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Species Differences in Regenerative Hyperplasia of Bladder Urothelium After Transurethral Cauterization*

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Summary. The early histological changes in bladder urothelium following transurethral cauterization were sequentially studied in female rats, mice, hamsters and guinea pigs. The changes differed markedly between species: papillary hyperplasia or papilloma in the rats and simple hyperplasia in the mice, hamsters and guinea pigs. The regenerative response to the fulgurative ulcer within a given species was similar to the early response previously observed by others following administration of known bladder carcinogens. This suggests that there may be a regulatory mechanism in the urothelium which is common to urothelial proliferations induced by both carcinogenic and non-carcinogenic stimulations, though differing between animal species.

Key words: Regenerative hyperplasia, Bladder cancer, Transurethral cauterization.

It is well known that BBN and FANFT are selective carcinogens of the urinary bladder in several species of animal [3, 5, 6, 10]. In animal models, focal hyperplasia of the bladder urothelium is thought to be a preneoplastic change [10]. It appears in the early phase of carcinogenesis and shows histological characteristics differing with the animal species [11]. In rats, the bladder urothelium shows papillary changes in the early stage of carcinogenesis following exposure to BBN or FANFT, and those changes finally become papillary carcinoma [5, 10]. On the other hand, the mouse bladder urothelium shows flat growth, which becomes an invasive cancer [3, 6]. Mouse bladder cancer tends to produce more metastatic lesions than rat bladder cancer. In hamsters, diffuse cell growth of the urothelium is observed, but no papillomatous growth is seen [11]. The bladder urothelium of guinea pigs does not increase in thickness following treatment with BBN [11]. The purpose of the present paper is to define whether such different urothelial reactivity between species exists in regenerative hyperplasia following non-carcinogenic stimulation such as transurethral fulguration [1].

Materials and Methods

Female Wistar rats, C3H/He mice, Golden Syrian hamsters, and guinea pigs (8 weeks old; obtained from Nippon hamsters, and guinea pigs (8 weeks old; obtained from Nippon Biological Materials Co., Tokyo, Japan) were used. Groups of 5 animals each were housed in stainless steel cages, in a room maintained at 27 °C and 50–60% relative humidity. They were provided with a commercial stock diet and tap water.

The method for transurethral fulguration of the urinary bladder was originally developed by Soloway [12], and has been described previously [1]. The animals were anesthetized with intraperitoneal pentobarbital (50 mg/kg) and placed on a grounding plate with a moistened gauze pad between the skin and plate. An electrode constructed of 4-0 surgical steel wire insulated with PE-10 tubing was inserted transurethrally. The wire was attached to the working electrode of a solid-state electrocoagulator unit (Kaiba Co., Japan). The tip of the electrode was advanced until there was resistance from the posterior bladder wall. The cautery unit was set at 4 and the charge was applied for two seconds. Three separate areas of the bladder mucosa were cauterized.

Three animals of each species were sacrificed with an overdose of intraperitoneal pentobarbital on the day of cauterization and 7, 14, 21 and 28 days after cauterization. The urinary bladders were inflated with a 10% phosphate-buffered formalin solution, excised, fixed in the same fixative, cut into 20–30 slices longitudinally, embedded in paraffin, and stained with hematoxylin and eosin. The histology was studied under a light microscope and evaluated according to the criteria proposed by Fukushima et al. [8] as cancer, papilloma, papillary or nodular hyperplasia or simple hyperplasia.

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Abbreviations: BBN, N-buty-N-(4-hydroxybutyl)nitrosamine; FANFT, N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide

Table 1. Effective number of animals and total number of fulguration injuries caused by transurethral cauterization

Animal species		Days after fulguration					
		0	7	14	21	28	Total
Wistar Rats	No. of animals	3	3	3	3	3	15
	No. of injuries	9	8	9	9	6	41
C3H/He Mice	No. of animals	3	3	3	3	3	15
	No. of injuries	9	8	9	9	6	41
Golden Syrian Hamsters	No. of animals	3	3	3	3	3	15
	No. of injuries	9	9	8	6	5	37
Guinea Pigs	No. of animals	3	3	3	3	3	15
	No. of injuries	9	9	8	7	6	39

The previous study had proven that the histological changes developed in a confined area of the fulguration [1].

