Chemoprevention of Experimental Bladder Cancer

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Abstract   The chemopreventive efficacy of several compounds was evaluated in the N-butyl-N-(4-hydroxybutyl)nitrosamine (OH-BBN)-induced urinary bladder cancer model using C57BL/6 x DBA/2F$_1$ (BDF) male mice. Compounds were administered in a defined semipurified diet (AIN-76-A) either as single agents or in combination. As single agents and at the doses employed, 2-α-difluoromethylornithine (DFMO), piroxicam, oltipraz, and sodium molybdate effectively inhibited the incidence of transitional cell carcinoma (TCC). 4-Hydroxyphenyl retinamide (4-HPR) was ineffective. Body weight gain and survival was not affected by the doses of agents used. Combinations of two agents which increased efficacy were 4-HPR + DFMO, DFMO + piroxicam, 4-HPR + oltipraz, and DFMO + oltipraz. Three-agent combinations which showed enhanced efficacy against TCC induction were 4-HPR + Na molybdate + DFMO, 4-HPR + DFMO + piroxicam, and 4-HPR + DFMO + oltipraz. Although the three-agent combinations were, for the most part, no more effective than the two-agent combinations at the doses employed, all combination regimens significantly reduced bladder cancer incidence even when single agent administration did not.   © 1992 Wiley-Liss, Inc.

Key words: bladder cancer, chemoprevention, MNU, model systems, mouse, OH-BBN, rat, retinoids

Urinary bladder cancer is a multifocal disease; experimental studies indicate that, at least in rodents, such lesions develop by a multistage process [1]. Of the several experimental models of urinary bladder cancer which exist, the N-butyl-N-(4-hydroxybutyl)nitrosamine (OH-BBN)-induced cancer model has been most widely used for chemoprevention studies [2,3]. As originally described, the carcinogen (OH-BBN) is administered in drinking water [4,5]. Although effective, this method of administration presents inherent risks to the safety of laboratory personnel and makes accurate quantitation of the amount of carcinogen ingested by each animal difficult. In our laboratory, these disadvantages are eliminated by administering OH-BBN via gastric intubation. This method of urinary bladder tumor induction has been used in both rats and mice [2,3].

Experiments using both male and female Fischer 344 (F344) rats showed that intragastric administration of OH-BBN induced transitional cell carcinomas of the urinary bladder [2,6] which were histologically similar to the human counterpart [7]. There is a short latency period before tumor appearance; the transitional cell carcinomas exhibit subepithelial and, occasionally, muscle invasion. In male C57BL/6 mice, intragastric administration of OH-BBN results in the induction of both squamous (60%) and transitional cell carcinomas [8]. These carcinomas are highly malignant; the majority of tumors show invasion of the bladder muscle wall and urinary bladder calculi are not found. However, since the majority of human urinary bladder carcinomas are of the transitional cell type, we have developed a mouse model in which the induced tumors are transitional cell carcinomas. The intragastric administration of OH-BBN to male C57BL/6 x DBA2-F$_1$ (BDF) mice results in the induction of highly invasive urinary bladder carcinomas which are morphologically similar to a human variant of advanced urinary bladder transitional cell carcinoma. The present report describes the effect of several chemopreventive agents alone or in combination

on the induction of these highly invasive carcinomas.

CHEMOPREVENTION WITH SINGLE AGENTS

Sporn *et al.* [9] were the first to demonstrate the inhibitory effect of retinoids on bladder carcinogenesis which in experimental animals is a multistage process involving initiation, promotion, and progression [10]. These workers found that 13-*cis*-retinoic acid not only inhibited the incidence, but also reduced the severity of bladder neoplasms induced in rats by the intravesical administration of *N*-methyl-*N*-nitrosourea (MNU). The intravesical administration of MNU in this study resulted in a high percentage of malignancies classified as squamous cell carcinomas. Moon and colleagues [11] conducted several studies to determine the efficacy of retinoids in experimental systems in which the tumors that developed were almost entirely transitional cell carcinomas. Early work by these investigators showed that 13-*cis*-retinoic acid reduced the incidence and severity of transitional cell carcinomas as well as a number of other proliferative lesions resulting from the

intragastric administration of OH-BBN to either F344 rats or BDF mice. Further studies indicated that the administration of this retinoid could be delayed for some time following the last carcinogen exposure and still inhibit bladder carcinogenesis.

In the attempt to improve upon the therapeutic index attained with 13-*cis*-retinoic acid, extensive studies were undertaken to identify retinoids with increased chemopreventive activity when administered in the diet at non-toxic levels [12]. Using the BDF/OH-BBN experimental model, a large number of synthetic *n*-alkyl amide derivatives of retinoic acid have been identified which possess a greater activity:toxicity ratio than that seen with 13-*cis*-retinoic acid (Table I). Particularly notable among the retinamides is *N*-4-hydroxyphenyl retinamide (4-HPR) which is, for the most part, less toxic than the other retinamides and is also an effective chemopreventive agent in organs (skin, mammary glands, lung) other than the urinary bladder.

