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TERATOGENIC ACTION OF PLATINUM THYMINE BLUE

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Summary

The teratogenic activity of the antitumor agent cisplatinum-2-thymine (platinum thymine blue) was investigated in rats. Pregnant Wistar-derived albino rats were given single ip injections of an aqueous solution of platinum thymine blue (PTB) at one day of pregnancy from day 5 through day 14 (sperm day=day 0). The dosages used ranged from 20 to 80 mg/kg maternal body weight. At autopsy (day 20) fetuses were recovered and subsequently examined for skeletal and soft-tissue abnormalities. PTB was embryolethal and teratogenic at several stages during rat gestation. Embryonic death occurred following all doses, and was dose dependent, except at day 5. The majority of malformed fetuses, however, were observed only after treatment at day 6 or 7 following injection with 50, 60, or 80 mg/kg. Eye defects were the predominant abnormality followed by hydrocephalus, gastroschisis, and ectopia cordis. The skeleton was only slightly affected. PTB is a potent inhibitor of DNA synthesis, but its mechanism of teratogenic action is unknown.

Rosenberg et al. (1) first reported the antitumor action of coordination complexes of platinum. Cis-dicholrodiamineplatinum II (cis-platinum) appears to have the most potent activity as a broad spectrum antitumor agent against tumors in different animal hosts (2). Some efficacy of cis-platinum II and its analogs against human tumors has been reported (3,4). The cis-platinum complexes cause a severe and prolonged inhibition of DNA synthesis with minimal effect on RNA and protein synthesis (5,6) presumably due to interaction of the platinum molecule with DNA (7,8,9). These platinum complexes have been reported to be mutagenic (10,11), carcinogenic (12) and teratogenic in mice (13).

Davidson et al. (14) discovered that cis-platinum II would react with pyrimidines and substituted pyrimidines to form water soluble deep blue complexes, the "platinum blues". Their postulated formula for the complexes is $PtC_5H_{14}N_4O_5$. The platinum blues have the advantage over cis-platinum II in high solubility in water and lack of kidney toxicity, while still being highly potent antitumor agents. The platinum pyrimidine blues also react with DNA (15, 16).

The platinum blues have not been tested for teratogenicity, but because of their antitumor activity and reactiveness with DNA, they are suspect. The present study was undertaken to investigate the effect of platinum thymine blue (PTB) on rat embryogenesis.

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Materials and Methods

Virgin female Wistar-derived rats from my colony were used. The animals were fed Teklad Rat Diet (Teklad Mills, Winfield, Iowa) ad libitum, with supplemental feedings of lettuce. Water was available at all times. The day of finding sperm in the vaginal smear was designated day 0 of pregnancy. Aqueous solutions of platinum thymine blue (Tousimis Research Corporation, Rockville, Maryland) were prepared for ip injection. Solutions were prepared so that the same volume was always injected, regardless of the concentration used (0.1 ml/20g body wt). The dose injected was either 20,40,50,60 or 80 mg/kg maternal body weight. The toxic level for PTB has been reported to be about 450 mg/kg in Swiss Webster mice (14). Controls received distilled water ip.

Pregnancy was terminated at day 20, resorption sites were counted and the fetuses recovered, weighed, and fixed in Bouin's fluid or 95% alcohol. Those fetuses fixed in Bouin's fluid were subsequently examined for external malformations and then free-hand sectioned with a razor blade. These sections are useful to detect gross malformations in the palate, brain, eyes, heart, kidney and sex organs. Abnormalities in other organs are not identified easily. Fetuses fixed in 95% alcohol were prepared for staining with alizarin red S for visualization of the skeleton. Statistical analyses of the results were performed by the Student's "t" test.

Results

Table I presents the results following treatment with PTB from day 5 through day 14 of gestation. A direct association between the dose injected and embryonic death (resorption) is apparent at all treatment days, except at day 5. A similar positive correlation occurs between the dose and the incidence of malformed survivors at days 6 and 7. Fetal malformations were observed following treatment at day 5 through 8, with the greatest number found at day 6 and 7. Malformed fetuses usually weighed less than normal fetuses, as reflected in significant reduction in fetal weights of litters containing many malformed fetuses. Treatment at days 10 and 12 also reduced fetal weight, but without concomitant malformations. Daily observations of the treated females failed to reveal any overt signs of PTB toxicity The weight gained during pregnancy was a reflection of litter size and varied with the number of resorptions.

