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Permeability of the Rat Bladder to Cisplatinum Under Different Conditions: Comparison with Mitomycin C and Adriamycin

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Summary. In 50 female Sprague-Dawley rats the absorption of Cisplatinum through the bladder wall was studied. Under different conditions, including cystitis and electrocoagulation of the bladder mucosa, the absorption was low and the measureable serum concentrations did not exceed 2.64 μg/ml. The influence of Tween 80 – a non-ionic surfactant – on the absorption is investigated. The results are compared with those found for Adriamycin and Mitomycin C under identical conditions.

Key words: Vesical absorption, Cisplatinum, Mitomycin C, Adriamycin, Tween 80, Rats.

Introduction

In the prophylaxis of recurrent bladder tumours by intravesical instillations, an improvement in results might be expected if the first instillation followed as soon as possible after transurethral resection (TUR). The problem of increased absorption of the instilled drug due to cystitis and electrocoagulation has not been clarified. In earlier investigations we demonstrated that the absorption of Mitomycin C [6] and Adriamycin (ADM) [5] is increased in acute cystitis, after electrocoagulation or in the presence of the detergent Tween 80, but in general the measured serum concentrations were still low.

Cisplatinum (Pt), mainly used in systemic chemotherapy of malignant disease, has now been introduced in clinical studies – among these an EORTC-study – for the instillation prophylaxis of recurrent bladder tumours.

The present investigation posed the following questions:

1. Are measurable serum concentrations of Cis-Platinum present in the rat after instillation into the rat bladder?
2. Is absorption influenced by cystitis, electrocoagulation or the instilled volume?
3. Is absorption influenced by the presence of Tween 80?

Materials and Methods

Fifty female Sprague Dawley rats weighing between 200 and 250 g were divided into five groups of 10 animals. Under Evipan-Na anaesthesia the ureters were ligated through a lower midline abdominal incision and Cisplatinum Platnoxan[1] was instilled through a urethral catheter. Two prevent urine leakage around the catheter, the urethra was ligated. Blood samples were taken from the inferior vena cava at 10, 30, 60, 120 and 180 min after instillation. The volume was replaced by saline solution. Cisplatinum was measured by automated flameless atomic spectometry [16] (HGA 76, Perkin Elmer, Überlingen, FRG) with a modified programme containing flow temperature stages and mini flow condition in the atomisation step. Pt-standards diluted with Pt-free serum were used for calibration. Quality control of the method was performed using four different concentrations of Pt serum solution and met all standards of clinical-chemical analysis. Only values measured between controls within the 2-fold standard deviation were accepted. $CV_{series} \leq 1.5\%$; $CV_{day\ to\ day} - 5\%$; carry over $< 10^{-3}$; Sensitivity: 0.05 μg/ml.

The five groups of animals were treated as follows:

Group 1

0.4 ml of Cisplatinum solution (2.5 mg Cisplatinum/ml normal saline solution) was instilled into the bladder.

Group 2

0.8 ml of Cisplatinum solution (1.25 mg Cisplatinum/ml) was instilled into the bladder.

Group 3

0.4 ml of a solution of 2.5 mg Cis-Platinum/ml 10% Tween 80 was instilled into the bladder.

* Parts of this study are included in a thesis

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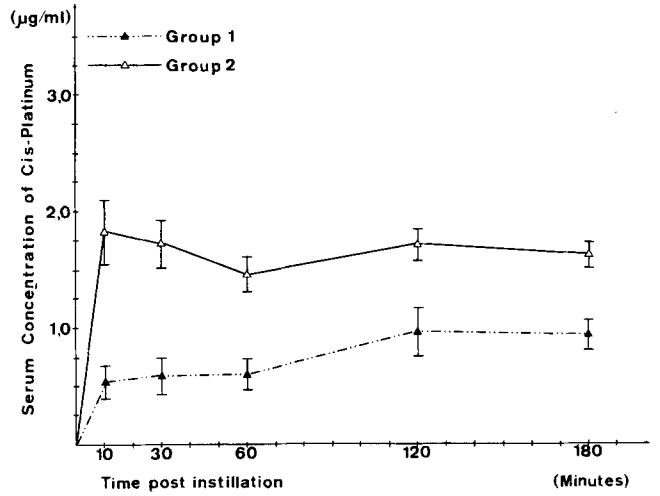