As shown in Table I, modifications of the basic retinoid structure can have a significant effect on anticancer activity. Adding an ethylamide or a hydroxyethylamide group to all-

TABLE I. Retinoids Evaluated for Chemopreventive Activity Against Urinary Bladder Cancer in Rats and Mice*

ACTIVE	INACTIVE
Retinoic Acid	Retinyl Palmitate
13-*cis*-Retinoic Acid	*N*-(4-Carboxyphenyl)retinamide
Retinyl Acetate	*N*-(4-Carboxypropyl)retinamide
N-Ethylretinamide	*N*-(3-Hydroxypropyl)retinamide
N-Ethyl-13-*cis*-retinamide	*N*-(2,3-Dihydroxypropyl)retinamide
N-(2-Hydroxyethyl)retinamide	*N*-(*n*-Butyl)retinamide
N-(2-Hydroxyethyl)-13-*cis*-retinamide	*N*-(4-Hydroxybutyl)retinamide
N-(4-Hydroxyphenyl)retinamide	*N*-(4-Hydroxybutyl)-13-*cis*-retinamide
N-(4-Hydroxyphenyl)-13-*cis*-retinamide	*N*-(5-Tetrazolyl)-13-*cis*-retinamide
N-(2-Hydroxypropyl)retinamide	Motretinid
N-(5-Tetrazolyl)retinamide	
Etretinate	

* Adapted from [11].

trans-retinoic acid results in compounds which are highly effective chemopreventive agents for bladder cancer, but much less toxic than all-*trans*-retinoic acid. Similarly, adding the ethyl-amide or hydroxyethylamide group to 13-*cis*-retinoic acid results in the formation of a less toxic retinoid, with equal or greater cancer-inhibitory activity. Conversely, changing the aromatic ring of the highly active ethyl retin-amide to a trimethylmethoxyphenyl group pro-duces a compound with little or no cancer-inhib-itory activity in the bladder.

It is clear that several retinoids effectively inhibit development of carcinogen-induced blad-der cancer in rats and mice. It is also apparent that several synthetic retinoids, particularly the retinamides, are less toxic and more effective than natural retinoids for this purpose. Al-though some retinoids may prevent initiation of bladder carcinogenesis, the majority are appar-ently more active during the progression phase of the carcinogenic response. If such compounds are to be used as chemopreventive agents for bladder cancer in humans, then the ability of retinoids to delay tumor growth in urothelium already neoplastically transformed is of critical importance.

Several other compounds of diverse chemical structure have been evaluated as potential che-mopreventive agents in a variety of tumor mod-el systems. These include not only the retinoids but also flavonoids, non-steroidal anti-inflam-matory agents, polyamine synthesis inhibitors, organosulfur compounds, flavoring agents, anti-oxidants, and vitamins. Of the several com-pounds which have been evaluated for chemo-prevention of urinary bladder carcinogenesis, oltipraz (5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione), DFMO (2-α-difluoromethylornithine) and piroxicam (4-hydroxy-2-methyl-*N*-2-pyrid-inyl-2H-1,2-benzathiazine-3-carboximide-1,1-dioxide) have proven the most effective.

Recent studies [13] from our laboratory have shown that piroxicam, a nonsteroidal anti-in-flammatory agent, is a potent inhibitor of OH-BBN-induced transitional cell carcinoma when administered in the diet of BDF mice. At 15 mg piroxicam/kg diet, tumor incidence was reduced 82%, from a 40% incidence in OH-BBN controls to 7% in the agent-treated mice. Similarly, at 30 mg piroxicam/kg diet, tumor incidence fell 70%, from 43% in carcinogen controls to 13% in

treated mice. The results of the study at the higher dose level also suggested that piroxicam may have inhibited invasion; however, the num-ber of tumors which developed was too low for an adequate evaluation.

In another study [14], the schistosomicidal drug oltipraz was supplied in the diet from 1 week prior to OH-BBN dosing until sacrifice six months later. At 250 mg/kg diet, the agent significantly reduced the incidence of transition-al cell carcinoma compared with carcinogen controls. Oltipraz also significantly reduced transitional cell carcinoma incidence when fed at 500 mg/kg diet for 76 days, then at 125 mg/kg diet until the end of the test period. Treat-ment at this higher dose level of oltipraz also appeared to decrease the depth of tumor inva-sion. At lower dose levels of 100 and 200 mg/kg diet, oltipraz alone had no effect on tumor inci-dence.