Table II presents the types of malformations induced by PTB. At all doses tested, four malformations predominated: anophthalmia/microphthalmia (ratio of 44/9), hydrocephalus, gastroschisis, and ectopia cordis. Several additional malformations were induced but in much reduced numbers. These included agnathia, cleft palate, club feet, diaphragmatic hernia, ectrodactyly, encephalomeningocele, exencephaly, renal agenesis, syndactyly and umbilical hernia.

Almost all of the malformed fetuses were induced after a dose of either 50 or 60 mg/kg, therefore, these two doses were used in another group of rats to look for skeletal abnormalities. A total of 206 fetuses were examined from dams treated at day 6, 8, 10, 12 and 14. The only malformation observed was rib fusion, which occurred in 5 of 30 surviving fetuses of dams treated with 60 mg/kg PTB at day 6. Absent ossification centers occurred with variable incidence in both control and treated fetuses. They were absent predominantly in the sternum and metacarpus. In 60 control fetuses, 20.3% had missing metacarpal ossification centers and 21.7% missing sternal ossification centers. In 206 treated fetuses, the percentages were 26.2 and 42.7 respectively. These results were not analyzed statistically.

TABLE I

Embryotoxicity Following Maternal Injection of PTB

Day Treated	Dosage (mg/kg)	No. Dams Treated	No. Implant. Sites	% Resorb.	Survivors Malformed No. (%)	Fetal Wt (g) mean ±SD
5	20	5	63	7.9	0	3.93 ±0.14
	40	5	72	13.8	0	3.83 ±0.25
	50	6	86	8.1	2 (2.5)	4.04 ±0.16
	60	6	88	12.5	1 (1.3)	3.76 ±0.08
	80	5	69	4.3	0	3.88 ±0.26
6	0[1]	5	68	0	0	3.82 ±0.28
	20	6	98	7.1	0	3.70 ±0.31
	40	6	80	30.0	11(19.6)	3.39 ±0.60
	50	6	81	51.8	11(28.2)	3.05 ±0.55[2]
	60	6	86	67.4	22(78.6)	2.78 ±0.54[3]
	80	4	67	98.5	1 (100)	3.14
7	0[1]	8	92	1.1	0	3.92 ±0.22
	20	3	37	29.7	0	3.80 ±0.35
	40	5	64	66.6	0	3.64 ±0.17
	50	6	81	60.5	3 (9.3)	3.64 ±0.21
	60	10	121	90.9	7 (63.6)	2.65 ±0.24[3]
	80	5	61	90.1	5 (83.0)	3.12 ±0.41[3]
8	0[1]	6	74	1.4	0	3.84 ±0.48
	20	3	33	15.1	0	4.04 ±0.08
	40	5	53	16.9	2 (4.5)	3.67 ±0.33
	50	4	60	50.0	0	3.62 ±0.48
	60	6	71	98.6	1 (100)	2.92
	80	3	49	95.5	0	3.23 ±0.23[4]
9	40	5	65	46.2	0	3.85 ±0.73
	50	3	41	75.6	0	3.36 ±0.68
	60	4	60	78.3	0	3.74 ±0.14
	80	3	46	100.0	-	
10	40	3	44	34.0	0	3.31 ±0.21
	50	4	39	94.9	0	2.43 ±0.66
	60	3	44	100	-	
	80	3	45	100	-	
12	50	6	78	50.0	0	2.90 ±0.29
	60	4	52	65.4	0	2.72 ±0.49
	80	3	39	100.0	-	
14	50	5	70	18.6	0	3.58 ±0.32
	80	3	34	100.0	0	

1. Controls were run at days most susceptible to teratogenic effects of PTB.
2. P = 0.05
3. P = 0.02 or less
4. Only 2 survivors

TABLE II

Major Malformations Following PTB Treatment[1]

Day of Treatment	Dosage (mg/kg)	Anophthalmia/ Microphthalmia	Hydrocephalus	Gastroschisis	Ectopia Cordis
6	40	5/11	4/11	3/11	1/11
	50	4/11	2/11	-	-
	60	20/22	10/22	7/22	5/22
	80	1/1			
7	50	2/3	2/3	2/3	-
	60	4/7	5/7	3/7	2/7
	80	4/5	4/5	1/5	1/5
8	40	2/2	-	-	-
	60	1/1	-	-	-

