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Organ Distribution of Adriamycin® After Intravesical Instillation With or Without Tween 80 in the Rat

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Summary. The absorption of Adriamycin® (ADM) into the systemic circulation and into different organs including the urinary bladder was investigated in the rat after intravesical instillation of ADM with or without a surface active detergent, Tween 80. The postinstillation plasma concentration of ADM increased significantly with increasing dose of the drug. Even though the leakage of ADM into the systemic circulation and into extravesical organs in general was slight a significant increase was observed after addition of Tween 80. The uptake of ADM into the urinary bladder wall was also significantly enhanced by Tween 80 thus the role of this agent in conjunction with intravesical chemotherapy should be further investigated.

Key words: Intravesical chemotherapy – systemic absorption – Adriamycin® – Tween 80

Introduction

The therapeutic potential of intravesical chemotherapy is well documented in superficial, exophytic tumours as well as in flat carcinoma in situ [2, 4, 5, 21]. Although treatment failure may occur during or after previous successful chemotherapy it is not uncommon to achieve a remission by changing to another intravesical cytostatic agent or BCG [3, 13, 19]. Drug resistance rather than suboptimal concentration of the instilled drug is the most likely explanation of a poor response to the therapy. Currently used doses of cytostatic agents guarantee an intraluminal concentration of the drug well above the therapeutic level with respect to the killing effect on the malignant urothelial cells [22]. Poor resorption of the drug into the tumour tissue within the urinary bladder is one possible reason for unsatisfactory efficacy of intravesical chemotherapy. Thus, methods to improve drug penetration into a bladder tumour without causing undesirable absorption of the drug into the systemic circulation and extravesical organs, are of clinical importance.

It is possible to improve the efficacy of one of the most widely used drugs for intravesical chemotherapy, Adriamycin® (ADM) by the addition of Tween 80 [8], a surface detergent, to the instilled solution. Whether the mode of action of Tween 80 is related to enhancement of the uptake of ADM or not is still unclear.

We therefore used an experimental model to estimate the uptake of ADM into the bladder wall after intravesical instillation with or without Tween 80. The distribution of the drug into the systemic circulation and extravesical organs was also measured.

Material and Methods

A total of 97 female rats (mean b.w. 220 g, range 188–256 g) were subjected to intravesical instillation of ADM under general anesthesia with intraperitoneal chloral hydrate (360 mg/kg b.w.) after bilateral ligation of the ureters. The rats received ADM dissolved in sterile water (40 rats) or in sterile water containing 10% Tween 80 (40 rats). The ADM concentrations used were 1 mg/ml, 2 mg/ml, 3 mg/ml and 4 mg/ml, respectively and the instillation volume was 0.4 ml. In the remaining 17 rats isotope labelled ADM (^{14}C-ADM, 91.8 μCi/mg) (kindly supplied by Farmitalia Carlo Erba, Milano, Italy) was used. The instillation volume was 0.4 ml and one fixed concentration of ADM (2 mg/ml) was used. Tween 80 was added to the ADM solution prior to the instillation in 8 of these 17 experiments. Blood samples were obtained by percutaneous puncture of the heart after one hour of instillation. The instillate was recollected by aspiration from the exposed bladder. The urinary bladder, lungs, liver, kidneys, spleen and heart were removed from the 17 animals that had received ^{14}C-ADM and collected in a freezer. The organs were then thawed and allowed to dry at room temperature for 24 h and combusted in a Tri-Carb Sample oxidizer. The radioactive material was collected in 21 ml CarboSorb/Permafluor (8:13 v/v) and counted in a liquid scintillation counter. The ADM concentration in plasma and in the instillate was measured by liquid chromatography [6] in all the experiments.

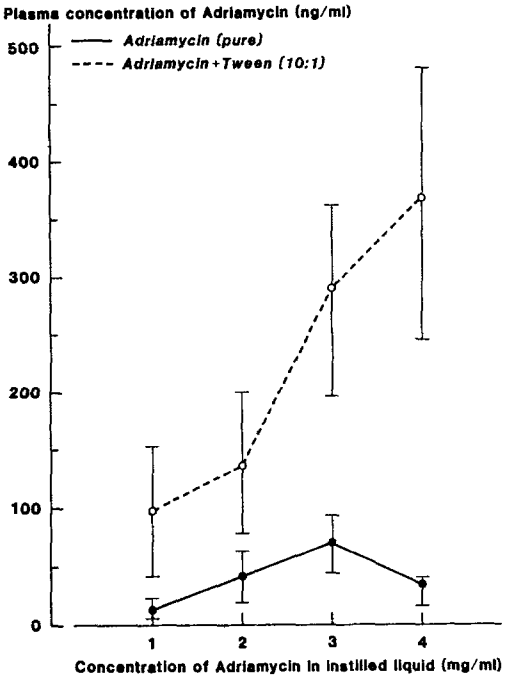

Fig. 1. Plasma concentration of Adriamycin® after 1 h of intravesical instillation in the rat (median values with 95% confidence interval)

Statistical Methods

Medians and confidence intervals were calculated by the Wilcoxon-Tukey method. Two independent samples were compared with the Mann-Whitney U-test. The association was evaluated by the Kendall' s tau test. Friedman two-way analysis of variance by ranks was used for the comparison of several related samples.

