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# Possible Contribution of Indomethacin to the Carcinogenicity of Nongenotoxic Bladder Carcinogens That Cause Bladder Calculi

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Nongenotoxic bladder carcinogens that form bladder calculi have been concluded to be of low carcinogenic risk to humans because bladder stones would be expelled or surgically removed before they had a chance to exert their carcinogenic effect. It is the aim of this report to examine the possible contribution of indomethacin to the carcinogenic risk posed by nongenotoxic bladder carcinogens that cause bladder stones. Indomethacin may act as a tumor promoter in the bladder by interfering with the synthesis of prostaglandins. Prostaglandins have a cytoprotective function in the gastric mucosa and possibly also in the urinary bladder. Diminished cytoprotection may be implicated in bladder carcinogenesis as β-naphthylamine, a human bladder carcinogen, also inhibits prostaglandin synthesis *in vitro*. The presence of other tumor promoters in the bladder may further ensure that tumors would be formed even if bladder stones were expelled. People who are exposed to nongenotoxic bladder carcinogens that are present in the environment and that form bladder stones, therefore, may be at an increased risk for developing bladder cancer if they are also exposed to tumor promoters, such as indomethacin.

***Keywords*** Bladder, Calculi, Carcinogens, Indomethacin, Nongenotoxic, Terephthalic acid.

## INTRODUCTION

About 80% of all bladder cancer in the United States occurs in men over the age of 60 (Burin et al., 1995; Kryger et al., 1996). Several causes of bladder

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cancer in humans have been identified including cigarette smoke, which is responsible for approximately 50% of all bladder cancer, chronic bladder infection, catherization, pharmaceuticals such as phenacetin-containing analgesics, chlornaphazine and cyclophosphamide, and genotoxic chemicals including 2-naphthylamine and aromatic amines (Hartge et al., 1987; LaVecchia et al., 1999). In addition, a number of nongenotoxic chemicals that cause bladder calculi in rodents also cause bladder cancer in the same animals, but their carcinogenic risk to humans is under intensive debate (Huff, 2002).

A mechanistic hypothesis was formulated for the possible contribution of indomethacin to the carcinogenicity of nongenotoxic bladder carcinogens that form bladder stones.

## MATERIALS AND METHODS

The published scientific literature on the tumor promoter effect of indomethacin and the carcinogenicity of nongenotoxic bladder carcinogens that form bladder calculi was reviewed (Gross, 1974; Heck et al., 1985; Holmang et al., 1995; Fukushima et al., 1992).

## RESULTS AND DISCUSSION

There has been intense interest in the relationship between bladder cancer and bladder calculi ever since it was shown that inert pellets (e.g., glass beads, paraffin wax) implanted into the bladder of rats caused bladder tumors (Jull, 1951; Clayson et al., 1970; Jull, 1979). Since then, at least 17 nongenotoxic chemicals have been reported to cause bladder cancer in rats and/or mice, mainly or exclusively transitional cell carcinoma (Table 1). Of these, sodium saccharin, terephthalic acid (TPA), uracil, melamine, diethylene glycol, and 4-ethylsulfonylnaphthalene-1-sulfonamide also formed bladder calculi in the same animals that had bladder cancer (Table 1). Studies in rats with TPA, melamine, and uracil have further shown that bladder cancer generally was absent in animals lacking calculi (Heck et al., 1985). In addition, a statistical analysis of the data from the melamine carcinogenicity study revealed a significant association between bladder cancer and bladder calculi (Melnick et al., 1984; Heck et al., 1985).

It has been suggested that the mechanism of carcinogenicity for nongenotoxic bladder carcinogens that form bladder calculi includes an initiation phase caused by mutagens in the urine and a promotion phase caused by irritation, inflammation, and resulting cell proliferation produced by bladder calculi (Clayson et al., 1970; Rodent Bladder Carcinogenesis Working Group, 1995). A threshold-dependent mechanism of carcinogenicity for nongenotoxic bladder carcinogens that form bladder calculi therefore was proposed (Rodent Bladder Carcinogenesis Working Group, 1995).

**Table 1:** Nongenotoxic bladder carcinogens in rats and mice.

| Bladder carcinogens | |
|---|---|
| **Rats** | **Mice** |
| Actaminophen[c] | Chloroprene[c] |
| 11-Aminodecanoic acid[c] | Nitrilotriacetic acid[c] |
| Chloroprene[c] | Salicylazosulfapyridine[c] |
| Diethylene glycol[a] | Uracil[a,b] |
| Melamine[a,b] | 4-Ethylsulfonylnaphthalene-1-sulfonamide[a,b] |
| Nitrilotriacetic acid[c] | |
| Nitrilotriacetic acid, trisodium salt, monohydrate[c] | |
| N-Nitrosodiphenylamine[b] | |
| Phenazone[c] | |
| *o*-Phenylphenate sodium[c] | |
| Saccharin, sodium[a,b] | |
| Terephthalic acid[a,b] | |
| *o*-Toluenesulfonamide[c] | |
| Uracil[a,b] | |

Nongenotoxic bladder carcinogens have been reported in rats and mice (Dybing et al., 1999; Huff, 1999).
[a]Causes bladder calculi.
[b]Carcinogenic only for the bladder.
[c]Carcinogenic for the bladder as well as at other anatomical sites.
None of the chemicals are mutagenic in the *Salmonella* mutation assay.

