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Master of Science Thesis

A photograph of the main building of Lund University, showing classical architecture with columns and a pediment.

**Investigation of side effects
during radioactive
(¹⁹¹Pt) cisplatin
treatment in wistar rats**

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Lund University, 2004**

LUJI-RADFYS-EX-6/2004

ABSTRACT

Cisplatin is a cytostatic agent that has been used for more than 30 years in the treatment of a wide spectrum of tumours. The clinical use is limited by its side effects, primarily renal toxicity. An enhanced anti tumour effect has been demonstrated for radioactive (^{191}Pt) cisplatin both in *in vitro* and *in vivo* models by our group.

The purpose of the present study was to compare the side effects from radioactive cisplatin treatment and conventional cisplatin treatment, to see if a treatment gain would be achieved. Rats were given an i.p. bolus injection of 5 mg/kg ^{191}Pt -cisplatin (85 MBq/mg, 113 MBq/mg, or 172 MBq/mg), 5 mg/kg conventional cisplatin, or physiological saline (controls). So far, 69 animals have been included in the study. To compare the different treatment groups the weight of the animals and plasma parameters, including creatinine, bilirubin, ALAT/ASAT and platelet count, was followed for six weeks after drug administration.

A significant increase in creatinine levels was observed three days post administration of radioactive cisplatin as well as for conventional cisplatin treatment. There were no statistically significant difference in creatinine levels between the two cisplatin-treated groups, suggesting that the acute side effects occurring on the kidneys was caused by cisplatin only. No other significant differences were seen between plasma parameters.

The weight of the cisplatin- and radioactive cisplatin-treated animals showed an initial decrease in weight from three days and for a week onward. Thereafter the weight gain followed the same pattern as for the control group. No significant difference was seen between the cisplatin-treated groups.

The absorbed dose from ^{191}Pt -cisplatin to rats was calculated for organs at risk. The highest absorbed dose was estimated to the kidneys (5.1-9.4 Gy) and the liver (1.3-2.3 Gy).

In conclusion, no increase in toxicity for the group treated with radioactive cisplatin was seen, compared with the group given non-radioactive cisplatin.

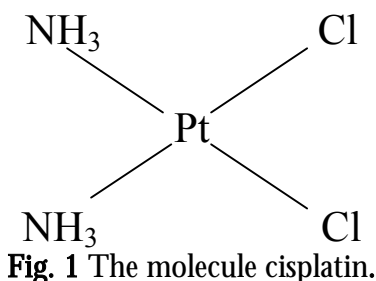
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INTRODUCTION

The anti-tumour activity of cis-dichlorodiammineplatinum (II), or cisplatin, was first discovered by Rosenberg *et al* (1969), and the cytostatic agent has been in clinical use since the middle 1970s. Cisplatin has during the years been used for treatment of various malignancies, such as ovarian, testicular, head-and neck, bladder, oesophageal and small cell lung cancers. However, extensive side effects are associated to the treatment and the use is today limited, but cisplatin is still used with curative intension mainly for treatment of testicular, bladder and head- and neck cancers. The major clinically problem associated to cisplatin treatment is damage to the kidney function, or nephrotoxicity (Osman *et al.*, 2000). Other side effects are ototoxicity, nausea and vomiting (Rosenberg, 1984). Liver toxicity rarely occurs, but may be observed when the drug is administrated at high doses (Zicca *et al.*, 2002).

The cisplatin molecule is seen in Fig. 1. The agent consists of a platinum atom surrounded by two chloride and two ammonia ligands, configured in cis-position. A method has been developed in Malmö to synthesise radioactive cisplatin, from the isotope ^{191}Pt , with high specific activity (Areberg J., 2000a). Since the main target for cisplatin in the cell is believed to be DNA, an enhanced anti-tumour effect is expected from this new combination of chemo- and radiotherapy. Many clinical studies report of a treatment gain when cisplatin is used as a sensitizer in external radiation therapy (Marcu *et al.*, 2003). It is nevertheless assumed that the maximal effect is given when both cisplatin and radiation is given at the same time (Begg, 1990). That condition would be satisfied for radioactive cisplatin. An advantage of radioactive agents is also that it is possible to study the uptake and localisation of the drug by using a gamma camera to detect the radiation emitted from the decay.



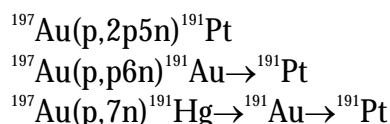
The appeared enhanced effect when combining cisplatin and radiation is evidently dependent on cisplatin concentration, radiation dose and cell type examined (Coughlin and Richmond, 1989). A synergistic interaction for ^{191}Pt -cisplatin has earlier been demonstrated *in vitro*, by our group, in a human cervical carcinoma cell line (ME-180) (Areberg *et al.*, 2000b). Results from a study performed *in vivo* (also by our group) in a mouse model showed that radioactive cisplatin was more effective than non-radioactive cisplatin in retarding tumour growth. In that study, an equal effect was achieved for 5 mg/kg (80 MBq/mg) of ^{191}Pt -cisplatin as for 9 mg/kg conventional, non-radioactive, cisplatin. The concentration of cisplatin could consequently be reduced to roughly 50% by using radioactive cisplatin.

The aim of the present study was to investigate the side effects from treatment with ^{191}Pt -cisplatin compared to common cisplatin treatment, to see if a significant treatment gain would be achieved. In detail, to study the toxicity profile of radioactive cisplatin at organ level in rat, and to estimate the absorbed dose to organs at risk.

MATERIAL AND METHODS

Production of ^{191}Pt

A technique for production of ^{191}Pt has previously been reported (Areberg *et al.*, 1999). The same production method was used in this study. The procedure was performed at the Svedberg Laboratory in Uppsala. Briefly, the gold foil (2 g/cm^2) target was irradiated with 65-75 MeV protons, with an integrated beam current of 50-100 μAh . This resulted in mainly three nuclear reactions:



The first of these three reactions has the highest cross section.

Two days after the irradiation took place, the separation procedure in the target was started. The gold target was heated to a temperature of 1030°C (close to melting) for about one hour, in order to remove the mercury isotopes from the target. Then, 10 mL aqua regia was added to the target, which was evaporated to near dryness, and then dissolved in 5 mL concentrated hydrochloric acid. This process was repeated 2-4 times in order to eliminate the nitric ions. ^{191}Pt was separated from the target solution by 3-4 liquid-liquid extractions of gold, using methyl isobutyl ketone. The final solution withholds an activity of approximately 10 GBq, and was in the form of $^{191}\text{PtCl}_4$.

^{191}Pt decays by electron capture (E.C.) to stable ^{191}Ir with a half-life of 2.802 days (Firestone and Ekström, 2004). The gamma spectrum from ^{191}Pt is dominated by 539 keV (13.7 %), 409 keV (8.0 %), 360 keV (6.0 %), 82 keV (4.9 %) and 172 keV (3.5 %) gamma rays and the characteristic X-rays emitted are between 63 and 74 keV.

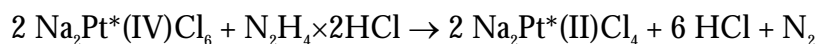
The synthesis of ^{191}Pt - cisplatin

The synthesis was first described by Hoeschele *et al* (1979, 1982) and the main steps of the procedure are described here. In the synthesis of ^{191}Pt -cisplatin, 34 mg of PtCl_4 was used as a carrier. In the formulas, the mixture of non-radioactive platinum and radioactive (^{191}Pt) platinum is denoted Pt^* . The synthesis was also performed without addition of the radioactive substance, i.e. "cold" synthesis.