Fig. 1. Serum concentrations of Cisplatinum after vesical instillation in groups 1 and 2

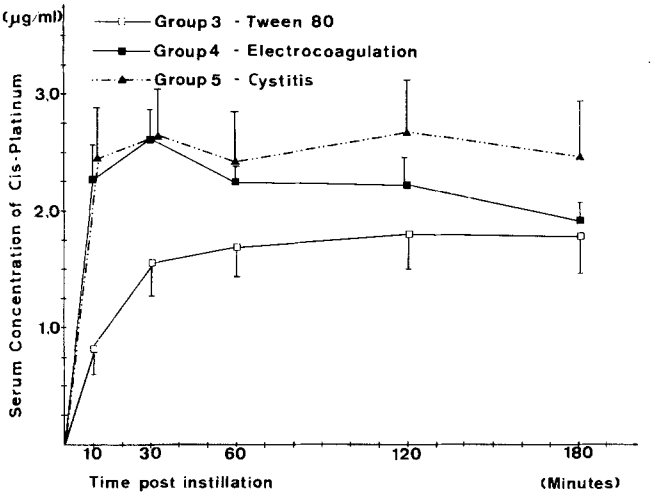

Fig. 2. Serum concentrations of Cisplatinum after vesical instillation in group 3, 4 and 5

Group 4

The bladder was exposed through a lower midline incision and the bladder dome opened. 30%–40% of the bladder surface area was coagulated by a ball electrode of 1.5 mm in diameter (Siemens Erlangen, FRG). The bladder was closed with a continuous suture and a tissue adhesive. 0.4 ml of Cisplatinum solution (concentration as in group 1) was instilled immediately afterwards.

Group 5

0.4 ml Xylene (Merck Darmstadt, FRG) was instilled into the bladder and left for 10 min. Thereafter it was removed and the bladder washed out thoroughly with normal saline solution. Then a suspension of *E. coli* containing 10^8 bacteria/ml was instilled. Urethral resistance was increased by placing a metal clip around the urethra in order to impair bladder emtying. Twenty-four hours later Cis-Platinum solution was instilled (volume and concentration as in group 1).

In all groups the animals were sacrificed at the end of 3 h. The bladders were distended with a 5% formalin solution and excised. Histological sections were stained with haematoxylin-eosin, PAS-reagent, and Hale stain. Statistical analysis was done with the help of a modified rank sum test [20].

Results

The serum concentrations of Cisplatinum in groups 1 and 2 are shown Fig. 1, and those of groups 3, 4 and 5 in Fig. 2. Absorption reached a mean maximum of 0.94 µg/ml after 2 h in group 1. In group 2 the absorption rate was higher, the mean maximum value of 1.83 µg/ml was reached after 10 min, with a slight decrease in the curve after 3 h to 1.62 µg/ml.

In group 3, no significant difference could be detected after 10 min as compared to group 1, but all the other serum concentrations were significantly higher, with a mean maximum value of 1.84 µg/ml after 2 h. In group 4, the mean maximum value of 2.63 µg/ml was measured after 30 min. Serum concentrations decreased to 1.90 µg/ml after 3 h. In group 5, the mean maximum value of 2.64 µg/ml was reached after 30 min and decreased to 2.43 µg/ml after 3 h.

Statistics

The serum concentrations of groups 1 and 2 are significantly different ($p < 0.05$). The values of group 3 are significantly different from those of group 1. The values in group 4 are significantly different from those of groups 1, 2 and 3. The values of group 5 are significantly different from those of groups 1 and 2.

