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Stage of Pregnancy-dependent Transplacental Passage of ^{195m}Pt after *cis*-Platinum Treatment*

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Abstract—*The placental transfer of the clinically important cytostatic drug* cis-*diamminedichloroplatinum(II) (*cis-*platinum, DDP) was investigated by application of* ^{195m}Pt-*labelled DDP to pregnant NMRI mice on day* 10, 11, 12, ..., *or* 17 *of gestation and by measurement of the radioactivity in the embryos or fetuses in intervals between* 0.5 *and* 48 hr *after application. Whereas on days* 10, 11 *and* 12 *after treatment only very small amounts of radioactivity could be detected in the embryos, from day* 13 *considerable and increasing amounts of radioactivity could be found; finally, within the fetal compartment on day* 17 *even an effect of enrichment of DDP over a uniform distribution between mother and embryos was observable. It is supposed that the ability of DDP to pass the placental barrier only after accomplished maturation of the placenta is due to active carrier-mediated transport processes.*

INTRODUCTION

cis-DIAMMINEDICHLOROPLATINUM(II) (*cis*-platinum, DDP) represents a new, clinically approved antitumor agent which differs from all other cytostatic drugs by its purely inorganic character. Clinical trials of the last few years have shown that DDP is most effective against urogenital cancers and head and neck cancers, as well as against lung cancers and brain cancers [1–4].

According to pharmacokinetic investigations, DDP, or its platinum-containing metabolites, are eliminated mainly by the kidneys. After injection the plasma level of DDP declines biphasically, the skin, liver and kidneys being the principal sites of localization of the drug [5–7]. However, DDP does not apparently pass the intact blood–brain barrier [8, 9], though it is cytostatically active against brain tumors [3, 4].

All these pharmacokinetic studies were performed with non-pregnant animals. No attention has been paid up to now to the behavior of DDP at the placental barrier. Therefore, in the present study we investigated the ability of DDP to traverse the placental barrier and to enter into the embryonal or fetal compartment, depending on the stage of pregnancy.

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MATERIALS AND METHODS

The ^{195m}Pt-labelled DDP used as tracer in this study was obtained as solution in normal saline from Oak Ridge National Laboratory, Oak Ridge, TN (for radiophysical and radiopharmaceutical properties of ^{195m}Pt and [^{195m}Pt]-DDP see refs [10, 11]).

The original solution was made up with saline and unlabelled DDP (prepared as described previously [12, 13]; elemental analyses within ±0.5% of the theoretical values) to achieve final concentrations of 1 mg DDP/ml and 25 μCi/ml. Of this solution, a volume of 0.01 ml/g body weight of mouse, corresponding to 10 mg DDP and 0.25 mCi/kg body weight, was injected intraperitoneally (i.p.) into pregnant NMRI mice on day 10, 11, 12, ..., or 17 of pregnancy (day of fertilization = day 0). In all, 168 animals were so-treated.

At the different gestation stages, the embryos of 3 mice were removed 0.5, 1, 2, 4, 8, 24 or 48 hr after administration, washed five times with normal saline and put into test tubes. By use of a gamma counter, the impulse rates (counts/min) of all the embryos of each mouse were determined, corrected for decay and apparatus errors and correlated with the weight of the embryos. Values were converted into values of absolute radioactivity per g embryo and related as a percentage of the dose of radioactivity administered per g mouse. The results are given in Table 1 and Fig. 1.