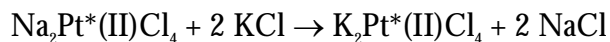
The "hot" synthesis was performed as follows; concentrated HCl was added to the $^{191}\text{Pt(IV)Cl}_4$. The mixture was then evaporated to near dryness on a heating plate. This process was repeated once after addition of 1 ml 1 M HCl. The solution was transferred to a centrifuge tube containing 33.7 mg Pt(IV)Cl_4 . Then, 200 μl of 2 M NaCl was added to the tube, and $\text{Na}_2\text{Pt}^*(\text{IV})\text{Cl}_6$ was formed by the following reaction:



The tube was shaken, and 20 μl of 1M HCl was added and the solution was then centrifuged for 10 min, 4000 rpm. The tube was cooled in ice bath. In the next step, 100 μl of 0.5 M hydrazine hydrochloride, $\text{N}_2\text{H}_4 \times 2\text{HCl}$, was added:

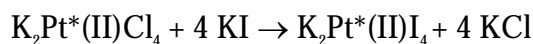


The reduction was performed under argon gas protection, to prevent oxidation. The solution was shaken, and nitrogen gas was produced, visible as small bubbles. The colour of the solution changed from yellow to orange. The tube was then placed in a 40°C water bath for 60 minutes. Next, the solution was cooled to normal room temperature and 105 µl of 2 M KCl was added, under continuous argon protection:



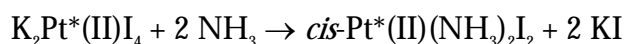
The tube was shaken and cooled in ice. Then 2-3 ml of ice-cooled ethanol (99.5 %) was sturdily added, by using a Pasteur pipette. A white precipitate was formed in a pink solution. The tube was cooled in ice bath for 10 min. After centrifugation (10 min, 4000 rpm), the supernatant was removed using a Pasteur pipette. The centrifugation was repeated after addition of 1 ml ice cooled acetone, to wash the precipitate. The supernatant was removed, and the precipitate was dried under a flow of argon gas.

The precipitate was dissolved in a minimal amount of distilled water, and the tube was shaken. Afterwards 150 µl of 4 M KI was added to form $\text{K}_2\text{Pt}^*(\text{II})\text{I}_4$:

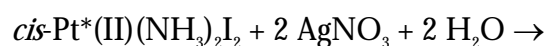


The colour of the solution turned dark red, argon was added, and the tube was closed and left in room temperature for 20 minutes.

In the next step 35 µl of 8 M ammonia, NH_3 , was added to the tube and $\text{cis-Pt}^*(\text{II})(\text{NH}_3)_2\text{I}_2$ was formed by the reaction:



The tube was placed in the 40°C, water bath for two minutes, and a dark brown precipitate was formed. The tube was centrifuged (10 min, 4000 rpm), and the supernatant was separated from the precipitate. The precipitate was rinsed by adding 500 µl of 0.001 M KI, the tube was centrifuged, and the liquid was removed from the mixture. Next step was to add 500 µl of 0.4 M AgNO_3 :

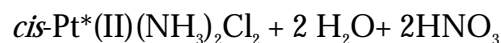


The tube was shaken, and placed in a 60°C water bath for 15 minutes. During this time the tube was shaken at least five times. A white coloured precipitate was formed and separated from the solution by the “standard” centrifugation process. The solution was transferred by a Pasteur pipette to a new centrifuge tube. The precipitate was washed with 500 µl of 0.01 M NaNO_3 , and the solution was added to the new tube.

To eliminate the Ag^+ ions in the solution, 80 µL of 1 M HCl was added and a white precipitate was formed. The tube was placed in a 60°C water bath for 5 minutes, and was then centrifuged (10 min, 4000 rpm). By adding a drop of 0.1 M HCl into the solution, the presence of Ag^+ ions was tested. No further precipitate should be formed.

The supernatant was then transferred to a new centrifuge tube. 100 µl of concentrated HCl was added to this new tube and the following reaction took place:





The tube was then placed in the 60°C water bath for 10 minutes. Cisplatin was formed as yellow crystals.

The tube was centrifuged for 10 min, and the supernatant was removed. 800 µl physiological saline was added to the cisplatin crystals, the tube was placed in boiling water, until the crystals were dissolved. The tube was then cooled to room temperature, and then in ice for 30 min. In this purification step cisplatin was again formed as yellow crystals. The supernatant was then removed, after 10 min centrifugation, and the crystals were dissolved in 10 ml of physiological saline (9 mg/ml).

Quality control

The radionuclide purity of the ^{191}Pt solution was checked using an HPGe (High Pure Germanium)-detector (DSG, Mainz-Hechtsheim, Germany) with a detector volume of 60 cm³ and an energy resolution of 2.5 keV (FWHM) at 1400 keV. The distance to the detector was 2.5 meters and the measuring time was 5 minutes.

High performance liquid chromatography (HPLC) was used to verify the chemical purity, and to determine the concentration of the synthesized cisplatin. As a reference for identification, a commercial cisplatin solution (Platinol™, Bristol-Mayers Squibb) with known concentration was used and the chemical yield of platinum was calculated. The mobile phase in the HPLC system (Thermo Separation Products, San Jose, USA) consisted of a 1.0 mM solution of benzalkonium chloride in water on a LiChroCart™ (Merck, Darmstadt, Germany) RP-18 reversed-phase column (250×4 mm). The flow rate in the HPLC system was 0.7 mL/min and the UV wavelength, used for detection, was 301 nm.

The activity yield, i.e. the ratio of the activity of the final product of the synthesized cisplatin compared to the starting activity of the $^{191}\text{PtCl}_4$ solution, was determined and measured by an activity meter (Capintec, CRC-15R).

Animals

Female wistar rats, weighing approximately 200 g at the start, were included in the study. The animals were provided with standard diet and water *ad libitum*. The study was approved by the Swedish National Board for Laboratory Animals.

Experimental design

The animals were divided into three groups where Group 1 (n=25) was control group, given physiological saline intraperitoneally (i.p.), while Group 2 (n=26) and Group 3 (n=18) were given 5 mg per kg body weight i.p. in a bolus injection of cisplatin (non-radioactive) and ^{191}Pt -cisplatin respectively. The administered activity of radioactive cisplatin was in the range of 80-150 MBq (three different batches). The amount of cisplatin and ^{191}Pt -cisplatin injected was chosen to be the same as in the study performed on nude mice examined to investigate the anti-tumoural effect of radioactive cisplatin (Areberg *et al.*, 2001).

The rats were anaesthetized with 0.1 ml Hypnorm™ (Jenssen animal health, Belgium) and blood samples were withdrawn from a tail vein. Baseline samples were taken 1 week before drug administration, then sampling was continued at 3 days post injection and once a week thereafter during six weeks. To monitor the effect of organs at risk plasma samples were analysed for creatinine, bilirubin, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activity, and platelet count.

The plasma creatinine is a measure of the glomerular filtration rate, which is an approximate method to quantify renal function. A reduced number of platelets in plasma are seen in connection with any type of bone marrow injury. An increased bilirubin level demonstrates liver tissue damage but can also be a result of hemolysis in the blood sample. The ALAT value is also

highly related to liver status, whereas the ASAT value can also be an indicator of muscle- and heart- disease. It is therefore useful to measure the concentration of both enzymes (ALAT/ASAT) if there is uncertainty about the presence of liver injury. (Black *et al.*, 1997).

The weight of the animals was followed during the sampling period, which continued six weeks after administration of the drug. Fifty percent of the animals in each group were dissected three weeks after administration and the remaining fifty percent of animals at six weeks after administration. Tissue samples of liver, kidneys, small intestine and bone marrow were collected. The samples will be analysed for pathology and platinum content as a complement to the blood samples in a later study.

Statistical method

Data were analysed by one-way analysis of variance (ANOVA). The two cisplatin-treated groups were compared separately at each time interval post administration and were also compared with the controls. The level of significance was set at $p < 0.05$. The test is based on the following assumptions (Araï, 1999):

1. The samples are randomly chosen.
2. The populations are gaussian distributed.
3. The populations are thought to have the same magnitude of the variance.

