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The Pathogenesis of Experimental Bladder Cancer[1]

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Summary

The pathogenesis of signal morphological lesions of the urinary bladder induced in several species following administration of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, bracken fern, or *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide is presented. Incidences of bladder neoplasia exceeding 80% were generated in the rat by each compound. Bladder neoplasia was induced in the following species by each substance: by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the mouse, hamster, guinea pig, and dog; by bracken fern in the guinea pig, mouse, and cow; and by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide in mouse, hamster, and dog. The guinea pig appeared resistant to the bladder oncogenicity of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. Different species displayed a gradient of bladder neoplastic responsiveness. Hyperplasia was a consistent early lesion and was usually focal. Early hyperplastic lesions regressed following removal of the carcinogenic stimulus, but later lesions appeared to be irreversible. These animal systems appear useful in providing opportunities for investigations relevant to human bladder cancer.

Introduction

The discovery in 1895 that human bladder cancer might be etiologically related to chemical exposure occurred about 40 years before the 1st successful attempt to produce vesical neoplasia in experimental animals (14). Between 1935 and 1965 many chemicals were reported as oncogenic for the bladder when tested in a variety of species (3, 14). However, vesical tumor yields were generally low, latent periods of generation were lengthy, and biological evidences of lesional aggressiveness were generally lacking. Thus, studies tended to be descriptive rather than mechanistic, and progress in understanding the pathogenesis of experimental bladder cancer was slow.

In the mid-1960's opportunities to accelerate the acquisition of knowledge about bladder carcinogenesis were provided by the demonstrations by several workers of the generation of bladder neoplasia in high yields with shortened latent periods and with clear evidence of local and distant metastases or transplantability. Several chemical agents and routes of administration were employed to generate these tumors (3, 14). Among those agents more widely utilized were BBN[3] (8), BF (24), and FANFT (12) fed to rodents. During the past decade the utilization of these systems has provided an increasing body of knowledge concerning the complex array of temporal events that precede and accompany the development of a spatial lesion recognized as neoplasia. The purpose of this abbreviated review is to present the current status of the acquired understanding of experimental bladder cancer that has thus far evolved through the use of the above systems.

Purposes and Minimum Desirable Characteristics of Bladder Cancer Models

The expectation of experimentalists in developing *in vitro* or *in vivo* models of cancer is that these systems will permit the expeditious study of some aspects of the natural history of human cancer that cannot be studied in man because of ethical, moral, or technical constraints. Such studies should lead to identification of key processes that are related temporally to the evolutionary disease pathogenesis. These investigations should describe what is going on and how and why it happens. Such knowledge would thus be useful in the rational design of modalities to intervene with the key processes leading to organic disarray.

An initial step in the exploitation of a model is to define desirable characteristics that the model should possess. The characteristics should be selected to mirror the human disease under study. If nothing else, this process would permit the investigator to recognize when he has achieved his desired goal.

The following seem to be minimum characteristics desirable for a proposed bladder cancer *in vivo* model. The lesion should be a proliferative epithelial response with a reproducible adjacent and supporting tissue reaction occurring with temporal regularity when generated by a defined stimulus. The lesion should be produced in a significantly greater incidence than a comparable lesion present in untreated control animals. For many purposes it would be useful to have lesions generated in an incidence exceeding 80%. A neoplasic response must demonstrate evidence of local tissue invasion, local and distant organ metastases, progressive growth with time, lethality to the host, and transplantability to allogeneic hosts.

