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Dimethylsulfoxide enhances the absorption of chemotherapeutic drug instilled into the bladder

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Summary. We examined the effect of dimethylsulfoxide (DMSO) on the absorption of a chemotherapeutic drug instilled into the bladder. Female Wistar rats with bladder tumors underwent intravesical instillation of normal saline (S group) or 50% DMSO (D group) prior to the administration of pirarubicin (tetrahydropyranyl-Adriamycin). The absorption of pirarubicin was estimated histologically by observing its fluorescence. In the S group, fluorescence of pirarubicin was observed only in the epithelial layer of normal or hyperplastic regions and in the cells of superficial layers of the tumor. In the D group fluorescence was observed in the entire bladder wall of normal or hyperplastic regions and extended to deeper regions of the tumors than in the S group. These findings indicate enhancement of the absorption of pirarubicin by pretreatment with DMSO.

Key words: Dimethylsulfoxide – Drug absorption – Intravesical chemotherapy

Intravesical chemotherapy has been used in the management of superficial bladder carcinoma, either as an adjuvant to surgery or as definitive treatment. The results of this treatment are not always satisfactory, however, several methods of enhancing the antitumor activities of chemotherapeutic drugs have been devised. Attempts have been made to increase the efficacy of this treatment by promoting the penetration of drugs into urothelial cells by the addition of Tween 80 [6] or urokinase [3].

Dimethylsulfoxide (DMSO) is an industrial solvent that has been in use since the nineteenth century, and it is known to have various pharmacological activities, such as enhancement of membrane permeability [2]. The aim of the present study was to determine whether pretreatment with DMSO would modify the absorption of a chemotherapeutic drug instilled into the bladder. Pirarubicin (tetrahydropyranyl-Adriamycin produced by Meiji Seika, Tokyo, Japan) was used as the chemotherapeutic drug in this study. The absorption of pirarubicin was estimated by observing the fluorescence of this drug in the same way as for Adriamycin [5].

Materials and methods

Fifteen female Wistar rats (4 weeks old) were given 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN, produced by Tokyo Kasei, Tokyo, Japan) in the drinking water for 10 weeks, then given tap water only for 15 weeks. The rats were anesthetized by i.p. injection of pentobarbital (40 mg/kg) and underwent transurethral insertion of catheter (3 F).

Prior to intravesical chemotherapy 1 ml normal saline (S group, 5 rats) or 50% DMSO (D group, 10 rats) was instilled into the bladder over 1 h. Intravesical administration of pirarubicin (1 mg) in 1 ml water was then performed for 30 min and the bladder was excised. The bladders were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin according to the usual steps. Sections 5 μm thick were made, mounted on glass slides, cleared with xylene and covered with Malinol (Muto Kagaku, Tokyo, Japan). The specimens were observed under a fluorescence microscope (Vanox-T produced by Olympus, Tokyo, Japan) with an excitation filter of B. Four slides per bladder were examined histologically.

Results

Normal epithelia and epithelial hyperplasia

In the S group, fluorescence of pirarubicin was observed in cell nuclei of the whole epithelial layer, but little fluorescence was observed in the submucosa or the muscle layer (Fig. 1). In the D group fluorescence was observed in the entire bladder wall, including the muscle layer (Fig. 2). These findings were observed in all the specimens examined.

Papilloma and carcinoma

In the S group fluorescence was observed only in cells taken from the superficial layers of the tumor (Fig. 3A, B). In 20 S group specimens the limit of absorption was up to