Results

The postinstillation plasma concentrations of ADM were significantly increased in the animals receiving instillation with ADM dissolved in 10% Tween 80 as compared to those with ADM dissolved in pure sterile water ($p < 0.02$) (Fig. 1). The plasma concentration increased with increasing dose ($\tau = 0.25$; $p = 0.008$ for ADM; $\tau = 0.49$; $p < 0.001$ for ADM + Tween 80). In general the leakage of ADM into the systemic circulation was discreet. Thus, median plasma values of ADM below 60 ng/ml were recorded for ADM without Tween 80 whereas an increase amounting to about 400 ng/ml or less (median) was found for ADM with Tween 80 even at the maximal concentration of ADM in the instilled solution (4 mg/ml).

The organ distribution pattern of ADM within the two series of rats (i.e. ^{14}C-ADM alone as compared to ADM in Tween 80 solution) ways very similar ($\tau = 0.833$; $p < 0.001$) (Table 1).

The concentration of ^{14}C-ADM was higher in the bladder and liver as compared to heart, spleen and lungs ($p < 0.05$) (Table 1). Less than 1% of the radioactivity instilled into the urinary bladder was found in the heart, spleen, lungs, kidneys and plasma. The uptake of ADM in the urinary bladder wall was almost twice as high after addition of Tween 80 to the instillate as compared with the experiments without Tween 80 (7.3% versus 3.9%; median values) ($p = 0.004$). Also the amount of ^{14}C-ADM recovered in the liver and heart was higher after instillation of ADM in the Tween 80 solution as compared to instillation in sterile water ($p < 0.001$ and $p = 0.03$, respectively).

Table 1. Tissue and plasma concentration of ^{14}C labelled Adriamycin® after 1 h of intravesical instillation in the rat

	^{14}C Adriamycin (% of radioactivity)	^{14}C Adriamycin + Tween 80 (% of radioactivity)
	Median value	Median value
Left kidney	0.26 (0.17–0.48)[a]	0.57 (0.33–0.75)[a]
Right kidney	0.28 (0.19–0.50)	0.61 (0.43–0.77)
Liver	1.59 (1.18–1.95)	4.13 (3.03–5.09)
Spleen	0.12 (0.07–0.18)	0.21 (0.14–0.27)
Heart	0.10 (0.07–0.17)	0.20 (0.15–0.24)
Left lung	0.10 (0.07–0.17)	0.21 (0.15–0.25)
Right lung	0.13 (0.08–0.21)	0.25 (0.17–0.28)
Urinary bladder	3.86 (2.96–5.40)	7.30 (5.30–10.90)
Plasma	0.52 (0.37–0.68)	0.58 (0.40–0.78)

[a] Figures in brackets represent 95% confidence interval

Discussion

Previous experimental studies have shown that the absorption of ADM from the urinary bladder into the systemic circulation was increased with increasing volume and concentration of ADM in the instillate [10], though the maximum plasma level of ADM was still moderate (below 350 ng/ml) even after addition of Tween 80 to the instilled ADM solution. The absorption of ADM after intravesical instillation in humans even shortly after transurethral resection is also very low and actually negligible compared to the plasma concentration of ADM registered after intravenous administration [14].

The present study showed that the concentration of ADM measured in a variety of parenchymatous organs after one hour of intravesical instillation with or without Tween 80 was low in relation to the total amount of the instilled drug. As regards the myocardium the amount of ADM was less than 0.2% of the total amount of the instilled drug. Hence, side effects such as cardiotoxicity that may jeopardize the therapy after intravenous administration of ADM are most unlikely to occur after intravesical instillation. Even in the case of long-term treatment with ADM the risk of absorption and accumulation of the drug into different extravesical organs seems to be negligible.

Clinical studies on ADM have proved the efficacy of the drug as given for therapeutic as well as prophylactic purpose

[18, 20]. Attempts to increase the tumouricidal effect of the drug after addition of urokinase [15] have been unsuccessful. In contrast, Tween 80 has been found to improve the response to intravesical ADM in bladder cancer patients having developed resistance to the drug [8]. Though the mechanism for this action of the substance is unclear it is noteworthy that Tween 80 has been shown to enhance significantly the cytotoxic activity of ADM in vitro [16]. In the present animal study we proved a significantly increased ADM concentration in the bladder wall after addition of Tween 80 to the instilled liquid. Logically, clinical response to topical chemotherapy is dependent both on drug penetration into the tumour and to the intrinsic chemosensitivity of the tumour cells to the drug [17, 22]. In vitro studies suggest that treatment failures with ADM might be due to inadequate tumour perfusion [22]. Bessman et al. [1] have claimed that the normal rat bladder is impermeable to instilled ADM since they failed to detect the drug in plasma or bladder wall after 6 h of instillation. However, our studies as well as those by others [9] prove that absorption of ADM instilled into the rat bladder occurs though only to a minor extent even after addition of Tween 80 to the instilled liquid. Clinical studies on absorption of ADM in patients with bladder carcinoma using tritium labelled ADM have shown the uptake of the drug to be almost as high in papillary tumour lesions as in normal appearing surrounding urothelium, whereas the uptake in the base of the tuours was only about 15–20% as compared to that in the exophytic part of them [11].

By adjusting the volume of the instilled drug in relation to the bladder capacity, it is possible to increase the contact area between the drug and the tumour tissue in patients with bladder carcinoma [7]. This regimen should promote penetration of topically administered cytostatic agents into and through the urothelial lining. As confirmed by the present study the uptake of ADM in the wall of the urinrary bladder of the rat can also be enhanced by the addition of Tween 80 to the instilled solution. The mode of action of Tween 80 seems to be promotion of the resorption of ADM, though enhancement of the tumouricidal effect of ADM might also be involved. It is concluded that the influence of Tween 80 on the efficacy of intravesical chemotherapy with ADM should be evaluated in controlled clinical trials in patients with superficial bladder carcinoma.

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