell proliferation, and indirectly affecting chemicals by alterations in concentrations of normal urinary components such as electrolytes, water and proteins. These latter effects are greatly modified by diet composition and consumption and also by water consumption. Several examples of these effects are presented. J. Nutr. 127: 826S–829S, 1997.

KEY WORDS: • urine • calculi • saccharin • sodium salts

It is axiomatic in bladder cancer research that urine is of fundamental importance (Clayson and Cooper 1970). Urinary tract carcinogens affect the urothelium via exposure through the urine rather than through the blood. Recently, however, urine itself has been identified as having relatively high concentrations of essential growth factors in urine, such as epidermal growth factor (EGF) (Momose et al. 1991). Animals exposed to carcinogens do not develop bladder tumors if the urothelium is not exposed to urine. Oyasu et al. (1981) showed that if a heterotopic bladder was exposed to a carcinogen, such as N-methyl-N-nitrosourea (MNU), followed by exposure to urine, bladder tumors developed. If the MNU was followed by exposure to saline, bladder tumors did not develop.

In addition to these direct influences of urine on bladder carcinogenesis, numerous indirect effects have been identified (Cohen 1995). These include pH, osmolality, volume, and others, many of which are highly responsive to variations in diet composition, diet intake and water consumption. It is these latter indirect influences of diet on the carcinogenic process that will be the focus of this article.

URINARY PHYSIOLOGY

Urine is a complex aqueous mixture containing a variety of electrolytes, organic and inorganic molecules, and macromolecules such as mucopolysaccharides and proteins (Cohen 1995, Hard 1995). The concentration of these substances, including water itself, is highly dependent on their intake in the diet and drinking water and on physiologic adjustments of the body to regulate them in the blood and tissues. There are enormous variations in concentrations of these substances during the day, among individuals depending on their overall physiologic status, and among species. Because diet and water intake greatly affect urinary composition, a diurnal variation occurs secondary to the time of day when they are ingested (Cohen 1995). For humans, ingestion of food and water is generally during daylight hours. There is increased excretion of various ingested substances into the urine, including an increase in urinary pH. In contrast, rodents are nocturnal animals, so variation in concentrations of substances in the urine tends to be opposite of that of humans with respect to time of day.

The fact that there is a marked diurnal variation in most components of the urine poses difficulties technically in evaluating the role of these factors in carcinogenesis (Cohen 1995, Fisher et al. 1989). To avoid these difficulties, fresh voided urine is strongly recommended for studies on urinary effects in carcinogenesis.

The effect of these variations can be seen in Figure 1 (Fisher et al. 1989). As discussed in greater detail below, the effects of saccharin in the urine are dependent on urinary pH remaining above 6.5. When sodium saccharin is administered in the diet at high concentrations, urinary pH remains above 6.5 during the food ingestion period, but it rapidly decreases to below 6.5 when the rat stops eating when the lights come on. Calcium saccharin, in contrast, tends to keep the pH below 6.5 and has only marginal effects on the urothelium. If urinary pH is measured during the daylight hours, no differences are observed in urinary pH following feeding sodium saccharin vs. calcium saccharin.

As seen in Figure 1, the type of diet also has enormous effect on urinary pH. The AIN-76A diet produces a markedly acidic urine, with pH consistently below 6.0 even during periods of diet ingestion (Fisher et al. 1989). When fed in AIN-76A diet, neither sodium saccharin nor calcium saccharin produces a urine above the critical level of pH 6.5 at any time during the day, and no urothelial effects occur (Garland et al. 1989).
EFFECT OF DIET ON GENOTOXIC CARCINOGENS

Numerous chemicals have been identified as carcinogenic toward the urinary bladder in both experimental animals and humans (Clayson and Cooper 1970, Cohen and Johansson 1992), including various aromatic amines, cyclophosphamide and related compounds. These chemicals require metabolic activation to reactive electrophiles, which are either excreted into the urine or produced by metabolism directly in the bladder epithelium. Because the ionic form affects whether the chemical can be absorbed across the asymmetric unit membrane of the bladder epithelium, the urinary pH can affect the potential carcinogenicity of these chemicals by modifying the proportion of ionized vs. unionized forms (Cohen 1995). For aromatic amines, acidic pH apparently enhances the carcinogenic activity compared with higher pH. This may be due not only to the potential for ionization but also to the potential acidic hydrolysis of N-glucuronides that are formed in the liver and excreted in the urine. Acid hydrolysis yields the corresponding highly reactive N-hydroxylamine.

EFFECT OF DIET ON NONGENOTOXIC URINARY BLADDER CARCINOGENS

In contrast to chemicals that are metabolically activated and yield DNA adducts, several substances have been identified that are not reactive with DNA and do not act as direct genotoxins. These produce their carcinogenic effect by increasing the number of DNA replications in the target organ, the urothelium, usually by causing toxicity with regenerative hyperplasia, but occasionally by producing a direct mitogenic effect (Cohen and Ellwein 1990 and 1991).