Absorbed dose estimation

In the present study it is of interest to investigate if there is any correlation between the absorbed dose to organs, and increased toxicity from radioactive (^{191}Pt) cisplatin as measured from plasma parameters. The absorbed dose was calculated for organs at risk by using the MIRD-formalism (Medical Internal Radiation Dose, Loewinger *et al.*, 1991). This standard scheme for radiopharmaceutical investigations take into account the uptake in the organs or tissues, each of which may contribute to the radiation absorbed dose in the tissue of interest. The basic equation in the formalism for calculating the mean absorbed dose, \bar{D} , to a *target organ*, (r_k), from a specific *source organ* (r_h) is written as

$$\bar{D}(r_k \leftarrow r_h) = \tilde{A}_h S(r_k \leftarrow r_h), \quad [1]$$

where \tilde{A}_h is the cumulated activity, or all the nuclear transitions that take place within the source organ during the time interval, t , of relevance. Thus,

$$\tilde{A}_h = \int A_h(t) dt, \quad [2]$$

where A_h is the activity in the source.

The cumulated activity is often expressed with the *residence time*, τ_h , which is an effective time that the administered activity, A_0 , resides in the source organ and is defined as

$$\tau_h = \frac{\tilde{A}_h}{A_0}. \quad [3]$$

Equation [1] above can now be modified to

$$\frac{D(r_k)}{A_0} = \sum_h \tau_h S(r_k \leftarrow r_h), \quad [4]$$

which include all source organs that contribute to the absorbed dose to the target organ. The S-value in the equations above is simply a factor that expresses the mean absorbed dose in the target per unit cumulated activity in the source. Further, the S-value cover the mean energy of type i radiation emitted per nuclear transition, denoted Δ_i , which consider the different kinds of radiation emitted and depends of the decay scheme. Only some fraction, ϕ_i , of the emitted energy will be absorbed by the target, which depends on the size, shape and distance between the target and source. The S-value can then be derived by consider the total mean energy emitted per transition:

$$S = \sum_i \Delta_i \frac{\phi_i}{m_k}, \quad [5]$$

where m_k is the target mass.

The absorbed dose, $D(r_k)$, was estimated, by using equation [4] above, for kidneys, liver and bone marrow as target organs. The residence time for these organs was calculated from values of uptake and retention of platinum reported in literature.

Two different approaches were used to estimate the S-value. The first method was to calculate the self absorbed dose by only considering the nonpenetrating (np) radiation, i.e. the electrons. In that case, the source organ is the only target organ, and the absorbed fraction was set to unity. For any other organ the absorbed fraction is set to zero. That is,

$$\begin{aligned} \phi_{np}(r_h \leftarrow r_h) &= 1 & \text{and} \\ \phi_{np}(r_k \leftarrow r_h) &= 0, & k \neq h. \end{aligned} \quad [6]$$

The second method to calculate S-values for the rats utilized Monte Carlo simulations. Hindorf *et al* (to be published) have recently developed a Monte Carlo program, (based on the code EGS4), for small animal geometry to derive S-values. In their work, an analytical mouse model was presented shown in Fig. 2. The mouse organs were approximated by simple geometric calculations, e.g. cylinders, ellipsoids and semi ellipsoids. An advantage of this type of mathematical phantom is that it is easy to modify and change the organs relative size, shape and locations.

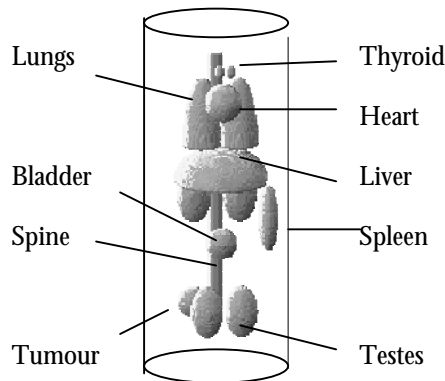


Fig. 2 The mouse model produced by Hindorf *et al* (2003).

The animal model used to calculate S-values in this study was scaled from the mouse model by changing the voxel size, which is equivalent to change the relative organ masses. The “new” phantom was based on mean weights for wistar rats (Table 1) reported in the literature (Melby and Altman, 1976), and was then used as input for the calculation of S-values. The Monte Carlo simulations were done assuming a homogenous activity distribution within the organs and each organ was simulated separately. All emitted photons and electrons from the ^{191}Pt decay (listed in Appendix 1) were included in the simulation of S-values. The cut-off energy, i.e. the limit at which the energy was considered to be locally absorbed, was set to 40 keV for electrons and 10 keV for photons. The simulated S-values were compared with data given for spheres of different masses from the RADAR database (Stabin and Siegel, 2003). Thus, a more accurate estimate of the rat-specific S-values for ^{191}Pt was determined

Table 1
Mean weights used in the calculation of S-values.

	Kidneys	Liver	Total body
Mean weight (g)	1,72	8,52	200
“Scaled” weight (g)	1,49	8,56	240

RESULTS

Production of ^{191}Pt

The radionuclide purity of the $^{191}\text{PtCl}_4$ solution was verified by HPGe-detector measurements. The peaks were identified as ^{191}Pt gamma or ^{191}Ir X-rays. No impurities were found. The activity was in the range of 4-7 GBq at the start of the synthesis.

The synthesis of ^{191}Pt -cisplatin

The chemical purity was analysed by HPLC. The quality control showed a single peak corresponding to that of cisplatin from Platinol™, which was used as a reference. The concentration was calculated from the height of the HPLC-peak, using the reference with known concentration. Chemical characteristics, i.e. activity yield and specific activity, from the batches included in the study are seen in Table 2. Also the chemical yield for platinum is presented in the same table.

Table 2

Chemical yield and the concentration (mg/ml) given in parentheses, activity yield (corrected for disintegration) and specific activity, measured before time of injection for the batches used in the study.

Batch no.	Chemical yield (%) (Concentration)	Activity yield (%)	Specific activity (MBq/mg)
1	35.3 (1.07)	50.0	113.0
2	34.6 (1.05)	44.0	85.0
3	24.4 (0.74)	30.4	172.0
4	21.8 (0.66)	<i>non-radioactive</i>	-
5	31.3 (0.95)	<i>non-radioactive</i>	-
6	20.1 (0.61)	<i>non-radioactive</i>	-
7	34.0 (1.03)	<i>non-radioactive</i>	-

Plasma parameters

Creatinine, ALAT, ASAT, bilirubin and platelet concentrations of plasma in rats at different times after i.p. administration of cisplatin (5 mg/kg), radioactive cisplatin (5 mg/kg) and physiological saline are presented in Table 3. All results are expressed as mean \pm 1 standard deviation (SD). Reference values are given for creatinine levels and platelets in rat (Chang *et al.*, 2002, Sanderson and Phillips, 1981) and for human plasma levels (Rustad, 2004). Also the results from the statistical analysis are seen in the same table.

A significant increase in creatinine concentration was observed for the radioactive cisplatin treated group as well as for the cisplatin treated group with peak value at three days post administration (Fig. 3). No statistically significant difference was seen between the cisplatin and radioactive cisplatin group during the six-week sampling period. The creatinine levels measured for the cisplatin-treated group are in agreement with data reported in the literature. For example, Chang *et al.* (2002) found similar creatinine levels after a single dose of cisplatin in wistar rats.

To study the effect on liver tissue, plasma levels of bilirubin and ALAT/ASAT were measured (Fig. 4,5,6). No significant changes could be observed during the study period, and no difference was seen between the cisplatin groups. These results indicate that radioactive cisplatin has no effect on liver function in rat.

The platelet count at different time intervals after treatment with cisplatin, ^{191}Pt -cisplatin and

saline are seen in Fig. 7. No statistically difference could be observed between the different sample groups, indicating that the bone marrow effect from treatment with radioactive cisplatin is limited.

Table 3

Data from analysis of blood samples collected from rats at different time intervals after administration of the drugs and reference values.