Thus, the histological changes were studied in each fulguration area, not for the bladder as a whole.

Results

All bladders removed from each species on the day of fulguration showed fulgurative mucosal ulceration accompanied by submucosal edema. The lesions healed and transmural scars were noticeable in all animals studied at each time point. Table 1 shows the effective number of animals and accumulated number of fulguration injuries (or scars) observed in animals killed at each time point.

The results of histological study on the reparative process of each confined area of fulguration are summarized in Table 2.

On Day 7, the rat urinary bladders showed exophytic changes; out of 8 lesions, 5 showed papillary hyperplasia, 2 showed papilloma and 1 showed simple hyperplasia. On Day 14, out of 9 lesions, 2 showed papillary hyperplasia, 5 showed simple hyperplasia, and 2 showed normal urothelial regeneration. These changes were reversible, and on Day 21, out of 9 lesions, 3 showed simple hyperplasia.

On Day 7, the mouse urinary bladders showed planophytic changes; out of 8 lesions, 5 showed simple hyperplasia. These changes were also reversible.

In the reparative process, the hamsters showed 4 simple hyperplasias out of 9 lesions on Day 7 and 1 out of 8 on Day 14.

The guinea pigs showed 1 simple hyperplasia out of 9 lesions on Day 7 and 1 out of 8 on Day 14.

Generally, except for the rat, the animals did not show any exophytic growth. As for simple hyperplasia, the greatest thickness of cell layers and the highest frequency were seen in the mouse urinary bladder. The histological findings for each species on Day 7 are shown in Fig. 1.

Table 2. Histology of each confined area of fulguration

Animal Species	Histology	Days after cauterization				
		0	7	14	21	28
Wistar Rats	Fulgurative injury	9	–	–	–	–
	Normal	–	0	2	6	6
	SH	–	1	5	3	0
	PH	–	5	2	0	0
	P	–	2	0	0	0
C3H/He Mice	Fulgurative injury	9	–	–	–	–
	Normal	–	3	6	8	6
	SH	–	5	3	1	0
	PH	–	0	0	0	0
	P	–	0	0	0	0
Golden Syrian Hamsters	Fulgurative injury	9	–	–	–	–
	Normal	–	5	7	6	5
	SH	–	4	1	0	0
	PH	–	0	0	0	0
	P	–	0	0	0	0
Guinea Pigs	Fulgurative injury	9	–	–	–	–
	Normal	–	8	7	7	6
	SH	–	1	1	0	0
	PH	–	0	0	0	0
	P	–	0	0	0	0

All animals were 8-week old females
Histological changes were classified as simple hyperplasia (*SH*), papillary hyperplasia (*PH*) and papilloma (*P*)
"Normal" indicates normal urothelial regeneration without hyperplastic changes

Discussion

Several non-carcinogenic chemical and mechanical agents are known to induce regenerative hyperplasia of the bladder urothelium in animals [7]. At the present time, there are no histological characteristics differentiating regenerative hyperplasia from focal hyperplasia in the early carcinogenic phase [2, 7]. This may support the hypothesis that the mitotic rate is increased due to a variety of factors such as carcinogenic agents and that is also increased during reparative processes, including wound healing [9]. At this early phase of proliferation, there is no difference between regenerative hyperplasia and focal hyperplasia, and only when an increased level of proliferation is maintained will the stem cell population continue to enlarge, associated with the increased likelihood of eventual tumor development [9]. Our results showed that there was a close resemblance between the different reactivities of various animal species to carcinogenic stimulation [11] and their reactions to non-carcinogenic stimulations such as transurethral fulguration. Regeneration of the rat bladder urothelium was exophytic, whereas in the other species it was planophytic.