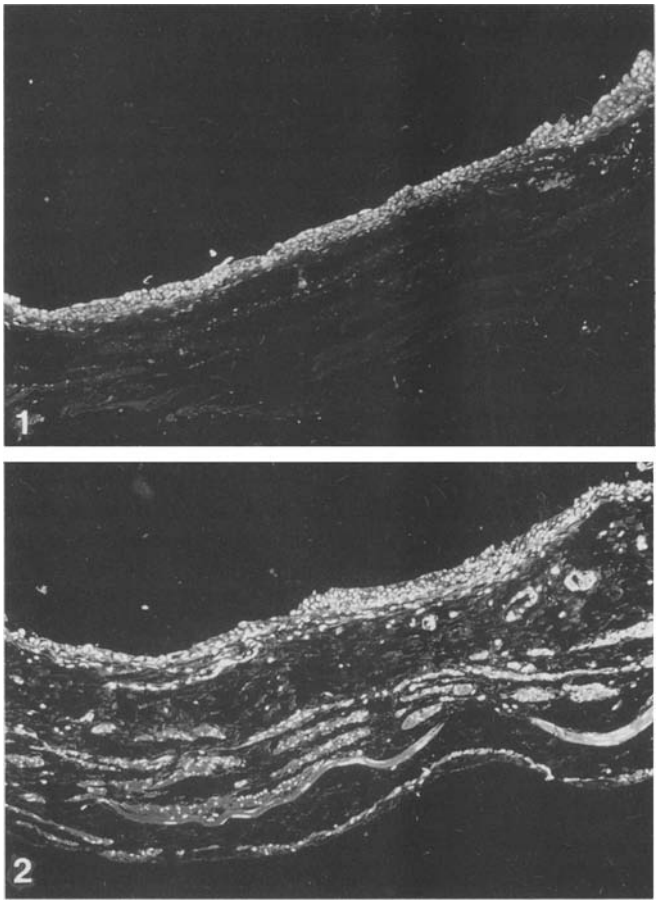

Fig. 1. Normal epithelia and epithelial hyperplasia in the bladder of an S group rat (saline). Fluorescence of pirarubicin is observed in cell nuclei of whole epithelial layer, but little fluorescence is observed in submucosa and muscle layer

Fig. 2. Normal epithelia and epithelial hyperplasia in the bladder of a D group rat (dimethylsulfoxide). Fluorescence of pirarubicin is observed in the entire bladder wall, including the muscle layer

about 10 layers of tumor cells from the luminal surface. In the D group fluorescence was always observed to extend to deeper regions (over 10 layers of tumor cells), and in relatively small tumors all epithelial cells had fluorescene of pirarubicin (Fig. 4A, B).

These findings indicate the enhancement of absorption of pirarubicin by pretreatment with DMSO; the enhancement was seen regardless of any pathological change.

Discussion

The actions of DMSO in enhancing the absorption of various drugs through the skin [8] or the bladder [1, 7] and increasing the cytotoxicity of chemotherapeutic drugs [10, 11] are known. Borzelleca et al. [1] reported that DMSO enhances the movement of sodium salicylate from the bladder. They observed a significant increase in salicylate movement with concentrations of 50% and 100% DMSO, and maximal movement of salicylate occurred within 3 h after instillation. Schoenfeld et al. [7] showed a threefold increase of cisplatin uptake from the bladder in an animal undergoing bladder instillation of cisplatin in 50% DMSO over 1 h. For the present study, 50% concentration and 1-h instillation time of DMSO were chosen on the basis of the data published in these reports. A 50% concentration of DMSO is also used clinically for the treatment of interstitial cystitis [9] and bladder amyloidosis [12] by intravesical instillation. A 30-min instillation time was selected for pirarubicin, because a fluorescence microscopic study on the absorption of Adriamycin through the rat bladder showed maximal absorption with 30-min instillation [5] and the molecular character of pirarubicin is similar to that of Adriamycin. Studies with different concentrations or instillation times of DMSO and pirarubicin have not been performed.

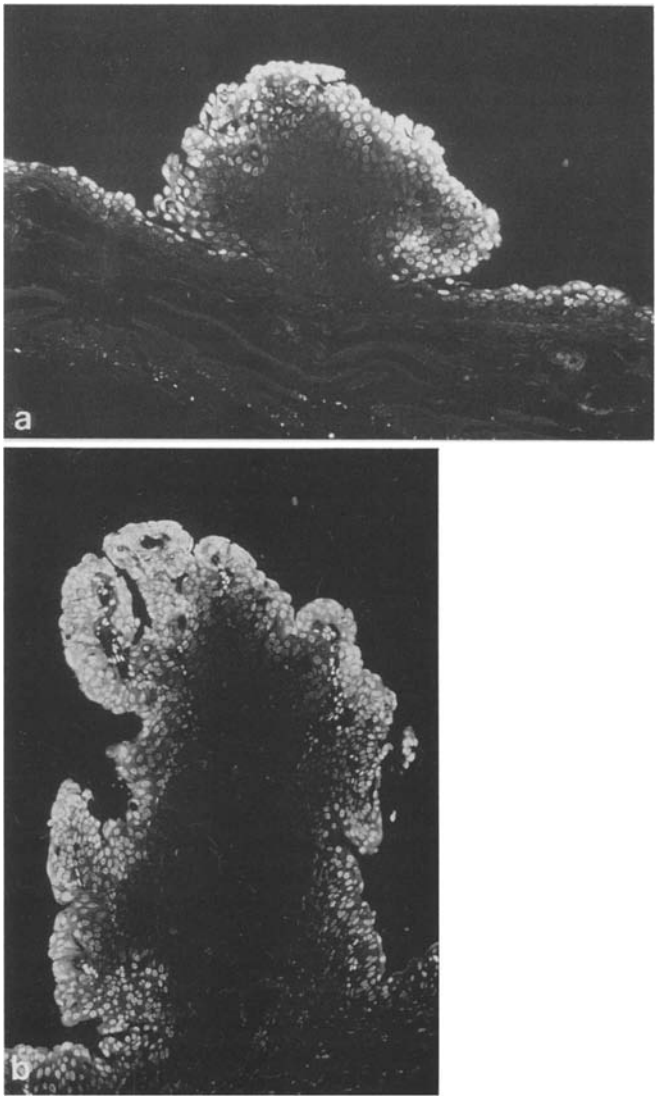

Fig. 3. a Papilloma and **b** carcinoma in the bladder of S group rats. Fluorescence was observed only in the cells of superficial layers of the tumor.