Table 1. Detection of ^{195m}Pt at various intervals after i.p. application of single doses of ^{195m}Pt-labelled cis-platinum (DDP) to pregnant mice on day 10, 11, ..., or 17 of pregnancy

	0.5 hr	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr
Day 10	0.27 ± 0.15	0.37 ± 0.12	0.40 ± 0.17	0.27 ± 0.15	0.23 ± 0.06	0.13 ± 0.06	0.13 ± 0.06
Day 11	0.33 ± 0.15	0.43 ± 0.15	0.70 ± 0.10	0.50 ± 0.26	0.40 ± 0.26	0.27 ± 0.12	0.10 ± 0.00
Day 12	0.37 ± 0.15	0.83 ± 0.06	0.83 ± 0.11	0.47 ± 0.11	0.43 ± 0.06	0.27 ± 0.20	0.20 ± 0.10
Day 13	1.47 ± 0.15	1.40 ± 0.20	1.83 ± 0.40	1.63 ± 0.30	0.97 ± 0.11	0.43 ± 0.06	0.30 ± 0.10
Day 14	5.67 ± 2.77	14.17 ± 3.44	8.90 ± 5.30	7.80 ± 3.87	10.47 ± 1.25	1.10 ± 0.53	0.30 ± 0.10
Day 15	12.20 ± 3.15	33.80 ± 5.00	22.90 ± 4.20	13.90 ± 1.45	6.40 ± 1.65	5.50 ± 1.65	0.93 ± 0.15
Day 16	23.70 ± 6.07	41.27 ± 8.36	21.10 ± 3.90	8.43 ± 3.92	5.87 ± 2.08	4.50 ± 3.30	2.00 ± 1.25
Day 17	71.63 ± 9.00	197.40 ± 27.70	150.00 ± 9.56	92.53 ± 14.26	28.10 ± 7.78	8.30 ± 2.26	3.13 ± 1.94

The evaluated parameter is the radioactivity per g embryo given as a percentage of the radioactive dose administered per g parent animal. The values represent the mean values and standard deviations of the data of three parent animals.

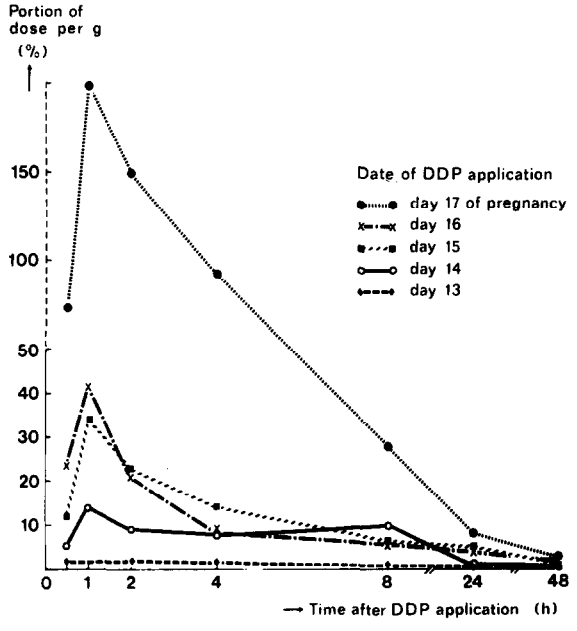

Fig. 1. Detection of ^{195m}Pt in embryos after application of [^{195m}Pt]-DDP to pregnant mice.

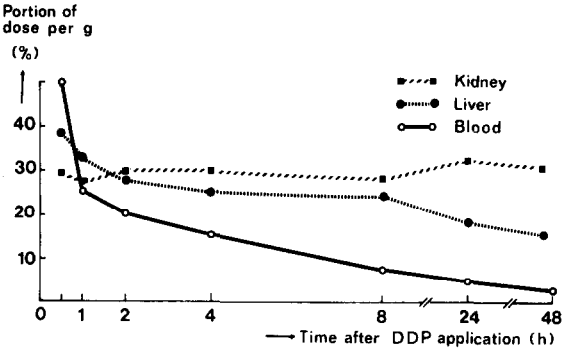

Fig. 2. Organ distribution of ^{195m}Pt after application of [^{195m}Pt]-DDP to pregnant mice.