Experiments performed in rodents generally make two basic assumptions with respect to extrapolation to humans (Cohen and Ellwein 1991): 1) the response in humans is reasonably similar to that in rodents and 2) the response at lower doses is similar to the response to the chemicals administered at higher doses in rodent experiments. For genotoxic chemicals, these two assumptions are reasonable, although the extrapolations may not be directly linear. For nongenotoxic substances, one or both of these assumptions may be incorrect. Urinary alterations greatly affect the potential of these chemicals to produce increased cell proliferation and therefore to effect carcinogenesis. Many of the experiments on the effects of diet on these chemicals have been performed utilizing various markers of increased cell proliferation as surrogate markers for tumor formation.

URINARY TRACT CALCULI

Calculi, which act as foreign bodies, can form in the urine when a critical concentration of a substance is reached, leading to precipitation (Clayson et al. 1995, Cohen 1995). Depending on the coarseness of the surface of the calculus, there are various degrees of erosion and ulceration of the urothelial surface with consequent inflammation and regeneration, ultimately leading to the formation of carcinomas. The effect seems to be much more extensive in rats than in mice, and rodents are considerably more susceptible to the toxic, regenerative and carcinogenic effects of calculi than are humans (Burrin et al. 1995, Clayson et al. 1995). This is presumably related to the fact that humans are upright, bipedal animals, and urinary calculi tend to produce urinary obstruction soon after their formation unless they are excreted. This contrasts to rodents, which are horizontal quadrupeds; the calculus can rest in the dome of the bladder for several months to the lifetime of the animal without completely obstructing urinary flow.

Calculi can form by a variety of processes in rodent urine (Clayson et al. 1995, Cohen 1995), including direct surgical implantation into the bladder lumen. Tumors eventually develop, with an increased incidence over time. Jull (1979) demonstrated that paraffin wax pellets implanted in mice produced an incidence of 10.6% after 12 mo, 26.8% after 18 mo and 53.8% by 24 mo.

Calculi can also be formed in the urinary tract by the administration of high doses of exogenous chemicals or by modification of normal physiologic processes in the body (Clayson et al. 1995, Cohen 1995). For example, surgically formed portacaval shunts lead to abnormalities in uric acid metabolism (Engelmann et al. 1987). Ultimately, urinary tract urate calculi form and tumors appear.

Substances that can lead to the formation of urinary calculi when fed in the diet are listed in Table 1 (Clayson et al. 1995). Some of these substances are essential ingredients in the diet (e.g., ascobic acid, calcium), some are present in diet and are also formed during endogenous intermediary metabolism (e.g., glycine, uracil, uric acid), and some are foreign substances (e.g., melamine, fosetyl-al). The critical variable is the amount that ultimately is excreted in the urine and the concentration achieved. If an insufficient amount of chemical is ingested or formed endogenously to produce calculi, there is

| Chemicals administered at high doses that lead to urinary calculi in rodents |
|-----------------------------|----------------------------------|
| Uracil                      | Acetazolamide                    |
| Melamine                    | Terephthalic acid                |
| Fosetyl-al                  | Dimethyl terephthalate           |
| Biphenyl                    | Nitrilotriacetate                |
| Glycine                     | Polyoxymethylene β-stearate      |
| Orotic acid                 | Diethylene glycol                |
| Oxamide                     | Calcium oxalate                  |
| Calcium phosphate           | Uric acid                        |
| Homocysteine                | 4-Ethylsulfonylnapthalene-1-sulfonamide |
no toxicity, regeneration or carcinogenic effect. Carcinogenic effects present at high doses do not occur at low doses (Cohen and Ellwein 1991). This is obviously critical because many of the substances listed in Table 1 are essential for our survival.

It is also obvious from the list of substances in Table 1 that urinary concentration and thus the potential for precipitation. For example, increased intake of calcium simultaneously with substances that alkalize urine can increase the risk of development of calcium oxalate or calcium phosphate calculi (Clayson et al. 1995, Cohen 1995). With respect to exogenous chemicals, modifying effects of the diet also can markedly affect the formation and excretion of the bladder. The effects also appear to be strain related, with Sprague-Dawley rats being less susceptible than F344 rats. Sodium saccharin (Sodium saccharin administered at high doses (5%) of the Clea, and related sodium salts restricted to high doses (>1% of the diet), but the effect is specific to rats (Ellwein and Cohen 1989). Humans do not have the necessary urinary composition to generate the calcium phosphate-containing precipitate (unpublished observations), and thus they are unable to produce urothelial hyperplasia or carcinogenicity.

**EFFECTS OF OTHER DIETARY COMPONENTS ON BLADDER CARCINOGENESIS**

Sodium saccharin administered through the neonatal time period in rats produces iron and folate deficiencies, with a corresponding anemia (Garland et al. 1993). Also, hypercholesterolemia and hypertriglyceridemia occur. These effects reverse for the most part as the animals progress to the age beyond weaning. To determine whether these effects were related to the urothelial hyperplasia associated with sodium saccharin administration, iron and/or folate were replaced in the diet. Increased iron reversed not only the iron deficiency but the folate deficiency and corresponding anemia, hypercholesterolemia and hypertriglyceridemia. Folate had less of an effect on the anemia and the lipid changes, and it did not affect the iron deficiency. Neither iron nor folate corrected the urothelial proliferation produced by sodium saccharin administration. On the contrary, they actually enhanced the proliferative effects.