This may suggest that there is a regulatory mechanism in the urothelium which is common to urothelial prolifera-

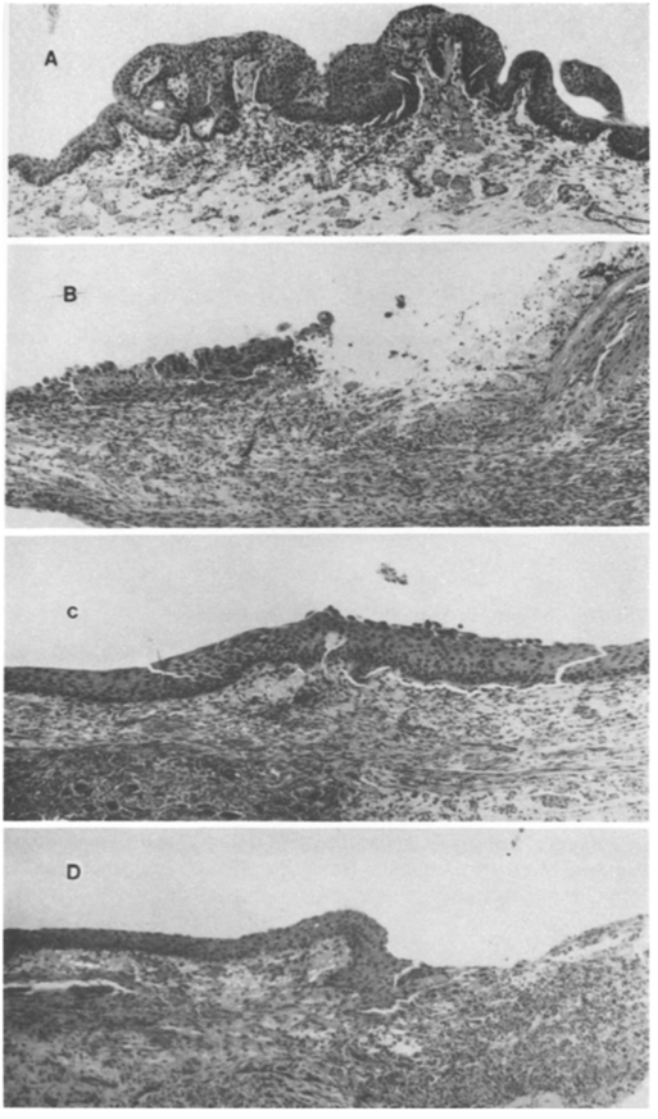

Fig. 1A–D. Histological studies of each species on Day 7. Hematoxylin and eosin stain (x150). **A**, Rat bladder urothelium. Figure shows a papilloma. **B**, Mouse bladder urothelium. Figure shows simple hyperplasia. **C**, Hamster bladder urothelium. Figure shows simple hyperplasia. **D**, Guinea pig bladder urothleium, which does not increase in thickness. *Right halves* of Figs. B and D show fulguration injuries of the urothelium

tions induced by both carcinogenic and non-carcinogenic stimulations, although differing between animal species. The difference in this regulatory mechanism might be responsible for the morphological differences in bladder carcinogenesis between animal species. To extend this hypothesis beyond observation by light microscopy of urothelial regeneration, studies should be performed to determine whether or not the rate of cell division corresponds to the degree of hyperplasia, including carcinogen-administered groups.

There are 3 clinical types of bladder cancer: papillary carcinoma, sessile or solid carcinoma, and carcinoma in situ. These types of cancer often coexist simultaneously and/or sequentially.

Thus, in the human bladder urothelium, several regulatory factors may coexist, i.e., qualitative abnormality in the epithelium for non-papillary cancer and proliferative or quantitative capability for papillary cancer.

Our results may offer a clue for solution of the problem of polymorphophysics of human bladder cancers.

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