Histology

As in our previous studies [5, 6], no abnormalities could be found in groups 1 and 2. In group 3, no increased staining of mucopolysaccharides could be found, contrary to Bridges' investigations [1] and confirming our previous results with ADM [5]. In group 4, complete loss of the epithelium occurred after electrocoagulation and the lamina propria was covered by an exudate and inflammatory cells. In group 5, severe, acute ulcerative cystitis with multiple bacterial colonies was found.

Discussion

Cisplatinum is stable in a 0.9% saline solution for at least 24 h at room temperature [7], but not in a 5% dextrose solution [4]. Mariani and co-workers [13] and Hincal and co-workers [9] investigated the compatibility and stability of Cisplatinum in different parenteral solutions including benzyl-alcohol and parabens. They were able to show the

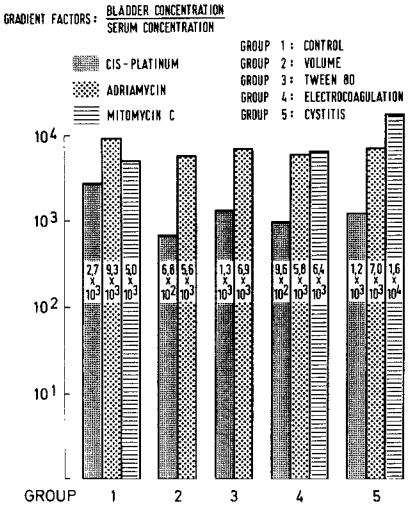

Fig. 3. Comparison of the vesicoplasmatic gradient factors $\left(\frac{\text{bladder concentration}}{\text{serum concentration}}\right)$ after vesical instillation of Cisplatinum, Adriamycin, Mitomycin C

stability of Cisplatinum solution under different conditions including the presence of dextrose or mannitol. The influence of Tween 80 on the stability of Cisplatinum solutions has not been investigated to date.

After instillation of Cisplatinum solution in the normal rat bladder (group 1), a low resorption could be noted with a vesicoplasmatic gradient factor of 2,700. Under the same experimental conditions the gradient factors were 9,300 for Adriamycin and 5,000 for Mitomycin C. In Fig. 3 the gradient factors for Adriamycin and Mitomycin C as determined previously [5, 6] are shown as compared to Cisplatinum in the different groups.

The measurable serum concentrations are increased after distension of the bladder due to an increased filling volume. The gradient factor is 680 in comparison to 5,600 for Adriamycin. The cause of the increased absorption is the increased circulation in the bladder wall during slight dilatation [14], but also the changes of the Asymmetric-Unit-Membrane [10, 11] and changes of the cell surface [19]. In the presence of Tween 80 the absorption curve of Cisplatinum shows a rapid increase of the serum concentration in the first 10–30 min and is different from the absorption curve of Adriamycin under the same conditions. With Adriamycin, very low concentrations are noted in the first 60 min. For Tween 80 a direct influence on the cell membrane was surmised thus increasing the absorption of substances [21, 12, 8, 15]. The potentiation of the drug effect of Actinomycin D [18] and Adriamycin [2, 3, 17] by Tween 80 is well-known. Theoretically, this effect can be caused by changes of membrane permeability for the cytotoxic agent, by changes of the physico-chemical behaviour of the drug, or even by some synergism between the cytotoxic agent and Tween 80.

As Adriamycin and Cisplatinum show different absorption patterns in the presence of Tween 80, a direct action of Tween 80 to the cytotoxic drug itself cannot therefore be excluded. Investigations on Cisplatinum concerning these questions are now in progress.

Conclusion

After instillation of Cisplatinum in the rat bladder under different experimental conditions the serum concentrations are comparable to the concentrations found for ADM and Mitromycin C under identical conditions. If the results presented could be confirmed in humans, they might be the basis for the instillation prophylaxis of recurrent bladder tumours by Cisplatinum. With local administration of Cisplatinum systemic side effects due to significant serum concentration are most unlikely. This would hold true even under conditions such as cystitis or electrocoagulation.

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