Unfortunately, present knowledge requires that the de-

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[3] The abbreviations used are: BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; BF, bracken fern (*Pteridium aquilinum*); FANFT, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide.

sired *in vivo* system must first display morphological bladder cancer before extensions backward in time can be made to such sentinel lesions. The substances BBN (1, 8, 15–17), BF (22–25), and FANFT (6, 9–12, 27) provide the stimuli to generate in a variety of species epithelial lesions bearing accepted morphological and, in some cases, biological evidences of neoplasia. In the rat, feeding BBN (1, 8, 15–17), BF (22, 24, 25), or FANFT (11, 12, 27) produces a high yield, generally exceeding 90%, of epithelial tumors. This neoplasia is usually preceded by epithelial hyperplasia (4, 11, 27) and may be accompanied by squamous metaplasia. Early epithelial lesions may be papillary or sessile. Eventually, penetration into subjacent bladder tissues occurs, as well as the formation of intravesicular lymphatic and venous emboli. Lesions of long standing may nearly occlude the bladder lumen, and thus it is somewhat surprising that more rats do not die as a result of this proliferative onslaught.

When animals begin to lose weight and exhibit signs of lower urinary tract dysfunction, most experimentalists kill their subjects to secure fresh tissues. Thus, no experiments dealing with bladder carcinogenesis have been conducted with the deliberate intention of permitting experimental animals to die as a result of their intentionally generated lesions, and it may not be entirely unexpected that so few animals display local, regional, or distant metastases. Nevertheless, such metastases have been observed and reported (4, 11, 22). Additionally, allogeneic unconditioned hosts have served as admirable recipients for these neoplastic transplants (11).

The histological characteristics of bladder neoplasia produced by BBN (16), BF (22), or FANFT (11, 27) are those of transitional cell carcinoma in the majority of cases. Occasionally, squamous cell carcinoma may be interspersed, or rarely it may be a singular manifestation. Exceedingly uncommon are adenocarcinoma, undifferentiated carcinoma, or nonepithelial tumors of the bladder. These histological characteristics observed are very similar to those reported for humans with bladder neoplasia residing in most portions of the world except certain areas of Africa.

Variable Species Susceptibility to BBN, BF, or FANFT

A number of animal species have been assessed for bladder carcinogenicity following exposure to BBN (1, 15, 17), BF (22, 23), or FANFT (6, 7, 9–12). From these studies it appears that much species response variation exists. Rats are most susceptible to BBN, followed by mice, hamsters, and guinea pigs (15, 17). With FANFT, the rat is most susceptible, followed by the mouse and hamster (6, 10, 12). Paradoxically, the guinea pig seems refractory to the bladder oncogenicity of FANFT (7). With BF, the rat appears most susceptible, followed by the guinea pig and mouse (22). Finally, the dog bladder is susceptible to the oncogenic effects of both BBN (15) and FANFT (9), while the cow responds well to BF (23).

It is tempting to speculate that differences, perhaps genetic, may regulate the metabolic activation pathways in these diverse species, leading subsequently to the macromolecular carcinogen adducts currently implicated as the essential molecular lesions preceding neoplasia. It has recently been suggested that such metabolic differences in aromatic amine acetylation-deacetylation reactions exist for all species measured, including man (19–21). This has formed the basis for a hypothesis, currently under test, seeking evidence that humans with bladder cancer are less capable of acetylating aromatic amines into apparently biologically innocuous molecular species (21). Such a hypothesis, if supported, could account in part for the apparent individuality of response exhibited by mammals to bladder carcinogens. Alternative hypotheses, as yet untested, might be generated to account for species and individual variable responses to bladder carcinogens.

Comparative Temporal Bladder Epithelial Response to FANFT, BBN, or BF

It is instructive to compare further the temporal appearance and known biological attributes that have been identified in studies in several species exposed to FANFT (4, 6, 9–12, 27). Such a comparison is presented in Table 1, which displays the characteristics identified at the light microscopic level. It is clear that morphological evidence of epithelial disturbance occurs early after initial exposure to FANFT for each susceptible species (4, 5, 9–12, 27).

Variability in time of appearance of carcinoma has been most rigorously studied in the rat, but some data are available for the other species (6, 9, 10, 15, 17, 23). Epithelial proliferative lesions penetrate adjacent bladder tissues and, on this basis, have been recognized as carcinoma. Recent biological data (4) strongly suggest that, by 10 weeks of exposure to FANFT, the bladder epithelium has developed characteristics that preclude reversibility following the removal of FANFT from the diet.

