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Serum and Tissue Levels of Platinum After Cisplatinum Instillation of the Rat Bladder*

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Summary. The absorption of chemotherapeutics after local application in the urinary bladder is important for optimum therapy. We therefore investigated the absorption of Cisplatinum (Pt) through the bladder wall in 100 female Sprague-Dawley-rats after instillation under different clinical and pharmacological conditions. Only low platinum (Pt)-concentrations of about 1 μg/ml could be detected in the serum using unmodified Cisplatinum solutions for instillation in the rat bladder indicating a rather low absorption rate for Cisplatinum. The extent of the absorption of Cisplatinum through the instilled bladder increased with the instilled volume. The absorption rate also increased after electrocoagulation and during cystitis with a maximum concentration of about 2.64 μg/ml. Correspondingly the vesicoplasmatic gradient factor clearly decreased. On addition of the detergent Tween 80, the serum Pt-concentration increased rapidly during the initial 30 min. Under these conditions Cisplatinum penetrated the bladder wall, about three times more effectively than in the absence of Tween while entering the systemic circulation only at about twice the normal rate without Tween 80. On addition of the polar organic solvent dimethylsulfoxide (DMSO) serum Pt-concentration increased continously during the following 2 to 3 h reaching maximum values of about 13.3 μg/ml. The greatest accumulation of Pt in the bladder tissue (about 1,600 μg Pt/g tissue) was also observed under these conditions. A comparison of the Pt-concentration in serum with that in the bladder wall showed the most favourable relation of high tissue penetration and low serum concentration using Tween 80.

Key words: Cisplatinum instillation – Platinum serum concentration – Platinum tissue concentration

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Introduction

Improved prophylaxis of vesical tumor recurrence is to be expected if intravesical instillation of chemotherapeutics is performed as soon as possible after the transurethral resection of the tumour. Although Cisplatinum (Pt) has mainly been used in systemic tumour chemotherapy, recently clinical studies have reported on the prophylaxis of vesical tumour recurrence by local application to minimize systemic toxicity. However, the problem of increased absorption due to a cystitis or to prior electrocoagulation remained to be clarified. It is also not known to what extent different galenic preparations affect the absorption rate and the enrichment of Cisplatinum in the bladder wall. We have measured the serum-Pt-concentrations after vesical instillation of Cisplatinum in rats and have studied their dependence on cystitis, electrocoagulation and on the instilled volume. Furthermore we examined the influence of the detergent Tween 80, the polar organic solvent dimethylsulfoxide (DMSO) and the proteases pronase and urokinase (occuring physiologically in urine) on platinum absorption.

Experimental

Animals

100 female Sprague-Dawley-rats (200–250 g) were divided into 10 groups (Table 1). After incision of the lower abdomen under hexobarbital anesthesia both ureters were ligated and Cisplatinum Platnoxan® (ASTA-Werke AG, Bielefeld, FRG) was instilled through urethral catheter. The urethra was also ligated to avoid urinary loss along the catheter. Blood samples were drawn from the V. cava inferior 10, 30, 60, 120 and 180 min after instillation. The corresponding volume was substituted with 0,9% saline.

Table 1 shows the experimental conditions for the different groups. All groups received their instillation according to the described procedure. In group 4 the urinary bladder was opened by incision and 30–40% of its inner surface was coagulated by a 1.5 mm diameter spherical electrode (Siemens, Erlangen, FRG). Subsequently the bladder was closed by a continuous suture and the use

Table 1. Description of the experimental groups

No.	Groups
1	– 0.4 ml cis-platin (2.5 mg/ml saline)
2	– 0.8 ml cis-platin (1.25 mg/ml saline)
3	– 0.4 ml cis-platin (2.5 mg/ml, 10% tween 80)
4	– 0.4 ml cis-platin (as >1<) elektrocoagulation
5	– 0.4 ml cis-platin (as >1<) cystitis
6	– 0.4 ml cis-platin (2.5 mg/ml, 50% dmso)
7	– 0.4 ml cis-platin (2.5 mg/ml) + urokinase (833 U/ml)
8	– 0.4 ml cis-platin (2.5 mg/ml) + pronase (0.5 g/ml)

of a tissue sticker. Cisplatinum was instilled immediately thereafter In group 5 after an initial instillation of 0.4 ml xylene (Merck, Darmstadt, FRG) for 10 min and subsequent washing of the bladder with 0.9% saline a suspension of *E. coli* (10^8 bacteria/ml) was instilled for 24 h. To reduce urinary excretion the urethral resistance was increased by use of a metal clip around the urethra. After 24 h Cisplatinum was instilled.