Plasma level, day after injection	Pre- treatment ^c	3 ^c	7 ^c	14 ^c	21 ^c	28 ^c	35 ^c	42 ^c
Creatinine								
(μmol/L)								
Saline	59.0±7.6	61.5±13.5	60.7±12.7	61.2±9.8	65.2±17.0	63.8±16.9	58.8±5.4	64.8±10.6
Cisplatin	57.0±11.3	145.9±75.2 ^b	95.0±43.7 ^b	75.0±18.0 ^a	75.6±15.3	69.8±11.6	66.3±11.3	65.6±14.0
¹⁹¹ Pt cisplatin	51.5±7.6	101.6±63.8 ^b	80.5±24.0 ^b	63.8±13.0	71.6±16.5	70.6±17.4	69.2±13.4	70.9±18.2
n	17-26	17-25	18-25	18-25	18-25	9-12	8-11	9-11
Ref, rat/ Human	53/ 63-105							
ALAT								
(μkat/L)								
Saline	0.78±0.19	0.74±0.17	0.73±0.18	0.87±0.14	0.78±0.24	0.96±0.38	1.03±0.51	0.90±0.25
Cisplatin	0.73±0.10	0.66±0.14	0.67±0.14	0.86±0.18	0.80±0.22	0.72±0.10	0.83±0.15	0.86±0.17
¹⁹¹ Pt cisplatin	0.69±0.11	0.72±0.12	0.59±0.15	0.86±0.16	0.72±0.10	0.82±0.15	0.81±0.10	0.80±0.19
n	17-26	12-25	18-25	18-25	18-25	9-12	8-11	9-11
Ref, human	<0.8							
ASAT								
(μkat/L)								
Saline	1.21±0.31	1.18±0.28	1.26±0.18	1.32±0.29	1.24±0.25	1.42±0.41	1.60±0.58	1.23±0.23
Cisplatin	1.24±0.30	1.33±0.18	1.21±0.22	1.32±0.16	1.22±0.18	1.24±0.11	1.31±0.16	1.22±0.17
¹⁹¹ Pt cisplatin	1.38±0.17	1.45±0.21	1.29±0.27	1.19±0.27	1.28±0.12	1.44±0.23	1.43±0.19	1.32±0.10
n	12-21	12-22	13-22	16-24	16-24	6-11	8-11	9-11
Ref, human	<0.8							
Bilirubin								
(μmol/L)								
Saline	6.8±1.9	6.7±2.0	5.8±2.9	6.0±1.9	5.7±1.6	6.9±2.5	5.4±1.8	7.5±1.8
Cisplatin	6.2±1.9	8.4±2.9	6.6±2.1	6.2±2.1	6.2±1.5	7.3±1.7	6.2±2.1	6.7±2.3
¹⁹¹ Pt cisplatin	6.8±2.0	7.7±2.0	7.8±2.8	6.2±1.9	6.8±2.1	7.9±2.6	7.2±1.9	6.0±2.4
n	12-22	12-23	17-24	18-25	17-25	9-12	7-11	9-11
Ref, human	<20							
Platelet								
(10 ⁹ /L)								
Saline	669±175	-	813±184	826±155	813±213	693±140	793±163	694±157
Cisplatin	721±196	-	704±164	894±220	789±147	653±167	733±124	733±111
¹⁹¹ Pt cisplatin	634±237	-	770±178	871±3	903±162	673±219	686±129	834±212
n	17-26		16-24	18-24	18-23	8-11	9-11	8-11
Ref, rat / human	628-713/ 125-340							

All values are the mean ± 1 SD. Statistical significance between means was performed using one-way analysis of variance (ANOVA).

^aSignificant different, P<0.05, compared to controls.

^bSignificant different, P<0.01, compared to controls.

^cNo significant difference between cisplatin and ¹⁹¹Pt-cisplatin (P>>0.05).

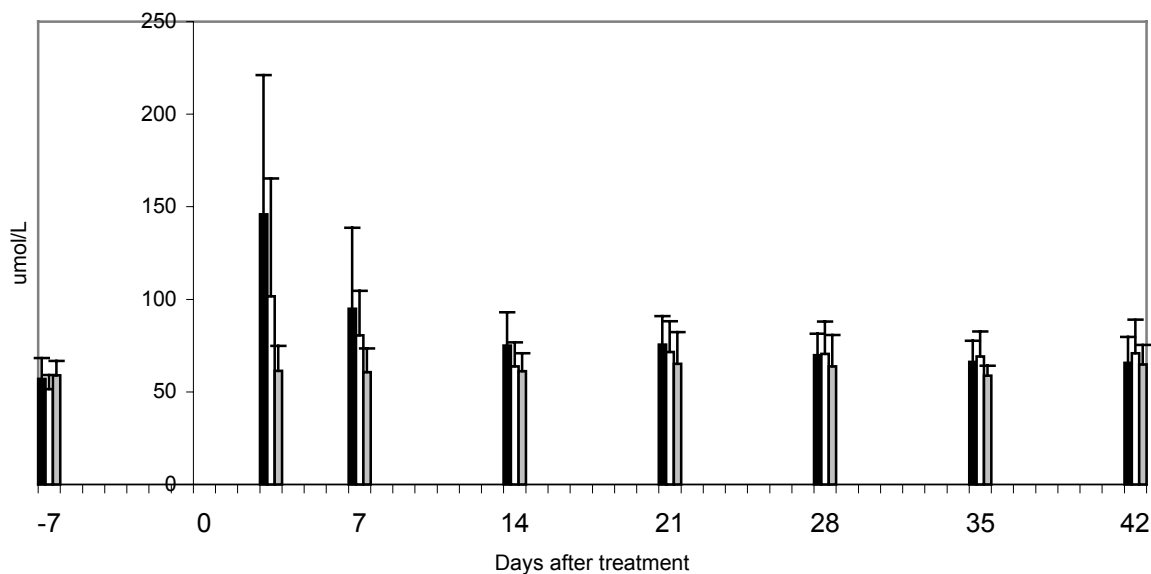


Fig. 3 Creatinin levels (± 1 Standard deviation) at different time points after administration of cisplatin (black bars), ¹⁹¹Pt-cisplatin (white bars) and saline (gray bars) in rat.

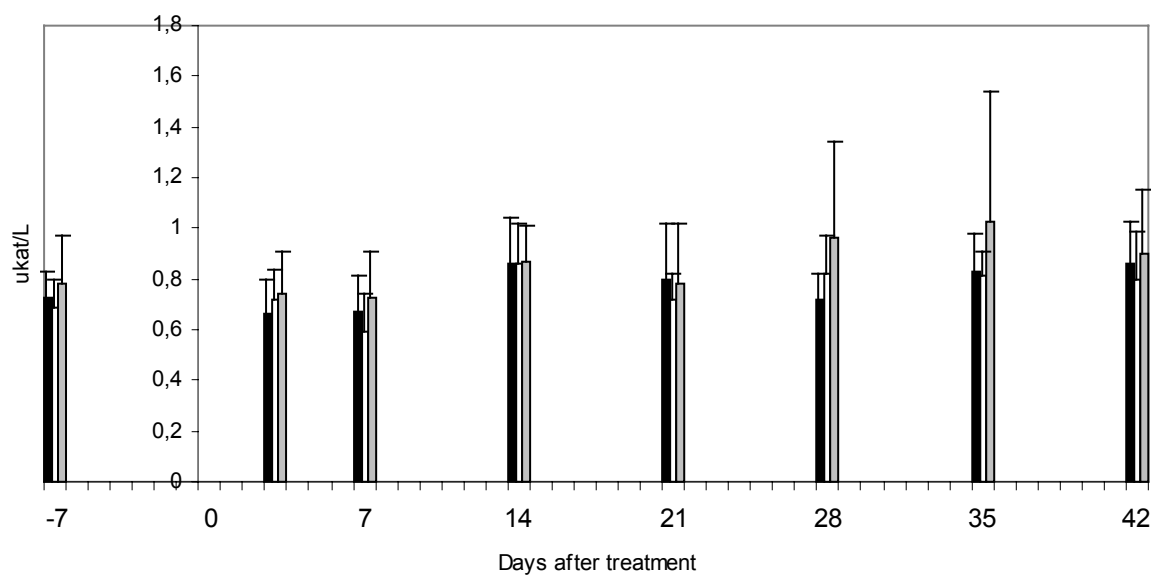


Fig. 4 ALAT levels (± 1 Standard deviation) at different time points after administration of cisplatin (black bars), ¹⁹¹Pt-cisplatin (white bars) and saline (gray bars) in rat.

General toxicity

The animal weight was followed as a general toxicity indicator. A significant difference in weight reduction of rats was seen three days after treatment of radioactive cisplatin and non-radioactive cisplatin, compared to controls, and for a week onward (Fig. 8). Thereafter both cisplatin groups regained weight and continued to follow the same growth pattern as the control group. No significant difference in animal weight was seen after the one-week-samples.