Table 1

Temporal comparison of bladder effects of FANFT fed to several species and of BBN or BF fed to rats

Depicted are the reported periods when manifestation of bladder effects have appeared following administration of the above carcinogens. These data have been developed from published reports cited in the text.

Carcinogen	Species	Minimum latency (wk)		Squamous metaplasia	Metastases	Incidence (%)	Transplantability
		Hyperplasia	Carcinoma				
FANFT	Rat	2-3	9-12	Yes	Yes	90	Yes
FANFT	Mouse	3	7-10	Yes	Yes	90	Yes
FANFT	Hamster	6	18	Yes	Yes	90	Unknown
FANFT	Dog	6	98	Yes	No	90	Unknown
FANFT	Guinea pig	None	None	None	None	0	None
BF	Rat	3	13	Yes	Yes	80	Unknown
BBN	Rat	4	13	Yes	Yes	90	Yes

Lesions thus produced by FANFT become progressive in mass and depth of extension. Local pelvic and distant metastases have been found for the rat, mouse, and hamster, but not the dog (6, 9–12). The incidence of invasive or metastatic bladder carcinoma exceeds 90% in those species surviving 10 or more weeks. Additionally, transplantability of bladder neoplasia to allogeneic recipients has been demonstrated for rats and mice (11, 26).

Studies of the temporal appearance of epithelial bladder lesions following exposure to BBN or BF (15, 16, 22) have been carried out in the rat (Table 1). The response observed has been closely similar to that reported for FANFT, suggesting that with bladder organotrophic stimuli of comparable potency the observed biological response may reflect an inherent property of the altered mucosa.

Temporal Comparison of Early Histological Rat Bladder Epithelial Alterations with Subsequent Biological Fate

A major criticism of morphological interpretation as a sole criterion of malignancy is that it may not accurately reflect the biological potential of the lesion under examination. Thus, any morphological entity may be associated with a measurable probability of regressing, remaining static, or progressing. Until recently (4), evaluations of experimental bladder lesions viewed by microscopy were based on morphological similarity to comparable human lesions coupled with the known biological fate of humans who harbored such lesions. This linkage to human biology, while suggestive, did not establish that the experimentally produced disease was an appropriate human mirror.

Recently, Cohen *et al.* (4) have presented evidence relating the morphological and biological fate of bladder lesions developed by FANFT in the Fischer rat model. Mild focal hyperplasia occurred within 2 weeks of exposure but regressed upon withdrawal of FANFT. Similarly, moderate focal hyperplasia observed at 6 weeks was reversible. At 8 weeks, marked focal hyperplasia was present and persisted as hyperplasia throughout the experimental duration. Finally, at 10 or more weeks of FANFT administration, transitional cell tumors were detected that became eventually progressive and invasive. The conclusion of these workers (4) was that hyperplastic lesions developing through 6 weeks of FANFT feeding appear to be reversible if FANFT is discontinued, but lesions appearing later seem to be irreversible, even if FANFT administration is discontinued. This novel study has provided strong relational documentation of the morphological and biological attributes of bladder lesions induced in rats and provides a powerful conceptual and experimental tool for further studies.

Conclusion

I have tried to portray the major thrust of current studies dealing with the pathogenesis of experimental bladder cancer. Studies concerned with this topic have progressed from a purely descriptive approach to mechanistic approaches and from questions dealing with "what?" to those dealing with "how?" Model systems are now available to permit the experimentalist to investigate the influence of various factors, presented concurrently or sequentially, on the temporal progression and augmented incidence of the development of bladder cancer. Examples of such studies recently presented can be cited (2, 5, 13, 28). These represent some of the newer studies designed to provide comprehension—to answer "why?" Knowledge of molecular events will ultimately serve as the basis for rational approaches to process intervention in the genesis and evolution of bladder cancer. Experimental systems will provide a leading role in this dynamic process, and, through the continuous interaction of scientists utilizing these approaches with their clinical counterparts, control of cancer is anticipated.

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