In all groups the animals were sacrified after an instillation period of 3 h. The urinary bladders were flushed with a 5% formol-solution, excised and frozen at –70 °C.

Analytical Procedure

Platinum concentrations were measured by atomic absorption spectrometry with uv-background compensation using a graphite furnace and a 4-stage program for temperature control with atomization at 2,650 °C (type HGA 76, Perkin Elmer, Überlingen, FRG). Serum samples were diluted with 10^{-4} n nitric acid.

Completely dried bladder tissue was hydrolized with 65% nitric acid and 20 µl samples were injected directly into the HGA 76. Standards with a comparable matrix were used for calibration. The measurements were automated and declared control samples were analyzed et each 10th position. The detection limit amounted to 60 ng Pt/ml of sample. Linearity ranged from 0.06 to 6 µg/ml in serum and 0.06–4 µg/ml in tissue.

Results

Figure 1 shows the serum-Pt-concentrations of the groups 1 and 2, Fig. 2 those of groups 3, 4 and 5 and Fig. 3 those of groups 6, 7 and 8. In group 1 the mean transvesical absorption reached a maximum of 0.94 µg/ml after 2 h.

In group 2 the absorption was greater and a maximum concentration of 1.83 µg/ml was observed after 10 min. This dropped slightly to 1.62 µg/ml after 3 h.

No significant difference was observed between groups 1 and 3 after 10 min, but thereafter all values were significanlty higher in group 3 with a maximum of 1.84 µg/ml after 2 h.

Group 4 showed a maximum of 2.63 µg/ml after 30 min which then decreased slightly to 1.9 µg/ml after 3 h.

In group 5 the mean maximum concentration amounted to 2.64 µg/ml after 30 min and decreased to 2.43 µg/ml after 3 h.

The addition of DMSO to the platinum solution produced a continous, almost linear steep increase in the mean serum-Pt-concentrations during the first 2 h. The resulting value of 13.3 ± 0.9 µg/ml ($\bar{x}$ ± s.d.) after 3 h is about 12 times higher than in the control group without DMSO.

The pronase- and the urokinase-modifications of the instilled Cisplatinum solutions produced insignificantly higher serum concentrations in comparison with the control group. The only differences were a continous increase during the whole treatment period of 3 h with a mean maximum value of 3 µg/ml.

Table 2 shows the mean ($\bar{x}$ ± s.d.) platinum concent of the rat bladder tissue of groups 1, 3, 6, 7 and 8.

After addition of DMSO (group 6) the resulting tissue concentrations of Pt were increased 7-fold as compared with the control group.

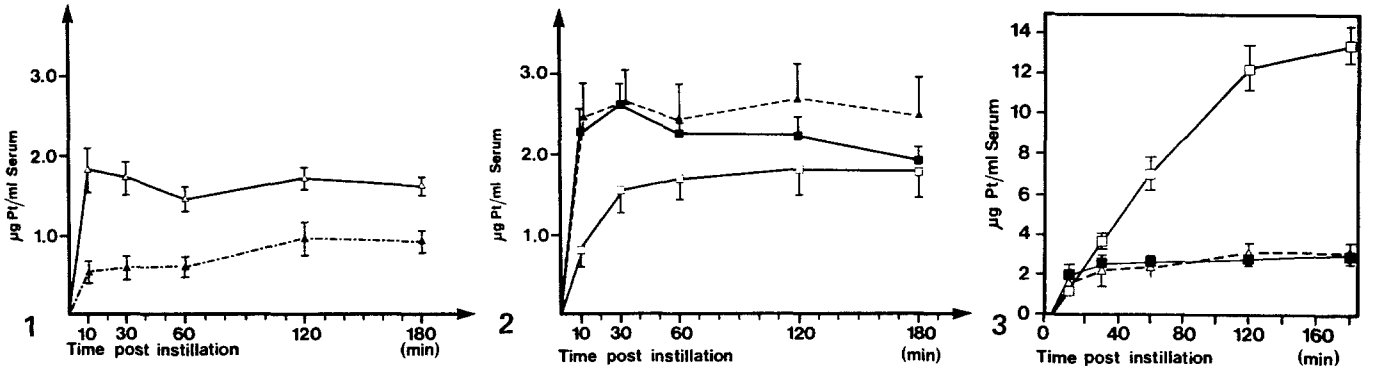

Fig. 1. Time course of the Pt-concentrations in the serum after intravesical instillation for the groups 1 (– – –▲– – –) and 2 (——△——)

