Early Changes Caused by N-Butyl-N-(4-hydroxybutyl)nitrosamine in the Bladder Epithelium of Different Animal Species

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Summary

Early changes in the bladder epithelium of male rats, mice, hamsters, and guinea pigs induced by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) were examined.

Light microscopy showed that the normal mucosal epithelium of the distended bladder of rats, mice, hamsters, and guinea pigs was 2 to 4 cells thick. However, on treatment with BBN, the bladder epithelium increased diffusely to 5- to 8-cell thickness in rats and mice and 4- to 6-cell thickness in hamsters. It did not increase in thickness in guinea pigs. On treatment with BBN for 8 weeks, focal hyperplasia developed in the bladder epithelium of rats and mice, but the bladder epithelium of hamsters and guinea pigs did not change. Histological examination showed that the areas of focal hyperplasia in rats and mice had reduced alkaline phosphatase activity and increased nonspecific esterase and β-glucuronidase activities. These activities did not change in the bladder epithelium of hamsters and guinea pigs treated with BBN.

Scanning electron microscopy showed that normally large, polygonal cells were present in the bladder epithelium of all these species. The luminal surface of the bladder of rats, mice, hamsters, and guinea pigs appeared to be covered with a network of fine ridges. After BBN treatment for 4 weeks, microvilli were seen on surface cells in rats, mice, and hamsters but not in guinea pigs. Cells in areas of focal hyperplasia in rats and mice appeared irregularly arranged and their surface was uneven with numerous microvilli.

By transmission electron microscopy, the cells on the surface of the normal bladder appeared clear, with numerous fusiform vesicles and free ribosomes in rats and mice but with few fusiform vesicles in hamsters and guinea pigs. On administration of BBN, the number of fusiform vesicles decreased in rats and mice but did not change in hamsters and guinea pigs. Areas of focal hyperplasia in rats and mice consisted of both dark and clear cells with few fusiform vesicles, and the cells had enlarged nuclei and nucleoli.

Thus, the difference in the susceptibilities of different animal species to urinary bladder carcinogenesis induced by BBN may be closely related to species differences in the changes induced in its fine structure.

Introduction

Animal models of urinary bladder cancer have been studied by several investigators (3, 4, 13). The nitroso compound BBN² has been shown to have a selective carcinogenic action on the urinary bladder in several animal species (2, 5, 7, 12), and there have been many reports on the histology, histogenesis, and ultrastructure of the induced cancers (1, 8, 9). A clear relation was found between the period required for carcinogenesis and the dose of carcinogen in rats. Recently, histological studies have shown differences in the susceptibilities of the urinary bladder epithelium of different animal species to carcinogenesis induced by carcinogens including BBN (7). Several investigators have suggested that focal hyperplasia of the bladder epithelium is a preneoplastic change (1, 8, 9, 13). The present paper reports on the relationship between early changes of the urinary bladder epithelium in different animal species and differences in the susceptibilities of these species to BBN carcinogenesis.

Materials and Methods

Male Wistar rats (Nihon Rat Co., Ltd., Saitama, Japan), ICR mice, golden Syrian hamsters, and guinea pigs (Nippon Animal Co., Osaka, Japan) (8 weeks old) were used. Rats, mice, and hamsters were given commercial stock diet MF and guinea pigs were given stock diet RC4 (Oriental Yeast Co., Tokyo, Japan). The carcinogen BBN (Izumi Chemical Co., Yokohama, Japan) was given as a 0.05% solution in the drinking water, as described previously (8). The animals were killed for histological, histochemical, and scanning or transmission electron microscopic observations 0, 4, 8, or 12 weeks after the beginning of the experiment. The methods used for light microscopy, histochemical examination, and transmission and scanning electron microscopy have been described previously (8).

Results

Light Microscopy. Normal bladder epithelium of rats usually consists of 3 layers of transitional cells. Its histological appearance is also essentially the same in mice, hamsters, and guinea pigs. On BBN administration for 4 weeks the bladder epithelium increases diffusely to a thickness of 5 to 8 cells in rats and mice and to a thickness of 4 to 6 cells in...
hamsters. Cell irregularities and mitotic figures are not seen in the areas of diffuse cell growth. The bladder epithelium of guinea pigs does not increase in thickness on treatment with BBN. On treatment with BBN for 8 weeks, focal hyperplasia developed in the bladder of rats and mice (Figs. 1 and 2). In areas of focal hyperplasia the epithelium was 8 or 10 cells thick. These areas were of 3 different histological types: polyoid, papillary, and solid. Proliferation of vascular stroma was rare in areas of focal hyperplasia, and few cell irregularities and no mitotic figures were seen. After 12 weeks, diffuse cell growth of the bladder epithelium was also seen in hamsters (Fig. 3), but no remarkable changes were seen in guinea pigs (Fig. 4). Papillomatous growth was seen in a few rats but not in mice.

**Histology.** The normal bladder cells of rats and mice had activities of alkaline and acid phosphatase, β-glucuronidase, monamine oxidase, and glucose-6-phosphatase, but no nonspecific esterase activity. In areas of focal hyperplasia some cells of the bladder epithelium showed low activities of acid phosphatase and β-glucuronidase and high activity of nonspecific esterase, while other cells showed normal activities. The activity of nonspecific esterase in hamsters and guinea pigs did not change with BBN treatment.