The survival rate, also an important general toxicity indicator, was 25/26 for the cisplatin-treated rats, 18/18 for the ¹⁹¹Pt-cisplatin-treated rats and 24/25 for the control group.

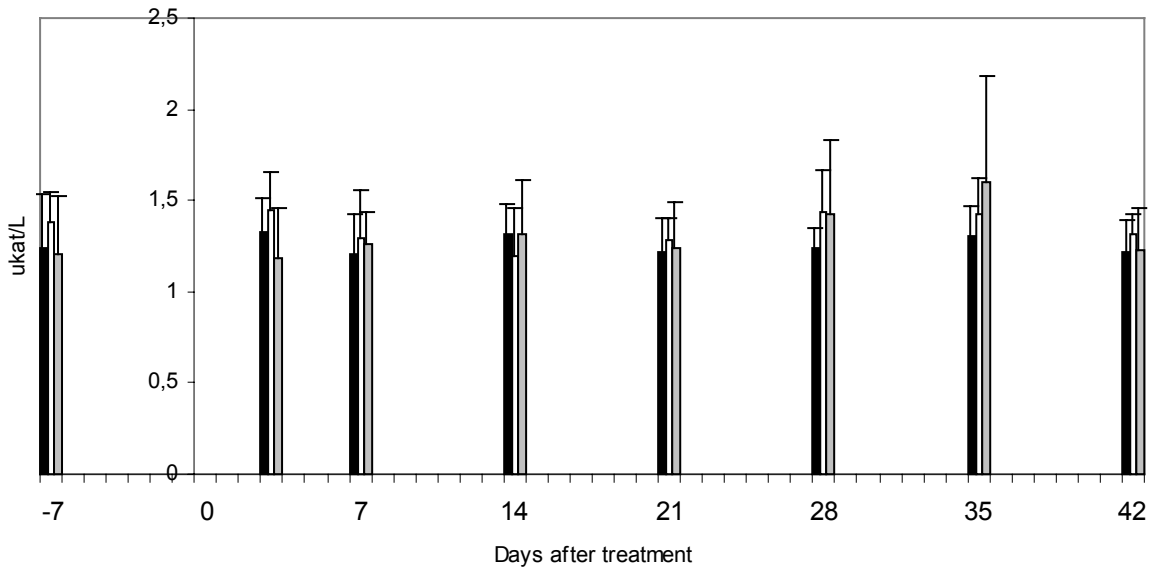


Fig. 5 ASAT levels (± 1 Standard deviation) at different time points after administration of cisplatin (black bars), ¹⁹¹Pt-cisplatin (white bars) and saline (gray bars) in rat.

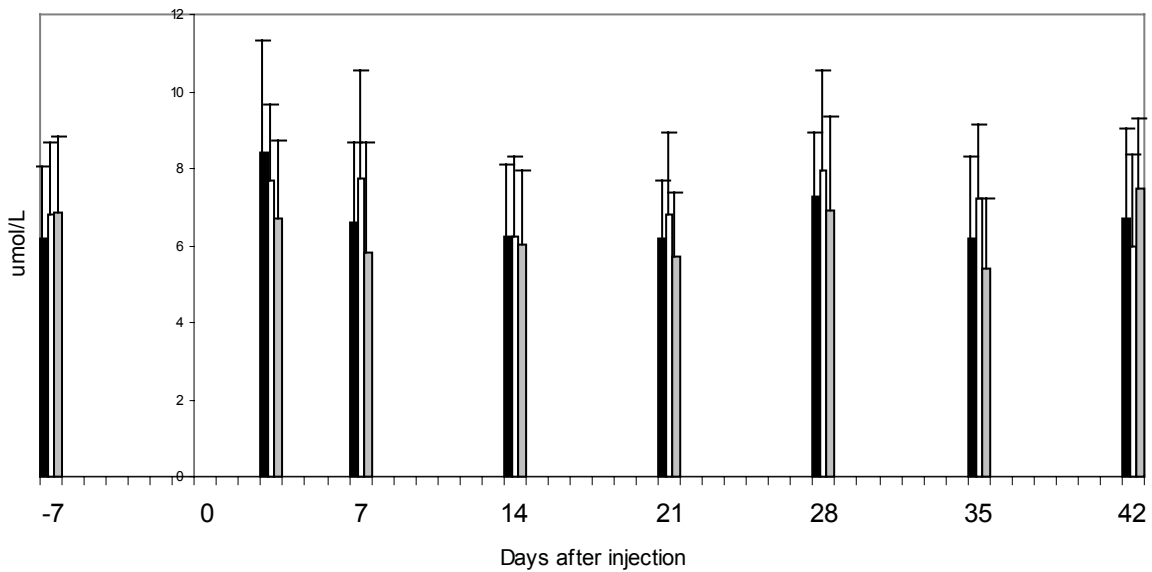


Fig. 6 Bilirubin levels (± 1 Standard deviation) at different time points after administration of cisplatin (black bars), ¹⁹¹Pt-cisplatin (white bars) and saline (gray bars) in rat.

Absorbed dose estimation

The Monte Carlo simulated S-values for the analytical rat model are given in Table 4. These values could be compared with S-values calculated considering only the non-penetrating radiation, which were determined to be $1.24 \cdot 10^{-2}$ mGy/MBq-s and $1.21 \cdot 10^{-2}$ mGy/MBq-s for the left and right kidney respectively, and $1.17 \cdot 10^{-3}$ mGy/MBq-s for the liver. These values are thus smaller than corresponding Monte Carlo-simulated S-values (seen in Table 4) when the target

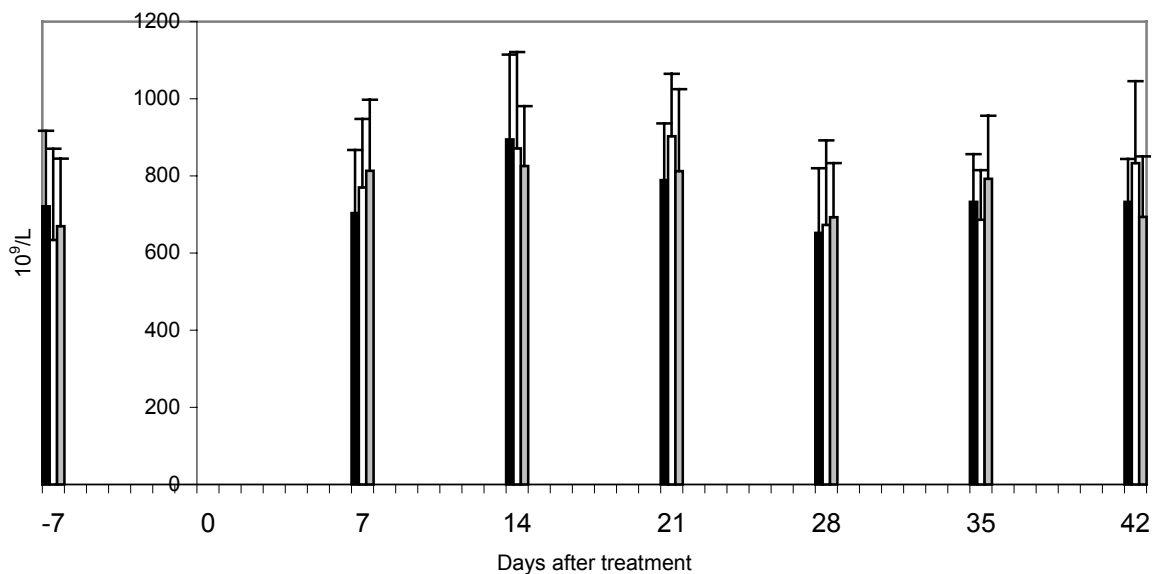


Fig. 7 Platelet count (± 1 Standard deviation) at different time intervals after administration of cisplatin (black bars), ^{191}Pt -cisplatin (white bars) and saline (gray bars) in rat.