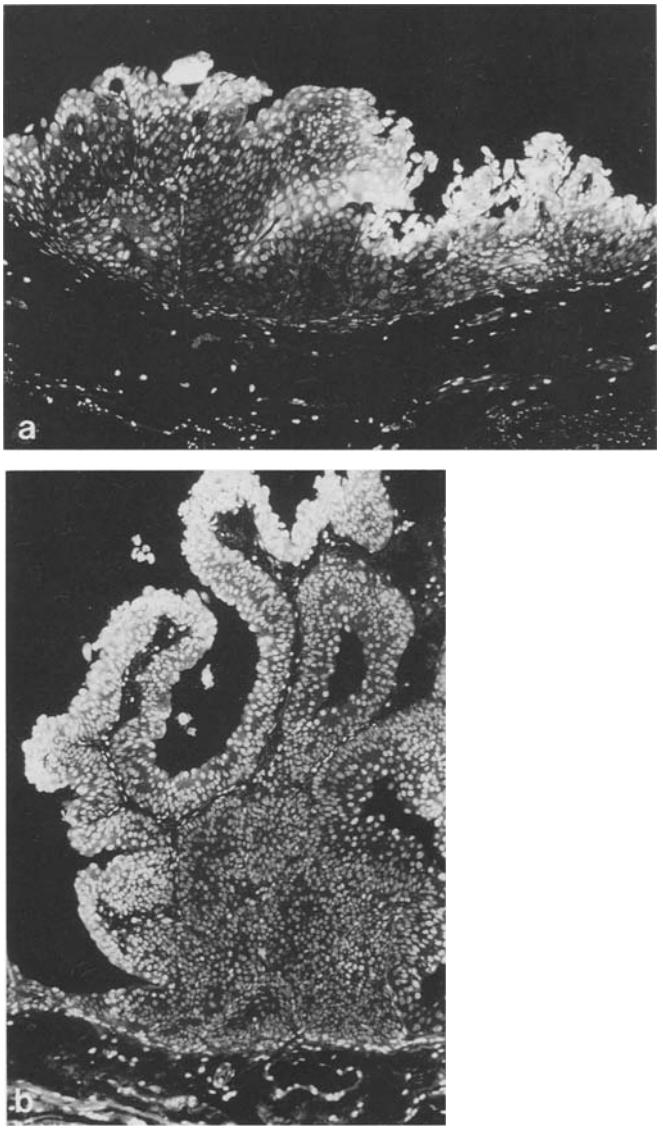

Fig. 4. **a** Papilloma and **b** carcinoma in the bladder of D group rats. Fluorescence was observed throughout the tumor close to the submucosal layer

The results of present study suggest that pretreatment with DMSO is useful in augmenting the penetration of pirarubicin into the bladder wall and the bladder tumor of rat. These data indicate the possibility of increasing the efficacy of intravesical chemotherapy by pretreatment with DMSO.

The question of the DMSO effect before a chemotherapeutic instillation into the bladder is of great interest. The absorption of Adriamycin depends on passive transport [4], and drug penetration into the bladder wall or bladder tumor is expected to be modified by some barrier of urothelium. The barrier of urothelium against drug penetration is constructed by the cell membrane and the junction of adjacent cells. It is suspected that DMSO modifies these of barrier structures. Borzelleca et al. [1] showed cytoplasmic granulation, shrunken appearance or absence of epithelial cells following treatment with DMSO. They reported that the enhanced transfer may result from an effect of DMSO on the integrity of the bladder mucosa, presumably brought about by modification of a barrier to drug movement. Turnbull [13] indicated that the destruction of epithelial tight junction by cyclophosphamide enhances the permeability of urothelium. A study in which we observed the permeability of lanthanum with the electron microscope also suggested the destruction of tight junctions by treatment with DMSO (data not shown). Walker et al. [14] reported that chemotherapy combined with DMSO did not enhance cytotoxicity in vitro, and denied any enhancement of drug absorption by DMSO. This in vitro study may indicate that DMSO does not play any role in modification of the cell membrane; however, the concentration of DMSO in this study (4%) is much lower than in our study.

In conclusion, we have shown that the permeability of the rat bladder and of bladder tumors is altered by exposure to DMSO, and we suspect that the mechanism of DMSO's action as a penetrant carrier is an effect on the barrier of the urothelium, such as destruction of tight junctions. Intravesical chemotherapy is usually used for the prophylaxis and treatment of superficial bladder carcinomas, but is not indicated for the treatment of invasive ones. The marked enhancement of drug absorption by DMSO seen in the present study suggests that intravesical chemotherapy combined with DMSO may be available for the treatment of invasive bladder carcinomas.

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