Fluorescence Microscopic Study on Absorption of Adriamycin through the Rat Bladder Epithelium

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Nakagawa, S., Kojima, M., Nakao, M. and Watanabe, H. Fluorescence Microscopic Study on Absorption of Adriamycin through the Rat Bladder Epithelium. Tohoku J. exp. Med., 1987, 153 (3), 227-232——The absorption of adriamycin (ADM) through the rat bladder epithelium was investigated histologically with a fluorescence microscope. ADM permeated through the epithelium into the lamina propria or the inner layer of the muscle within 15 min after instillation, but no further infiltration into the deeper part of the bladder wall was observed thereafter. In addition, fluorescence histological evidence on the uptake of ADM by the endothelial cells of the blood vessels in the lamina propria suggested the systemic diffusion of ADM to the whole body via the circulatory system of the blood. The method reported in the present study is considered to be promising in checking the penetrability of ADM both in the normal bladder wall and bladder tumors.—adriamycin; bladder instillation; fluorescence microscopy; rat

Intravesical adriamycin (ADM) instillation is widely used as a topical therapy for superficial bladder tumors, and its clinical usefulness is also confirmed by many groups (Banks et al. 1977; Mishina et al. 1979).

Considerable attention has been focussed on the absorption of ADM through the bladder epithelium, and several approaches using radioimmunoassay (Van Vunakis et al. 1974), fluorescence assay (Pavone-Macaluso et al. 1976) or 3H-ADM (Nijima 1978; Jacobi and Kurth 1980) have been developed. These reports, however, were concerned exclusively with a quantitation of ADM in the plasma, urine or bladder tissue, and offered no data regarding the absorption in histological aspects.

In this article, we dealt with the absorption of ADM in the rat bladder by means of histology with a fluorescence microscope.
MATERIALS AND METHODS

A total of 30 male Wistar rats weighting 180–200 g (7 weeks old) were used in the present study.

The abdominal cavity of each animal, anesthetized with intraperitoneal injection of sodium pentobarbital (35 mg/kg), was opened and the bilateral ureter and the urethra were ligated. A polyethylene tube (PE50) was then inserted through the apex and was fixed to the bladder wall. Following complete evacuation of urine, 0.3 mg of ADM dissolved in 0.3 ml of sterilized saline (pH 5.5–6.0) was injected through the tube. After leaving for a period of 5 (Group A), 10 (Group B), 15 (Group C), 30 (Group D) and 60 min (Group E; 5 rats each per group), the bladder was washed out with saline, and then with a fixative solution of 4% paraformaldehyde in phosphate buffer (pH 7.4). In the controls (Group F, 5 rats), saline was left instead of ADM for 60 min.

The bladder removed from the animals was further fixed in the same solution, and was embedded in paraffin according to the usual steps. Each section (5 μm thick) was mounted on a glass slide, cleared with xylene and was covered with Entellan. The specimens were observed under an Olympus fluorescence microscope fitted with an epillumination system composed of an excitation filter of B (IF490), a dichroic mirror DM 500+0515, and a barrier filter of 0530. Alternate slices were then stained with hematoxylin-eosin.

RESULTS

Under illumination with 490 nm emission light, the ADM absorbed through the bladder epithelium was readily distinguished as a substance emanating an orange fluorescence from the surrounding tissue, in which the muscular and the connective tissues emitted a weak green autofluorescence. The ADM was confined exclusively within the cellular nuclei, and specific orange fluorescence was scarcely visible either in cytoplasm or the intercellular space. In the control case, orange fluorescence was not observed in the bladder tissue.

At 5 min after the initiation of instillation (i.e., Group A), the localization of ADM was confined almost within the bladder epithelium through on occasions the underlying superficial layer of the lamina propria was labelled with ADM. No orange fluorescence was observed in the deep layer of the lamina propria or the muscle layer (Fig. 1).

At 10 min (Group B), ADM was observed in the superficial layer of the lamina propria in addition to the epithelium. The cell components scattered in the superficial region of the deep layer of the lamina propria often contained orange fluorescent ADM and the endothelial cells of the blood vessels distributed there.

Fig. 1. Fluorescence micrograph of the bladder wall 5 min after the instillation of ADM. ×160
Fig. 2. Fluorescence micrograph of the bladder wall 10 min after the instillation of ADM. ×160
Fig. 3. Fluorescence micrograph of the bladder wall 15 min after the instillation of ADM. ×160
Fig. 4. Fluorescence micrograph of the bladder wall 60 min after the instillation of ADM. ×160
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were also labelled with ADM (Fig. 2).

When the instillation continued for 15 min (Group C), specific orange fluorescence extended to the deep region of the deep layer of the lamina propria and was occasionally observed in the inner layer of the muscle layer adjacent to the deep layer of the lamina propria (Fig. 3).

With longer duration of administration, i.e., 30 min (Group D) to 60 min (Group E), the distribution pattern of ADM recognized was almost the same as was at 15 min (i.e., Group C). ADM was scarcely observed in the middle and the outer layers of the muscle layer (Fig. 4).

Fluorescence microscopic findings of each group are summarized in Table 1.

**DISCUSSION**

ADM has a characteristic emission of a fluorescence with peaks at 560 and 582 nm when excited with a 488 nm beam (Bachur 1975). This distinctive feature made it possible to distinguish ADM at the histological level by use of a fluorescence microscope.

ADM is one of the most useful anticancer drugs in the instillation therapy for superficial bladder tumors (Mishina et al. 1981). When intravesically instilled, ADM is absorbed by tumor cells and then exerts its cytotoxic effects by intercalating with DNA (Zunino et al. 1972). It is, therefore, of particular importance to examine how ADM permeates in bladder tumors.

Fundamental experiments on the absorption through the bladder epithelium (Mishina et al. 1986) suggested that the permeability of ADM was restricted within the mucosa and the lamina propria. In the present study, it was demonstrated clearly that ADM permeated deeply into the lamina propria or the inner layer of the muscle within 15 min of instillation and thereafter ADM was confined mostly to that region. These data may agree with the conclusion stated above. Anticancer drugs permeated into the lamina propria were considered to cause both general and local side-effects (Mishina et al. 1986). The fluorescence histological findings that orange fluorescent ADM was observed in the endothelial cells of the

<table>
<thead>
<tr>
<th>Bladder wall</th>
<th>Time after 5</th>
<th>Time after 10</th>
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<tr>
<td></td>
<td>No. of rat</td>
<td>No. of rat</td>
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<tr>
<td></td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Epithelium</td>
<td>+ + + + +</td>
<td>+ + + + +</td>
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<tr>
<td>Superficial layer of lamina propria</td>
<td>± - ± - -</td>
<td>+ + + + +</td>
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<tr>
<td>Deep layer of lamina propria</td>
<td>- - - - -</td>
<td>- + ± + ±</td>
</tr>
<tr>
<td>Inner layer of muscle layer</td>
<td>- - - - -</td>
<td>± - - - -</td>
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<tr>
<td>Outer layer of muscle layer</td>
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Blood vessels in the lamina propria 10 min after instillation may offer the evidence for the systemic diffusion of ADM to the whole body via the circulatory system of the blood.

The fluorescence histological method used in the present study is harmless and is readily applicable to check the penetrability of ADM in human bladder tumors. This method proves to be a promising approach to the development of an ideal instillation therapy effective on bladder tumor cells without side-effects.

References