Treatment of BDF mice with the polyamine synthesis inhibitor DFMO (2000 or 4000 mg/kg diet) during the period of OH-BBN administra-tion provided significant protection against the induction of transitional cell carcinomas. Tumor incidence was significantly reduced (~50%) whether or not DFMO administration was con-tinued beyond the OH-BBN dosing period. In contrast, DFMO treatment initiated 1 week after instillation of the final dose of OH-BBN did not inhibit tumorigenesis. Nonetheless, lesions tended to be less invasive in all OH-BBN-treated groups receiving the higher dose of DFMO as compared with the controls. With the exception of a slight but significant suppression of weight gain in the high-dose group, no signif-icant toxic effects were observed in mice after 180 days on the DFMO-supplemented diet [14].

COMBINATION CHEMOPREVENTION

Although significant reductions in carcinoma incidence and multiplicity have been achieved with the use of experimental chemopreventive drugs, the anticarcinogenic activity of com-pounds presently available is incomplete, *i.e.*, cancer incidence is not reduced to zero with administration of these agents at non-toxic levels. The toxicity of anticarcinogenic agents remains an important factor and limits their potential use. If such toxicity can be reduced,

higher doses of the compounds could be administered without adverse effects, raising the possibility of greater anticarcinogenic efficacy.

Two distinct approaches have been taken in order to develop chemopreventive regimens with increased efficacy and reduced toxicity. The first has involved the design of synthetic congeners of active compounds in the attempt to dissociate chemopreventive activity from toxicity. This approach has been used most extensively with retinoids. Synthetic retinoids have been designed which are less toxic yet have equal or greater anticarcinogenic activity than the natural vitamin A compounds [12]. The second means by which the efficacy of cancer chemoprevention may be increased is to co-administer anticarcinogenic agents. Such combination chemoprevention protocols seek to increase the efficacy of cancer prevention either through an additive or synergistic interaction between two chemopreventive agents [15,16], or through the reduction of agent toxicity, thereby permitting administration of active compounds at higher dose levels.

Such combination chemoprevention is exemplified by studies of experimental mammary carcinogenesis. For example, the combination of the retinoid 4-HPR and tamoxifen act synergistically to afford greater protection against mammary cancer than that of either agent alone [17]. Similar synergistic inhibition has been demonstrated with retinoids and other modifiers of mammary carcinogenesis [18].

Recently we have extended the combination chemoprevention approach to urinary bladder carcinogenesis induced with OH-BBN. As indicated above, oltipraz administered alone at dose levels of 100 and 200 mg/kg diet had no effect on tumor incidence. However, treatment with the combination of 640 mg DFMO/kg diet and 100 mg oltipraz/kg diet was efficacious, although DFMO alone at 640 mg/kg diet was inactive. The combination of 1280 mg DFMO/kg diet and 200 mg oltipraz/kg diet reduced transitional cell carcinoma incidence significantly compared with carcinogen controls, but the effect was no greater than that of DFMO alone; weight gain was suppressed compared with carcinogen controls. The depth of tumor invasion was also decreased with this combination treatment. Combinations of oltipraz, 4-HPR, and DFMO were efficacious and without apparent toxicity. Nonetheless, the three-agent combinations could not be considered more effective than either DFMO alone at 1280 mg/kg diet or the lower dose combinations of oltipraz and DFMO.

The chemopreventive potential of piroxicam, on the other hand, did not improve in combination with DFMO, 4-HPR, or both [14]. The agents were each administered in the diet at 0.4 and 0.8 of the maximum tolerated dose (MTD). The 0.4 MTD levels (per kg diet) were 15 mg piroxicam, 600 mg DFMO, and 156 mg 4-HPR. The 0.8 MTD levels (per kg diet) were 30 mg piroxicam, 1200 mg DFMO, and 313 mg 4-HPR. Combinations of piroxicam with DFMO were no more effective than piroxicam alone, and combinations of piroxicam with 4-HPR were less effective than piroxicam alone. Also, the combination of all three agents at the 0.4 MTD level was less effective than piroxicam alone. The three-agent combination at the 0.8 MTD dose level was highly effective in reducing tumor incidence (91%) compared with carcinogen controls (from 43% to 4%). However, the effect of this combination was not significantly different from that achieved by the 15 mg/kg diet dose of piroxicam administered alone. Thus, it would appear that piroxicam *per se* is highly effective in the chemoprevention of bladder cancer and that its effect is not enhanced by the addition of other bladder chemopreventive agents to the treatment regimen.

SUMMARY

It is readily apparent from the studies cited above that a number of retinoids effectively inhibit urinary bladder carcinogenesis. In view of the demonstrated safety of 4-HPR in clinical trials [19], this would be the retinoid of choice for a clinical study of the chemoprevention of superficial bladder cancer. The broad spectrum of chemopreventive efficacy of piroxicam, DFMO and oltipraz in various *in vivo* carcinogenesis systems and the demonstrated effect of these compounds on the inhibition of transitional cell carcinoma formation would also make these compounds likely candidates for future clinical trials. Of these three prospective clinical chemopreventive agents, it appears that piroxicam may provide the most beneficial effects based on these studies.

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