**Scanning Electron Microscopy.** The epithelium on the luminal surface of the normal bladder of rats, mice, hamsters, and guinea pigs appeared to have a network of fine ridges. These were formed by regularly arranged cells that were mainly hexagonal but, in places, pentagonal or rhombic in shape with no microvilli or cilia (Fig. 5). On BBN treatment, microvilli were seen on the surface in rats, mice, and hamsters after 4 weeks but not in guinea pigs even after 12 weeks (Fig. 6). After 8 or 12 weeks, the elevated ridges along cell junctions in areas of focal hyperplasia in rats and mice appeared wider and, in places, hemispherical cells with microvilli protruded from the surface. In some areas a cobblestone arrangement of cells with many long, ringlet-shaped microvilli was seen and the cell borders were sunken into the surface (Figs. 7 and 8).

**Transmission Electron Microscopy.** In general, there were 3 layers of epithelial cells of the urinary bladder in rats, mice, hamsters, and guinea pigs: large, well-differentiated cells on the luminal surface and smaller, less differentiated cells forming the basal and intermediate layers. The luminal membrane of surface cells of the normal bladder epithelium had a 3-layer unit membrane, named an asymmetrical unit membrane, and the cells contained a number of fusiform vesicles. These cells had no microvilli. In rats, mice, and hamsters, there were many fusiform vesicles in the surface cells (Figs. 9 and 10), but in guinea pigs there were only a few (Fig. 11). On BBN treatment for 4 or 8 weeks the number of the vesicles decreased in rats, mice, and hamsters (Fig. 12), but it did not change in guinea pigs. The cells in areas of focal hyperplasia in rats and mice still contained fusiform vesicles, but the papilloma cells in rats did not.

**Discussion**

In this work, early changes in the urinary bladder epithelium of rats, mice, hamsters, and guinea pigs induced by BBN were examined by several techniques.

It has been suggested that focal hyperplasia of the bladder epithelium is a precancerous lesion. Recently, we have found differences in the sensitivities of different animal species to BBN (7). In confirmation of this, in this work administration of BBN was found to induce focal hyperplasia in rats and mice but not in hamsters or guinea pigs.

Scanning and transmission electron microscopy showed differences in the numbers of fusiform vesicles and formation of microvilli in the surface cells of the urinary bladder epithelium as early changes induced by BBN in the epithelium in several animals.

The function and morphology of fusiform vesicles and the asymmetric unit membrane were examined by Koss (10). During carcinogenesis induced by BBN, it was observed that the effects of the carcinogen on the bladder epithelium are important in urinary bladder carcinogenesis (8). The present results suggest that the large number of vesicles in the epithelium of rats, mice, and hamsters results in a large area of luminal surface in contact with carcinogen, whereas the small number of vesicles in guinea pig epithelium results in a relatively smaller area of exposed surface. Recently, it has been reported that the urinary concentration of BBN metabolites may be important in induction of bladder cancer in animals (11). Thus the number of fusiform vesicles may be related to the area of the surface of the urinary bladder epithelium in contact with BBN or its metabolites.

Focal hyperplasia as a neoplastic change in urinary bladder cancer in rats is a reversible or irreversible change, as in liver (6, 8, 9). In the present investigations early changes in focal hyperplasia were found to be the development of microvilli and the loss of fusiform vesicles in the surface epithelium of the bladder. Bladder cancer should be treated during early stages such as during neoplastic changes so that these neoplastic areas can revert to normal bladder epithelium. Further analysis of intrinsic and extrinsic factors may provide an effective method for inhibiting tumor growth.

**References**

8. Ito, N., Hiasa, Y., Toyoshima, K., Okajima, E., Kamamoto, Y., Makiura,
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Fig. 1. Two areas of focal hyperplasia induced by BBN in a rat bladder epithelium. H & E, x 250.

Fig. 2. An area of focal hyperplasia in the epithelium of mouse bladder induced by BBN. The nonfocal hyperplastic area shows diffuse cell growth. H & E, x 250.

Fig. 3. Diffuse cell growth in the epithelium of a hamster bladder induced by BBN. Cell infiltration into the subepithelial layers is seen. H & E, x 250.

Fig. 4. Lack of change in the bladder epithelium of a guinea pig after treatment with BBN for 12 weeks. H & E, x 250.

Fig. 5. Scanning electron microscopy of the normal bladder of a guinea pig, showing large polygonal surface cells with a network of fine ridges. The cells have no microvilli. x 470.

Fig. 6. Scanning electron microscopy of the epithelium of mouse bladder after treatment with BBN for 8 weeks, showing many microvilli. x 6300.

Fig. 7. Scanning electron microscopy of an area of focal hyperplasia of the bladder epithelium in a rat induced by BBN for 4 weeks. Protruding hemispherical cells are seen with microvilli and wide elevated ridges along the cell junctions. x 800.

Fig. 8. Scanning electron microscopy at higher magnification of an area of focal hyperplasia in rat bladder. Many microvilli are seen. x 4900.

Fig. 9. Transmission electron microscopy of surface epithelial cells of normal rat bladder, showing the many fusiform vesicles. x 19,600.

Fig. 10. Transmission electron microscopy of normal surface cells of the urinary bladder in a mouse, showing many fusiform vesicles in the cytoplasm. x 5500.

Fig. 11. Transmission electron microscopy of normal surface cells of the urinary bladder of normal guinea pig, showing the small number of fusiform vesicles. x 9100.

Fig. 12. Transmission electron microscopy of surface cells of the bladder of a BBN-treated rat. Only a few microvilli and fusiform vesicles are seen. x 6000.
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