Rat and Mouse Urothelial Carcinogenesis
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ABSTRACT: Experimental urothelial carcinogenesis has an important role in studying urinary bladder cancer biopathology and to evaluate different chemotherapeutic drugs. Several rodent models of bladder cancer development have been established with these purposes. The aim of this article is to provide a critical assessment of rat and mice models available using chemical carcinogens.

KEYWORDS: Urinary bladder, rat, mice, carcinogenesis

Introduction
The bladder is one of the most common sites of cancer in the urinary tract. Bladder tumours are manifestations of a multifocal disease whose natural history has not been completely elucidated and the reaction of bladder tumours to radio and chemotherapy is unpredictable (1). In developed countries, the majority of bladder cancers are sporadic carcinomas that arise from the urothelium, the well specialized transitional epithelium that lines the urinary bladder. Urothelial cell carcinoma is three times more frequent in men than in women (2). Since the Surgeon Dr Rehn original suggested in 1895 a role for aniline dye in the etiology of bladder cancer, this is historically the neoplastic disease most strongly linked to professional and environmental contact to chemicals (3). Animal models of cancer have been significant for demonstrating that carcinogenesis is a multistep process comprising three key stages: initiation, promotion and progression (4).

Rodent Bladder
The structure and function of rodents’ lower urinary tract is remarkably comparable to that of humans (5). It extends from the renal pelvis through the ureters, urinary bladder, and into the urethra (6). Excluding the urethra, the urinary tract consists of four layers: mucosa, lamina propria, muscular and serosa (7-9). The mucosa of the lower urinary tract has been referred to as a transitional cell epithelium or as an urothelium. In the urinary bladder it is made up of three cell layers: superficial, intermediate and basal (5, 7). Normal urothelium from all species has an extremely low rate of turnover and virtual absences of mitoses (10, 11) but a high regenerative capacity, showing rapid proliferation during development and in response to damage or injury (12). The incidence of spontaneous tumours in rodents plays an important practical role in the design and analysis of carcinogenicity bioassays (1). However, most naturally occurring strains of rodents do not develop spontaneous bladder cancer, more than 99% are predominantly associated with advancing age (13-15). An exceptionally high incidence of urothelial and ureteric neoplasms have been reported in two rat strains, Brown/Norway (BN/RijHsd) and Dark Agouti (DA/OlaHsd), which were associated with the presence of calculi (16-17).

Chemical carcinogens
The induction of bladder cancer in dogs by 2-naphthylamine, reported by Hueper in 1938, established the experimental basis of bladder carcinogenesis (18). Early attempts to induce tumours in mice bladders by means of chemicals were unsuccessful until Armstrong and Bronser (1944) induced papillomas and carcinomas through the oral administration of 2-acetylaminofluorene (AAF) in CBA strain mice (19). In the 1960s and early 1970s, organospecific chemically-defined bladder carcinogens were discovered for rodents (1). These chemicals and their application provided the readily available reproducible models necessary for detailed studies of the biochemical, pathobiological and immunological mechanisms involved in the pathogenesis of bladder cancer (15). Over the past few decades, research efforts have focused on the development of rodent models that permit the reproducible induction of bladder cancer with minimal or no induction of tumours in other organs. Three chemicals have been proved to be particularly effective, in that, when administered via the appropriate route, at the appropriate dose and in the appropriate strain of animal, all pro-
duce 100% incidence of bladder tumours; these chemicals are N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT), N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) and N-Methyl-N-nitrosourea (MNU) (20). These compounds are complete carcinogens, the total dose has a greater effect when administered as several fractions, i.e. the effect of the fractions is synergistic rather than additive. The grade of cellular atypia and the extent of invasion increase as the dose of carcinogen increases as well as when the experimental period is extended (21, 22). The nitrofuran FANFT is highly specific to the urinary bladder in the rat, mouse, hamster and dog. It is a genotoxic compound and can act as an initiator or as a promoter. It is metabolically activated into reactive electrophiles, produces DNA adducts and ultimately produces mutation (7, 22, 23). FANFT is deformylated in 2-amino-4-(5-nitro-2-furyl) thiazole by liver and kidney enzymes before excretion (24). Tumours induced by this compound are predominantly transitional cell carcinoma (TCC), with a large proportion exhibiting squamous cell differentiation. However, hyperplasia, dysplasia and CIS have also been observed (22, 23, 25). FANFT is incorporated in the diet and induction of bladder cancer requires 8 to 11 months (23, 26). However, the use of this compound presents safety concerns for the researchers involved and the environment (1). BBN is one of the most suitable urinary bladder carcinogens for animal models, since its carcinogenic potential is essentially limited to this organ and is probably the most commonly referenced experimental bladder carcinogen (27, 28). Bladder tumours induced by BBN in rats and mice resemble their human counterparts both grossly and histologically (28). BBN is a metabolite of the symmetric dibutylnitrosamine (DBN) (29). In rats, both were demonstrated to be urinary bladder carcinogens, with BBN being specific to the urinary bladder, because DBN also induced tumours of the liver, lung, kidney and oesophagus (30, 31). MNU is the only carcinogen recognized to act directly on the urothelium following spontaneous pH-dependent decomposition without requiring metabolic activation. At present MNU is the only urothelial carcinogen known to produce bladder cancer at a single dose (22). MNU is a fine yellowish crystalline powder stabilized by addition of 5% acetic acid (32). Because MNU is intrinsically unstable, variations in carcinogenic potency can arise unless care is taken during its storage, preparation and use (22). It is a genotoxic compound that can act as an initiator or as a promoter and cause persistent methylation of the DNA (33, 34). The MNU model of bladder cancer has particular advantages for the experimental analysis of complete carcinogenesis, since the carcinogen can be administered directly in quantifiable pulse doses, via intravesical instillation (35). The disadvantage of this procedure is that in some animals bladder concretions and/or urocystitis may develop (32, 36). Bladders treated with intravesical MNU develop progressive neoplastic changes, and the tumours become progressively less differentiated with time. These lesions progress from hyperplasia, atypia, CIS, and papillary carcinoma to large bulky muscle invasive tumours that completely fill the bladder lumen, obstruct the ureteres and kill the animal (33, 37). 4-Ethylsulfonylnaphthalene-1-sulfonamide, benzidine, 3, 3´-dichlorobenzidine, 2-naphthylamine, 4-amino-hiphenyl, 2-acetylaminofluorene, phenacetin, and sodium O-phenylphenate are additional compounds which are carcinogenic for the urinary bladder (7, 36). Bracken fern (Pteridium aquilinum) and pellet implantation are also other possibilities to induce urothelial tumours on rodents (7, 8, 38, 39). Chemical induction of bladder cancer in rodents usually requires 8-12 months. However, the administration of chemicals in water or diet although effective present inherent risks to the safety of laboratory personnel. Moreover, it is difficult to quantify the amount of carcinogen ingested by each animal. This disadvantage of carcinogen dosing may be eliminated by the administration of the carcinogen via gavage.