Fig. 2. Time course of the Pt-concentrations in the serum after intravesical instillation for the groups 3 (——□——), 4 (——■——) and 5 (– – –▲– – –)

Fig. 3. Time course of the Pt-concentrations in the serum after intravesical instillation for the groups 6 (——□——), 7 (——■——) and 8 (– – –△– – –)

Table 2. Mean Pt-concentrations ($\bar{x}$ ± s.d.) in serum and rat bladder tissue of the groups 1, 3, 6, 7 and 8

Group	µg Pt/g tissue [x ± 1 s.d.]	µg Pt/ml serum [x ± 1 s.d.]
1 (PT)	211 ± 72	1.1 ± 0.3
3 (PT + TWEEN)	674 ± 125	4.0 ± 0.3
6 (PT + DMSO)	1,611 ± 154	13.3 ± 0.9
7 (PT + UK)	488 ± 67	2.9 ± 0.3
8 (PT + PR)	425 ± 98	3.0 ± 0.5

When Tween 80 was added to the instillate serum-Pt-concentrations were about twice those of the control group whereas the tissue content showed a 3 fold greater penetration of platinum into the bladder tissue. The addition of pronase or urokinase caused a rise of serum-Pt of about 50% compared with the control group and increased the amount of Cisplatinum in the tissue by a factor of 2.

Discussion

Cisplatinum is stable in 0.9% saline for at least 24 h at room temperature [5], but not in 5% dextrose [2]. Mariani et al. [8] and Hincal et al. [6] investigated the stability and the compatibility of Cisplatinum in different parenteral solutions and found it to be stable under a variety of conditions. However, the influence of Tween 80 and of the other modifications presented in this paper on the absorption behaviour of Cisplatinum have not previously been investigated.

The instillation of a pure cisplatinum-solution into the normal rat bladder (group 1) is followed by a very poor absorption with a vesicoplasmatic gradient of 2,700. Earlier investigations under similar experimental conditions gave a gradient factor of about 9,300 for adriamycin and 5,000 for mitomycin C [3, 4].

The serum concentrations clearly increased after extension of the bladder wall as a concequence of a higher instillation volume. In this case (group 2), the gradient was 680 for platinum in comparison to 5,600 for adriamycin [4]. Possible causes for this increased absorption rate are an enhanced circulation in the bladder wall due to the slight dilatation, physiological changes of the "asymmetric unit membrane" [9] and changes of the cell surface [12].

In the presence of Tween 80 the serum concentration of Pt rise rapidly during the first 10–30 min and differs from the absorption curve of adriamycin under similar conditions [4], which shows very low blood levels during the first 60 min. A direct influence of Tween 80 on the cell membrane leading to an enhanced absorption of drugs has been proposed [7, 10, 12, 14].

The potentiation of the pharmacological effect of Actinomycin D [13] and Adriamycin [1, 11] by Tween 80 is well known. This effect can be explained by changes of the membrane permeability for the chemotherapuetic or by changes of the physico-chemical properties of the drugs as well as synergism between Tween 80 and the drug. Since adriamycin and cisplatinum show different absorption curves with Tween 80, a direct action of Tween 80 on the chemotherapeutic agent cannot be excluded.

The aim of this study was to obtain further information on the influence of different application modes on the absorption behaviour of Cisplatinum. It is important to derive pharmacological conditions for the instillation procedure which avoid undesired systemic effects of this highly toxic drug, i.e. a maximal concentration in the bladder wall with a minimal transfer of Cisplatinum into the systemic circulation. Therefore it seemed useful to also investigate other additives to the Cisplatinum-solution (DMSO, urokinase, ronase) which cause electron microscopic changes of the urothel [12]. In the case of DMSO the changes of the urothelium are visible by normal light microscopy. The results show that serum-Pt-concentrations are increased by DMSO addition so that although larger amounts of cisplatinum penetrate the bladder wall there will be increased systemic toxicity.

Addition of urokinase or pronase did not lead to increased Pt-absorption and higher serum concentrations. Of the substances tested Tween 80 showed the most favourable relationship of a high penetration into the bladder wall and a low transfer to the systemic circulation, i.e. low systemic toxicity.

In conclusion, our data suggest that undesired systemic effects after instillation of Cisplatinum into the bladder are unlikely since resulting serum concentrations are releatively low even in the case of cystitis or a traumatic condition such as electrocoagulation. The addition of suitable chemical agents, such as Tween 80, should allow a better therapeutic efficacy of Cisplatinum through its higher enrichment in the treated tissue without increasing systemic toxicity. Nevertheless these results should be confirmed by clinical investigations.

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