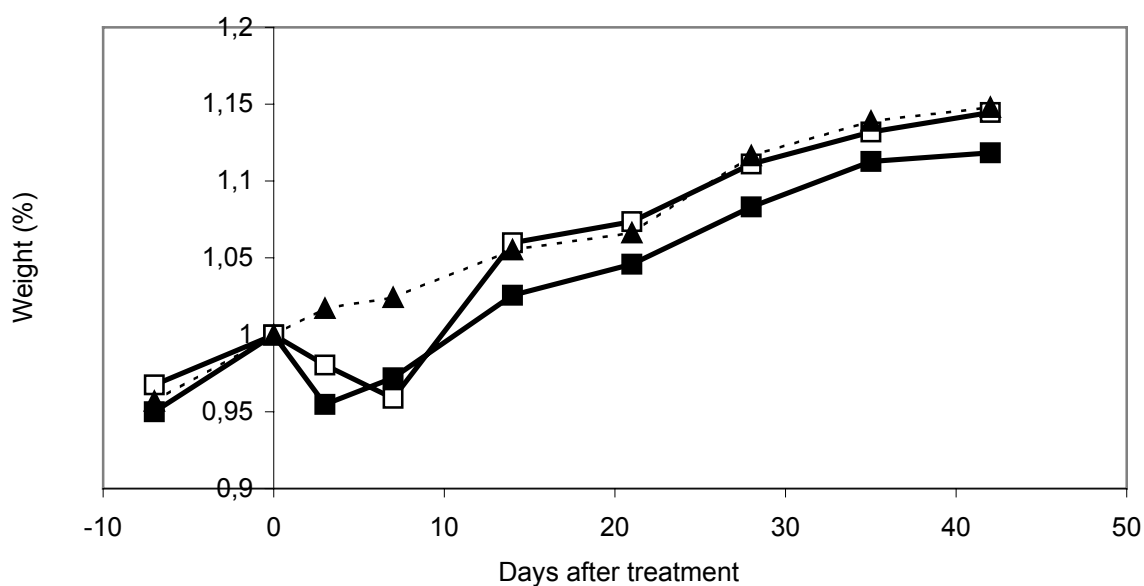


Fig. 8 Rat weight in mean values in relation to starting weight after an i.p. bolus injection of 5 mg/kg cisplatin (—■—), 5 mg/kg ^{191}Pt -cisplatin (—□—) and physiological saline (--▲--) respectively.

organ is the source organ it selves. Since the S-values are strongly dependent on the mass the S-values received from the Monte Carlo method are overestimated, since the masses were approximated during the scaling process. A correction for this overestimation was however performed when the absorbed dose was calculated by using equation [3].

Table 4
Simulated S-values [mGy/MBq-s] for the radionuclide ¹⁹¹Pt to our rat model.

Source / Target	Left kidney	Right kidney	Liver	Remainder
Left kidney	$1.47 \cdot 10^{-2}$	$3.76 \cdot 10^{-5}$	$1.41 \cdot 10^{-5}$	$1.66 \cdot 10^{-5}$
Right kidney	$3.77 \cdot 10^{-5}$	$1.47 \cdot 10^{-2}$	$1.41 \cdot 10^{-5}$	$1.66 \cdot 10^{-5}$
Liver	$1.40 \cdot 10^{-5}$	$1.42 \cdot 10^{-5}$	$1.38 \cdot 10^{-3}$	$1.30 \cdot 10^{-5}$
Remainder	$1.66 \cdot 10^{-5}$	$1.66 \cdot 10^{-5}$	$1.31 \cdot 10^{-5}$	$6.32 \cdot 10^{-5}$

The residence time was calculated from biological distribution data of platinum. The platinum concentration in rat has been reported to be highest in the liver and kidneys (Litterst *et al.*, 1979). Further, moderate concentration of the drug was reported in the skin, muscle, bone and small intestine. These organs were not included as target organs in the present study.

The residence time for kidneys, liver and “remainder of the body” are given in Table 5. The absorbed dose per administrated activity are given in the same table, where the absorbed dose to the bone marrow was approximated to be close to the “remainder of the body”, assuming a homogenous distribution of cisplatin. By applying the administered activity to these data, the kidney received the highest absorbed dose; 5.1-9.4 Gy. The absorbed dose to the liver and bone marrow was calculated to be in the range of 1.3-2.3 Gy and 0.2-0.4 Gy respectively. To study the influence of the cross absorbed dose the ratio of the self-dose to total dose was calculated (Table 5). As can be seen, the major absorbed dose contributes from electrons (auger radiations and conversion electrons) inside the target organ due to the small animal geometry.

The accuracy in calculated data are limited, mainly due to that the residence times are not from own rat data. In the applied literature data (Harrison *et al.*, 1983), the platinum concentration was reported for male wistar rats (5 animals per time point, at 1h, 24 h, 48 h, 72 h and 14 days after administration) weighing approximately 200 g at the start of the study. However, another route of administration, intravenous injection (i.v.) was used, which generally contribute to a more rapid drug uptake than the i.p. administration used in this study. Moreover, the kinetics may be highly variable from one rat to another.

Table 5

The estimated residence time, the absorbed dose per administered activity and the ratio of the self-dose to total dose for kidneys, liver and “remainder”.

<i>Organ</i>	Residence time (h)	Absorbed dose (mGy/MBq)	Selfdose to total dose
Kidneys	2.38	61.4	0.96
Liver	2.92	15.1	0.95
Remainder of the body	10.2	2.6	0.94

DISCUSSION

Radioactive cisplatin is a new advance in combining radio- and chemotherapy. The aim of the present study was to investigate the side effects of non-targeted normal tissues during ^{191}Pt -cisplatin treatments in a rat model, which included absorbed dose estimations to organs at risk. The radiation dosimetry of radioactive cisplatin has previously been studied for mice (Areberg *et al.*, to be published). From that study, if an injection of 5 mg ^{191}Pt -cisplatin (100 MBq/mg) per kg body weight is considered, the absorbed dose to the liver (1.8 Gy) and bone marrow (0.3 Gy) is in agreement with the absorbed dose calculated for rat tissues in Table 5. However, the absorbed dose to the kidneys was considerably higher in the present study compared to the absorbed dose to the mouse kidneys (1.7 Gy). This is partly explained by differences in S-values, partly to a longer residence time used for the rat kidneys.

To determine the effect from radionuclide therapy, comparisons are often made directly to absorbed dose response relationships achieved by external beam therapy. In external radiation therapy the absorbed dose tolerances to kidneys and liver are considerably higher, 23 Gy and 30 Gy respectively (Hall, 2000), than the absorbed doses found here. On the other hand, the traditional MIRDO formalism used in this study, express the mean absorbed dose to an organ in a macroscopic manner, and the absorbed dose within the organ, or on cellular level, may be highly variable. The biological consequence of this fact is not well known (ICRU, 2002).

Most tissues are less tolerant when radiotherapy is combined with other treatment modalities (Meredith, 2002). In order to study the toxicity level for radioactive (^{191}Pt) cisplatin compared to conventional cisplatin, concentrations of plasma parameters were evaluated. Preliminary findings showed that nephrotoxicity, manifested as a significant increase in creatinine concentration, appeared to be the major side effect. This effect was seen for both radioactive cisplatin treatment as well as for conventional cisplatin treatment, and is described as acute tubular necrosis (Parlakpınar *et al.*, 2002). The cisplatin-induced nephrotoxicity, extensively investigated, is not fully understood and many different suggestions are reported. Clinically, hydration therapy is used to reduce the strong side effects occurring on kidneys, leading to a more rapid excretion of platinum.

No statistically significant difference in creatinine concentration was however observed between the radioactive cisplatin treatment group and the cisplatin treatment group. Therefore, acute toxicity of radioactive cisplatin treatment in kidneys was not seen to be different from that of conventional cisplatin treatment. Rongen *et al.* (1994) found that acute toxicity in rats was caused by cisplatin only, when a single dose of cisplatin (5.5 mg/kg) was given before irradiation with 300 kV X-rays to kidneys. The same results for radioactive ^{191}Pt -cisplatin were seen here. Late effects of the combined treatment modalities were reported from Rongen *et al.* (1994) within 20 weeks after a *single dose* of 11 Gy. Milder effects were however found after doses of 8 Gy. Late effects from ^{191}Pt -cisplatin, not primarily investigated in this study, are suggested to be dose dependent, and the absorbed dose to tissues is a pointer for the degree of late effects.