Spectrum of lesions observed in rat and mouse

Urothelial carcinogenesis in the rat goes through a sequence of morphologic changes beginning as simple hyperplasia. It then progresses to nodular and papillary hyperplasia. These progress to papillomas and can eventually progress to higher-grade, noninvasive carcinomas and ultimately to invasive neoplasms (5, 42). Many exophytic tumours induced in rats are polypoid, often pedunculated and with an inverted papillary growth pattern. Nodular hyperplasia, in mice, is considerably more common than papillary proliferations and nodular hyperplasia frequently occurs with a complete absence of papillary hyperplasia (41). Thus, the rat model strongly resembles papillary neoplasms and the mouse model resembles flat urothelial lesions, both identified in man (42). In Figure 1 and 2, we present the spectrum of urothelial lesions observed on rat and mouse urinary bladder obtained following oral exposition to BBN, respectively.

Conclusion

We have reviewed experimental data related to the induction of bladder cancer in rat and mouse. Using bladder cancer models it is possible to evaluate intravesical therapy (chemotherapy and immunotherapy) and systemic chemotherapy. By monitoring the responses to chemical carcinogens using experimental models, it has been possible to recognize many of the mechanisms through which tumours developed. Animal tumours also offer an opportunity to study the chromosomal changes associated with the early development of bladder cancer.
Figure 1. Urothelial lesions observed in rat during urothelial carcinogenesis induced by BBN; A-Normal urothelium (200X, H&E), B-Simple hyperplasia (200X, H&E), C-Dysplasia (200X, H&E), D-Nodular hyperplasia (200X, H&E), E-Carcinoma in situ (H&E), F-Papiloma (200x, H&E), G-Papillary tumour of low malignant potential (200X, H&E), H-Low-grade papilloma (200X, H&E), I-High grade papillary carcinoma (200X, H&E), J-Invasive carcinoma (200X, H&E), K-Invasive carcinoma with squamous differentiation (400X, H&E), L-Squamous metaplasia (100X, H&E).
Figure 2. Urothelial lesions observed in mouse during urothelial carcinogenesis induced by BBN; A-Normal urothelium (200X, H&E), B-Simple hyperplasia (200X, H&E), C-Papillary hyperplasia (200X, H&E), D-Nodular hyperplasia (200X, H&E), E-Dysplasia (200X, H&E), F-Carcinoma in situ (200X, H&E), G-Invasive carcinoma (200X, H&E), H-Invasive carcinoma (400X, H&E), I-High grade papillary carcinoma (100X, H&E), J-Squamous metaplasia (200X, H&E)
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