The International Agency for Research on Cancer (IARC) has concluded that nongenotoxic bladder carcinogens that form bladder stones are of low carcinogenic risk to humans because humans would expel any bladder stones or have them surgically removed before they have had a chance to exert their carcinogenic effect (Consensus Report, 1999). However, if this were true, then any substance that can interfere with the bladder urothelium's ability to heal after being irritated and inflamed by bladder stones, or the presence in the bladder of any substances with tumor promoter activity, would increase the carcinogenic risk by ensuring that the carcinogenesis process would continue to completion and that tumors would be formed even if bladder stones were expelled.

Indomethacin, a drug that is often prescribed for the relief of symptoms in patients with rheumatoid arthritis or gout, is excreted intact in the urine in significant amounts (Holmang et al., 1991). The authors have shown further that the drug, an inhibitor of prostaglandin synthesis, enhanced *N*-[4-5(-nitro-2-furyl)-2-thiazolyl]formamide (FANFT)-induced urinary tract carcinogenesis in female Sprague–Dawley rats (Holmang et al., 1995). In addition, indomethacin-treated rats exhibited urothelial hyperplasia, a characteristic feature of tumor promoters (Fukushima et al., 1980). That acetaminophen, another inhibitor of prostaglandin synthesis and the main metabolite of the

bladder carcinogen phenacetin, also induced urothelial hyperplasia and bladder tumors in Leeds and Sprague–Dawley rats suggests that indomethacin may act as a tumor promoter in the bladder by interfering with the synthesis of prostaglandins (Flower, 1974; Johansson, 1981; Flaks et al., 1985).

Prostaglandin PGI2 and PGE2 have a cytoprotective function on the gastric mucosa and possibly also in the urinary bladder where prostaglandin analogues have been used to treat symptoms caused by schistosomal bladder ulcers (Konturek et al., 1984; Mohiuddin et al., 1984). Indomethacin has been shown to impair the protective function of the bladder urothelium from the hostile environment of urine by interfering with the synthesis of prostaglandins (Cetinel et al., 2003). Diminished cytoprotection in the bladder may be implicated in bladder carcinogenesis as β-naphthylamine, a human bladder carcinogen present in cigarette smoke, also inhibits prostaglandin synthesis *in vitro* (Jeremy, 1985). This may explain the increased incidence of bladder tumors in cigarette smokers (Jeremy, 1985).

Inhibitors of prostaglandin synthesis, including indomethacin, are not the only substances with tumor promoter activity to which people may be exposed on an acute or chronic basis. Drugs such as phenacetin, sodium-*o*-phenylphenate, allopurinol, and diphenyl, as well as the food additives sodium L-ascorbate, butylated hydroxyanisole, butylated hydroxytoluene, and sodium saccharin and the natural product DL-trytophan, all are promoters of bladder carcinogenesis (Ito et al., 1984). Because people may be exposed to one or more tumor promoters intentionally (i.e., through their medications or in their diet or drinking water), the presence of these substances in the bladder may ensure that bladder tumors will be formed after the bladder urothelium had been inflamed by bladder stones formed by certain nongenotoxic bladder carcinogens present in the environment and to which people may be exposed.

Inflammation of the bladder is one of the underlying conditions responsible for formation of bladder stones in humans. It is often the result of infections caused by bacteria or other infectious agents. However, bladder stones also can be the cause of the inflammation. For example, people with gout develop bladder stones composed almost entirely of uric acid, a chemical that is irritating to the bladder. Calcium oxalate stones are by far the most common in adults in developed countries with nearly 90% of bladder stones of Japanese adults composed of mixtures of calcium oxalate and calcium phosphate (Burin et al., 1995). Materials that have been reported in stones on rare occasions include steatin, sulfonamides, and indigo (Burin et al., 1995). Also, formation of bladder stones may be associated with various pharmaceuticals such as methoxyflurane, allopurinol, and corticosteroids (Cheng, 1980). Chemicals such as TPA, melamine, diethylene glycol, and 4-ethyl-sulfonylnaphthalene-1-sulfonamide have been shown to cause calculi in the bladder of rodents that, in turn, irritate and inflame the bladder and ultimately lead to tumor formation. Presumably, these chemicals also can cause bladder stones in humans

because the human bladder is anatomically and pathologically similar to that of rodents.

## CONCLUSION

People who are exposed to one or more tumor promoters through their medications or in their diet or drinking water that are excreted in the urine may be at an increased risk of developing bladder cancer if they are also exposed to nongenotoxic bladder carcinogens that form bladder stones. In addition, because many people who are exposed to carcinogenic chemicals in the environment or in their workplace also may be acutely or chronically treated with tumor-promoter or carcinogenic drugs, any proposed risk assessment methodologies for chemical mixtures must not only focus on chemical–chemical mixtures but also address carcinogenic risks posed by chemical–drug combinations.

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