Cisplatin has shown to be cytotoxic to tumours and kidneys but not to the liver (Parti and Wolf, 1990). A disadvantage with radioactive cisplatin is thought to be an increased risk of developing hepatic toxicity. Such an effect was not confirmed in this study, where the plasma bilirubin and aminotransferase activity (ALAT, ASAT) indicated no injurious effect on the liver. In addition, the absorbed dose delivered to the liver was calculated to be limited. In human, however, the liver has proved to be the organ that shows the highest uptake of platinum (Areberg *et al.*, 1999), which evidently leading to a higher absorbed dose to the human liver.

Although, the result of this study indicate that there are no significantly higher side effects if using radioactive (^{191}Pt) cisplatin compared to conventional cisplatin and necessitates further experimental and clinical studies.

ACKNOWLEDGEMENTS

I would like to thank

Kristina Norrgren, for the excellent guidance and for all help with writing,
Sören Mattsson, for giving me the opportunity to work on this project,
Lars Einarsson, at the Svedberg Laboratory in Uppsala, for the production of the ^{191}Pt ,
Bodil Roth, for the HPLC-analyses,
Gerry Jönsson, for the work with the rats,
Cecilia Hindorf, for the rat model and Monte Carlo simulations,
Johan Areberg, for the worth considering comments on the work,
and all staff at the Department of Radiation Physics, Malmö University Hospital,
for creating such a nice atmosphere!

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APPENDIX 1

Output data for ¹⁹¹Pt dosimetry in the MIRD format (from the database generated by NNDC, National Nuclear Data Center, Brookhaven National Laboratory, 2004).

Radiations	y(i) (Bq-s) ⁻¹	E(i) (MeV)	y(i)*E(i)
ce-L,γ1	7.87×10 ⁻⁰³	2.851×10 ⁻⁰²	2.24×10 ⁻⁰⁴
ce-M, γ1	2.40×10 ⁻⁰³	3.876×10 ⁻⁰²	9.29×10 ⁻⁰⁵
ce-L, γ2	3.68×10 ⁻⁰³	3.617×10 ⁻⁰²	1.33×10 ⁻⁰⁴
γ3	4.88×10 ⁻⁰²	8.240×10 ⁻⁰²	4.02×10 ⁻⁰³
ce-K, γ3	2.72×10 ⁻⁰¹	6.287×10 ⁻⁰³	1.71×10 ⁻⁰³
ce-L, γ3	1.97×10 ⁻⁰¹	6.898×10 ⁻⁰²	1.36×10 ⁻⁰²
ce-M, γ3	4.97×10 ⁻⁰²	7.922×10 ⁻⁰²	3.94×10 ⁻⁰³
ce-N+,γ3	1.42×10 ⁻⁰²	8.171×10 ⁻⁰²	1.16×10 ⁻⁰³
ce-L, γ4	2.28×10 ⁻⁰³	7.173×10 ⁻⁰²	1.64×10 ⁻⁰⁴
γ5	3.28×10 ⁻⁰²	9.652×10 ⁻⁰²	3.17×10 ⁻⁰³
ce-K, γ5	1.88×10 ⁻⁰¹	2.041×10 ⁻⁰²	3.84×10 ⁻⁰³
ce-L, γ5	3.28×10 ⁻⁰²	8.310×10 ⁻⁰²	2.73×10 ⁻⁰³
ce-M, γ5	7.58×10 ⁻⁰³	9.334×10 ⁻⁰²	7.07×10 ⁻⁰⁴
ce-N+, γ5	2.32×10 ⁻⁰³	9.583×10 ⁻⁰²	2.22×10 ⁻⁰⁴
γ6	3.20×10 ⁻⁰²	1.294×10 ⁻⁰¹	4.14×10 ⁻⁰³
ce-K, γ6	7.10×10 ⁻⁰²	5.329×10 ⁻⁰²	3.79×10 ⁻⁰³
ce-L, γ6	1.52×10 ⁻⁰²	1.160×10 ⁻⁰¹	1.76×10 ⁻⁰³
ce-M, γ6	3.52×10 ⁻⁰³	1.262×10 ⁻⁰¹	4.44×10 ⁻⁰⁴
ce-N+, γ6	1.13×10 ⁻⁰³	1.287×10 ⁻⁰¹	1.45×10 ⁻⁰⁴
ce-K, γ8	1.49×10 ⁻⁰³	6.477×10 ⁻⁰²	9.64×10 ⁻⁰⁵
γ10	3.52×10 ⁻⁰²	1.722×10 ⁻⁰¹	6.06×10 ⁻⁰³
ce-K, γ10	3.91×10 ⁻⁰²	9.608×10 ⁻⁰²	3.75×10 ⁻⁰³
ce-L, γ10	6.34×10 ⁻⁰³	1.588×10 ⁻⁰¹	1.01×10 ⁻⁰³
ce-M, γ10	1.46×10 ⁻⁰³	1.690×10 ⁻⁰¹	2.47×10 ⁻⁰⁴
ce-N+, γ10	4.65×10 ⁻⁰⁴	1.715×10 ⁻⁰¹	7.97×10 ⁻⁰⁵
γ11	1.02×10 ⁻⁰²	1.790×10 ⁻⁰¹	1.82×10 ⁻⁰³
ce-K, γ11	7.36×10 ⁻⁰³	1.028×10 ⁻⁰¹	7.57×10 ⁻⁰⁴
ce-L, γ11	1.83×10 ⁻⁰³	1.655×10 ⁻⁰¹	3.03×10 ⁻⁰⁴
ce-M, γ11	4.51×10 ⁻⁰⁴	1.758×10 ⁻⁰¹	7.93×10 ⁻⁰⁵
γ12	4.16×10 ⁻⁰³	1.877×10 ⁻⁰¹	7.81×10 ⁻⁰⁴
ce-K, γ12	2.91×10 ⁻⁰³	1.116×10 ⁻⁰¹	3.25×10 ⁻⁰⁴
ce-L, γ12	6.20×10 ⁻⁰⁴	1.743×10 ⁻⁰¹	1.08×10 ⁻⁰⁴
ce-K, γ14	8.84×10 ⁻⁰⁴	1.328×10 ⁻⁰¹	1.17×10 ⁻⁰⁴
γ16	8.24×10 ⁻⁰³	2.197×10 ⁻⁰¹	1.81×10 ⁻⁰³
ce-K, γ16	1.11×10 ⁻⁰³	1.435×10 ⁻⁰¹	1.60×10 ⁻⁰⁴
ce-L, γ16	7.48×10 ⁻⁰⁴	2.062×10 ⁻⁰¹	1.54×10 ⁻⁰⁴
ce-K, γ18	6.01×10 ⁻⁰⁴	1.476×10 ⁻⁰¹	8.87×10 ⁻⁰⁵
γ20	7.76×10 ⁻⁰³	2.679×10 ⁻⁰¹	2.08×10 ⁻⁰³
ce-K, γ20	6.26×10 ⁻⁰⁴	1.918×10 ⁻⁰¹	1.20×10 ⁻⁰⁴
ce-L, γ20	3.19×10 ⁻⁰⁴	2.545×10 ⁻⁰¹	8.12×10 ⁻⁰⁵
γ21	1.65×10 ⁻⁰²	2.687×10 ⁻⁰¹	4.43×10 ⁻⁰³
ce-K, γ21	1.32×10 ⁻⁰³	1.926×10 ⁻⁰¹	2.54×10 ⁻⁰⁴
ce-L, γ21	6.69×10 ⁻⁰⁴	2.553×10 ⁻⁰¹	1.71×10 ⁻⁰⁴

(Output data for ¹⁹¹Pt dosimetry continued).