**PROPOXUR, A URINARY MITOGEN**

High doses of propoxur administered to rats in the diet produce bladder proliferation and ultimately an increased incidence of tumors (Cohen et al. 1994). In contrast to the substances producing calculi or precipitate in the urine, propoxur apparently requires a urinary pH of approximately 6.5 or higher. Thus, acidification of the urine, whether by administration of the AIN-76A diet or administration of sodium saccharin in other diets simultaneously with high concentrations of NH₄Cl, prevents the formation of the precipitate and prevents tumorigenesis of the bladder. The effects also appear to be strain related, with Sprague-Dawley rats being less susceptible than F344 rats. Sodium saccharin administered in the AIN-76A diet produces a greater effect than administration of it at comparable doses in Purina 5002 or NIH-07 diet (Fisher et al. 1989, Garland et al. 1989). When administered in AIN-76A diet, there is effect whatsoever (Garland et al. 1989), because AIN-76A diet produces marked urinary acidification (see above). If the casein used in AIN-76A diet is replaced with ovalbumin as the protein source, the urinary pH is higher (unpublished observations).

The ultimate mechanism of carcinogenesis in rats secondary to sodium saccharin administration seems to be the formation of a calcium phosphate precipitate in the urine (Cohen et al. 1995). This is dependent on high concentrations of protein and apparently mucopolysaccharides (particularly heparan sulfate) that are present in rat urine. Concentrations of protein are greater in males than in females. Formation of this precipitate apparently requires a urinary pH of approximately 6.5 or higher. Thus, acidification of the urine, whether by administration of the AIN-76A diet or administration of sodium saccharin in other diets simultaneously with high concentrations of NH₄Cl, prevents the formation of the precipitate and prevents tumorigenesis of the bladder. The effects also appear to be strain related, with Sprague-Dawley rats being less susceptible than F344 rats. Sodium saccharin administered in the AIN-76A diet produces a greater effect than administration of it at comparable doses in Purina 5002 or NIH-07 diet (Fisher et al. 1989, Garland et al. 1989). When administered in AIN-76A diet, there is no effect whatsoever (Garland et al. 1989), because AIN-76A diet produces marked urinary acidification (see above). If the casein used in AIN-76A diet is replaced with ovalbumin as the protein source, the urinary pH is higher (unpublished observations).

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**EFFECTS OF OTHER DIETARY COMPONENTS**

Diet markedly affects the potential for saccharin and its various salts to cause an effect on the urothelium in rats (Ellwein and Cohen 1989). Administration of sodium saccharin in the Agway Prolab 3200 diet produces a greater effect than administering it at comparable doses in Purina 5002 or NIH-07 diet (Fisher et al. 1989, Garland et al. 1989). When administered in AIN-76A diet, there is no effect whatsoever (Garland et al. 1989), because AIN-76A diet produces marked urinary acidification (see above). If the casein used in AIN-76A diet is replaced with ovalbumin as the protein source, the urinary pH is higher (unpublished observations).

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seems to have a direct mitogenic effect on the bladder. The studies performed in rodents to evaluate the carcinogenicity of propoxur were initially performed using Altromin 1321 diet (Altromin GmbH, Germany), which produces a high urinary pH (commonly ≥ 8.0). When propoxur was administered in AIN-76A diet, there was no proliferative or tumorigenic effect. Co-administration with NH₄Cl in Altromin 1321 diet decreased urinary pH to ≤7.0 and resulted in marked inhibition of the urothelial proliferative effects. The mechanism by which this occurs is unknown, but it could be related directly to the chemistry of propoxur or potentially be related to the effects of urinary pH on the interaction of urinary growth factors with their receptors. For example, EGF binds to a significantly greater degree to its receptor on the urothelium if the pH is 7.0 or higher. Lowering of the pH could potentially inhibit this interaction despite normal quantities of EGF and its receptor being present.

SUMMARY AND CONCLUSION

Urinary bladder carcinogenesis can be produced by a variety of chemicals, both genotoxic and nongenotoxic. The urine acts as a vehicle to transport these chemicals and/or their metabolites to the urothelium for their effect, and urine also contains growth factors that enhance the proliferation and tumorigenicity of the bladder epithelium. In addition, urine pH and numerous components of the urine, such as calcium, protein and other substances, can markedly alter the urinary tract effects of many of these carcinogens, either directly or indirectly. Because many of these factors are markedly affected by diet and water consumption, it is not surprising that diet and water consumption play a significant role in enhancing or inhibiting the carcinogenic process in the urinary bladder. These factors need to be carefully elucidated to more rationally evaluate potential risks to humans based on extrapolation from studies in rodents.

LITERATURE CITED