To pursue the distribution of ^{195m}Pt within the mice, the liver, the kidneys and a blood sample of those mice treated on day 11 of pregnancy were removed and measured in the gamma counter. The relative radioactivity in relation to the administered dose per g animal was calculated and plotted in Fig. 2.

For further control purposes, 14 pregnant NMRI mice on day 11 as well as 14 animals on day 16 of gestation received an i.p. injection of unlabelled DDP (10 mg/kg mouse). The embryos of two animals were removed after 0.5, 1, 2, 4, 8, 24 and 48 hr each and measured in the gamma counter.

RESULTS

Whereas the impulse rates of the embryos of control animals were of the same magnitude as the background counts (5–20 pCi/g), the radioactivity in the embryos of the [^{195m}Pt]-DDP-treated mice, which indicates the presence of ^{195m}Pt in any molecular species (the differentiation between *cis*-platinum and other platinum-containing metabolites is not possible), exceeds in every case the control values by factors of 10–50000.

Table 1 and Fig. 1 summarize the values of radioactivity measured in the embryos as percentages of the radioactive dose administered per g animal. Both representations show that the content of ^{195m}Pt in the embryos differs markedly and is dependent on the stage of pregnancy: on days 10, 11 and 12 less than 1% of the per g radioactivity injected can be detected in the embryonal tissue. However, in all cases the registered values surmount the control values by a factor of 10–100. Moreover, even the low values of days 10, 11 and 12 clearly show the tendency to increase concentration of ^{195m}Pt in the embryonal compartment with advanced gestation.

Beginning on day 13, the relative values of radioactivity in the fetuses exceed the 1% limit, rise to 30–40% on days 15 and 16 and reach 100–200% on day 17. This means that on days 15 and 16 an amount of radioactivity can be found in the embryos which is comparable to that in the liver and kidneys of the parent mice (cf. Fig. 2),

whereas on day 17, near the end of the pregnancy, continued accumulation of ^{195m}Pt within the embryos produces a higher content of Pt in the embryos than in the liver or kidneys of the parent animals.

With regard to the time course of the embryonal content of ^{195m}Pt after injection of [^{195m}Pt]-DDP at the various gestation stages, in all cases the amount of radioactivity found in the embryos increases during the first hour after administration, when it reaches a maximum. After this time the radioactivity decreases in a rapid and continuous manner. However, even at 48 hr after treatment a small portion of the injected radioactivity is still detectable within the embryonal or fetal compartment.

DISCUSSION

The main finding of the present tracer study is that the entrance of DDP or its platinum-containing metabolites into the embryonal or fetal compartment is highly dependent on the age of pregnancy and that only during the fetal period, i.e. following day 12 of the murine gestation, when placenta formation is completed, is the drug able to pass through the placental barrier to a considerable extent.

This result is surprising and has no correspondence with the behavior of other clinically approved cytostatic drugs, which traverse the placenta, enter the embryonal compartment in early, medium and late periods of gestation [14–16] and induce teratogenic effects mainly during the sensitive phases of embryonal development (before day 12 in murine pregnancy). As a consequence of the minimal placental transfer of DDP during the organogenesis of the embryos, it may be expected that strong differences between DDP and other cytostatic drugs exist with respect to the induction of teratogenic and embryotoxic effects. Further teratological experiments are necessary to confirm or refute this postulation.

The nearly total incapability of DDP to traverse the placental barrier before placentation is accomplished is paralleled by the inability of DDP to pass the blood–brain barrier to any great extent [8, 9, 17]. With regard to the very limited transfer of DDP through the blood–brain barrier and through the placental barrier before day 13, it can be supposed that it is the inorganic character and the un-ionized, but dipolar, nature of the DDP molecule that are responsible for the inability to pass these membrane systems. Moreover, the present data suggest that after the placental maturation is finished, the noble metal complex is transferred by an active, carrier-mediated transport which finally leads to the enrichment of DDP or its platinum-containing metabolites within the fetal compartment at the end of the gestation period.

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