γ24	3.36×10 ⁻⁰²	3.512×10 ⁻⁰¹	1.18×10 ⁻⁰²
ce-K, γ24	4.97×10 ⁻⁰³	2.751×10 ⁻⁰¹	1.37×10 ⁻⁰³
ce-L, γ24	8.16×10 ⁻⁰⁴	3.378×10 ⁻⁰¹	2.76×10 ⁻⁰⁴
γ25	6.00×10 ⁻⁰²	3.599×10 ⁻⁰¹	2.16×10 ⁻⁰²
ce-K, γ25	8.82×10 ⁻⁰³	2.838×10 ⁻⁰¹	2.50×10 ⁻⁰³
ce-L, γ25	1.41×10 ⁻⁰³	3.465×10 ⁻⁰¹	4.89×10 ⁻⁰⁴
ce-M, γ25	2.32×10 ⁻⁰⁴	3.567×10 ⁻⁰¹	8.28×10 ⁻⁰⁵
γ28	9.60×10 ⁻⁰⁴	4.090×10 ⁻⁰¹	3.93×10 ⁻⁰⁴
γ29	8.00×10 ⁻⁰²	4.094×10 ⁻⁰¹	3.28×10 ⁻⁰²
ce-K, γ29	8.14×10 ⁻⁰³	3.333×10 ⁻⁰¹	2.71×10 ⁻⁰³
ce-L, γ29	1.31×10 ⁻⁰³	3.960×10 ⁻⁰¹	5.20×10 ⁻⁰⁴
ce-M, γ29	2.06×10 ⁻⁰⁴	4.063×10 ⁻⁰¹	8.39×10 ⁻⁰⁵
γ33	3.36×10 ⁻⁰²	4.565×10 ⁻⁰¹	1.53×10 ⁻⁰²
ce-K, γ33	2.45×10 ⁻⁰³	3.804×10 ⁻⁰¹	9.33×10 ⁻⁰⁴
ce-L, γ33	4.00×10 ⁻⁰⁴	4.431×10 ⁻⁰¹	1.77×10 ⁻⁰⁴
γ36	6.00×10 ⁻⁰⁴	4.947×10 ⁻⁰¹	2.97×10 ⁻⁰⁴
γ37	1.37×10 ⁻⁰¹	5.389×10 ⁻⁰¹	7.37×10 ⁻⁰²
ce-K, γ37	5.39×10 ⁻⁰³	4.628×10 ⁻⁰¹	2.49×10 ⁻⁰³
ce-L, γ37	9.08×10 ⁻⁰⁴	5.255×10 ⁻⁰¹	4.77×10 ⁻⁰⁴
γ38	3.68×10 ⁻⁰³	5.416×10 ⁻⁰¹	1.99×10 ⁻⁰³
ce-K, γ38	1.84×10 ⁻⁰⁴	4.655×10 ⁻⁰¹	8.55×10 ⁻⁰⁵
γ39	5.28×10 ⁻⁰⁴	5.688×10 ⁻⁰¹	3.00×10 ⁻⁰⁴
γ40	1.18×10 ⁻⁰³	5.765×10 ⁻⁰¹	6.78×10 ⁻⁰⁴
γ41	7.60×10 ⁻⁰⁴	5.836×10 ⁻⁰¹	4.44×10 ⁻⁰⁴
γ42	1.36×10 ⁻⁰³	5.879×10 ⁻⁰¹	8.00×10 ⁻⁰⁴
γ44	1.41×10 ⁻⁰²	6.241×10 ⁻⁰¹	8.79×10 ⁻⁰³
ce-K, γ44	4.87×10 ⁻⁰⁴	5.479×10 ⁻⁰¹	2.67×10 ⁻⁰⁴
K1 X-ray	6.69×10 ⁻⁰¹	6.490×10 ⁻⁰²	4.34×10 ⁻⁰²
K2 X-ray	3.90×10 ⁻⁰¹	6.329×10 ⁻⁰²	2.47×10 ⁻⁰²
K X-ray	2.89×10 ⁻⁰¹	7.360×10 ⁻⁰²	2.12×10 ⁻⁰²
L X-ray	5.07×10 ⁻⁰¹	9.180×10 ⁻⁰³	4.65×10 ⁻⁰³
Auger-K	5.85×10 ⁻⁰²	4.960×10 ⁻⁰²	2.90×10 ⁻⁰³
Auger-L	1.08	7.060×10 ⁻⁰³	7.64×10 ⁻⁰³
Listed X,γ , and γ [±] Radiations			2.91×10 ⁻⁰¹
Omitted X, γ, and γ [±] Radiations**			2.56×10 ⁻⁰³
Listed β, ce, and Auger Radiations			6.56×10 ⁻⁰²
Omitted β, ce, and Auger Radiations**			8.09×10 ⁻⁰⁴
Listed Radiations			3.57×10 ⁻⁰¹
Omitted Radiations**			3.37×10 ⁻⁰³

* Average Energy (MeV).

^a Maximum Energy (MeV) for subshell.

** Each omitted transition contributes <0.100% to y(i)×E(i)

POPULÄRVETENSKAPLIG SAMMANFATTNING

Radioaktivt läkemedel för cancerbehandling

Läkemedlet cisplatin är ett cellhämmande medel som med framgång använts kliniskt sedan mitten av sjuttioalet, framför allt vid behandling av testikelcancer, äggstockscancer och tumörer i huvud-näsa-halsregionen. Behandling med cisplatin kan dock medföra allvarliga biverkningar, där njurfunktionsnedsättning begränsar doseringen. Andra vanligt förekommande biverkningar är hörselnedsättning, muskelsvaghet, yrsel och illamående.

En hypotes är att man kan förstärka cisplatins effekt på tumörer genom att göra det radioaktivt, och utnyttja den effekt som den joniserande strålningen från det radioaktiva sönderfallet orsakar på cellen. Denna teori har bekräftats i olika studier, bl.a. på nakna möss, där ^{191}Pt -cisplatin visade sig vara ett effektivare cellhämmande medel än cisplatin utan radioaktiv märkning. Den basala frågeställningen är nu om en *terapeutisk vinst* föreligger, d.v.s. om den förstärkta effekten från radioaktivt cisplatin, jämfört med konventionell cisplatinbehandling, är större hos tumörvävnad än för normalvävnad.

Om man vill studera eventuella organskador så måste ämnet först testas i djurförsök. Oftast kan man sedan utgå från att liknande effekter uppträder hos människan. I denna studie undersöktes hur riskorganen (njurar, lever och benmärg) påverkades av behandling med radioaktivt cisplatin, jämfört med behandling med icke-radioaktivt cisplatin, i en råttmodell. Djuren delades upp i tre grupper, där "Grupp 1" fungerade som kontroller (injicerades med vanlig koksaltlösning), "Grupp 2" injicerades med cisplatin och "Grupp 3" injicerades med ^{191}Pt -cisplatin. Blodprover erhöles därefter en till två gånger i veckan, och analyserades med avseende på njurfunktion, leverfunktion och benmärgspåverkan.

Statistisk analys (ANOVA) av blodprovparametrar visade ingen signifikant skillnad mellan radioaktiv cisplatinbehandling och vanlig, icke-radioaktiv, cisplatinbehandling. Det förekom heller ingen skillnad i generell toxicitet, d.v.s. i mortalitet och viktreduktion. Den främsta biverkningen i samband med ^{191}Pt -cisplatinbehandling visade sig vara akut njurpåverkan, vilket demonstrerades med kraftigt förhöjda kreatininvärden. Dessa förhöjda nivåer kunde dock även ses för konventionell cisplatinbehandling, vilket antyder att det är cisplatin ensamt som ger upphov till denna akuta skada.

Denna undersökning visar att de bieffekter som har studerats, inte är mer förstärkta för ^{191}Pt -cisplatin än för konventionellt cisplatin, vilket kan innebära att radioaktivt cisplatin utgör ett alternativ till dagens behandlingar av cancer. En behandling med radioaktivt cisplatin skulle eventuellt medföra att mängden cisplatin kunde reduceras med bibehållen effekt, vilket där igenom skulle minska biverkningarna. Alternativt skulle samma mängd radioaktivt cisplatin som icke-radioaktivt kunna ge en effektivare behandling.