1. Results expressed as number of fetuses with the malformation/total number of fetuses with malformations.

Discussion

The results of this experiment demonstrate that the antitumor agent platinum thymine blue has both lethal and teratogenic effects in the rat. With the dosages used, a positive correlation was demonstrated between embryonic death (resorption) and the dose of PTB administered, except at day 5 when the incidence of resorptions was similar for all doses. The reason the day 5 embryo is more resistant to the lethal effects of PTB than later embryonic stages is not known, but may be related to its developmental stage (preimplantation) and the distribution of PTB. After administration, platinum antitumor agents reach an initial high concentration in kidney, liver, skin, bone, ovary, and uterus (17). A rapid elimination follows with 70-90% of the administered dose appearing in the urine within the first few days. The platinum remaining in skin, muscle, and bone forms a reservoir that is slowly depleted, with platinum still detectable in rat urine 30 days after a single exposure (17). The transient high concentration of platinum in the uterus may explain its greater lethality to the implanted embryo. This lethal effect is pronounced at all implanted stages exposed in this experiment. (Table I).

Malformations were induced following maternal treatment at day 5, 6, 7, or 8 with all doses except the 20 mg/kg dose, which failed to induce a malformation at any tested time in gestation. The greatest numbers of abnormalities followed treatment with 60 mg/kg PTB at day 6 or 7. There were a total of 66 malformed fetuses produced following treatment at all days. Maldevelopment of the eye was the predominant defect noted. Forty-four fetuses had apparent anophthalmia (histological sections were not done) and 9 had microphthalmia. Hydrocephalus

was present in 27 fetuses, and 18 fetuses had defects in development of the abdominal wall (2 fetuses with ectopia cordis only, 9 fetuses with gastroschisis only, and 8 fetuses with ectopia cordis and gastroschisis together).

Differentiation of the central nervous system and the mesoderm was most severly affected by PTB-treatment. In the Wistar rat, the optic evaginations from the diencephalon appear at day 9-10 and contact the surface ectoderm at day 10-11. The lens placodes are formed at day 11-12 and detach from the surface at day 13-14. During the same period of time, mesodermal cells migrate ventrolaterally from the somites to give rise to the developing muscle and connective tissue in the lateral body wall folds. These folds meet to form the ventral body wall at day 13-14, followed by the normal umbilical herniation. These events are dated from a presumed fertilization during the night of mating and, although approximate, probably vary no more than plus or minus one-half day (18). Thus, critical stages in the embryogenesis of the eye and ventral body wall occur within the same time period (day 9 to 13), and an insult during this period could be expected to affect the development of both structures. It is difficult to postulate the onset of hydrocephalus. It could be early or late in development, but could certainly be expected to be induced between days 9 and 13. Why other organs, also in critical stages of embryogenesis, are not affected remains an enigma of teratology.

Lazar et al. (13) reported cis-diaminedichloroplatinum II to be teratogenic in the Swiss Webster mouse. They reported reduced fetal weights and minor skeletal malformations of sternebrae, ribs, and vertebrae. Only one fetus with multiple malformations, including cleft palate and deformed extremities, was described. In the present study, PTB-delayed ossification was found only in the sternum and metacarpus. However, five of the 30 surviving fetuses exhibited rib fusions, a true morphological defect. Rib fusions were found only in fetuses from dams treated at day 6 with 60 mg/kg of PTB. There are four variables that could explain the discrepancies between the two studies. First, Lazar et al. used lower doses (3 or 8 mg/kg); second, the mouse was the experimental animal; three, the drug was administered only at day 8 (roughly comparable to day 9 in rat gestation); and four, cis-platinum was used, not PTB.

The cis-platinum complexes have been reported to induce a severe and prolonged inhibition of DNA synthesis with minimal effects on RNA and protein synthesis (6). The platinum pyrimidine complexes also interact with nucleic acids and proteins (2). The complexes appear to produce a partial denaturation of DNA and an almost complete destruction of protein secondary structure (16). The specificity of their complexing with nucleic acids is further demonstrated through the use of platinum complexes as nuclei acid stains for electron microscopy (19). Biological and chemical investigations of the platinum pyrimidine blues have been hampered because they are a mixture of oligomers of varying degrees of stability in solution, and only recently has the synthesis of a crystalline platinum blue been successful (15). The structural features elucidated are believed to be shared by all platinum blues. It is not unexpected that PTB proved to be a teratogen. Many chemotherapeutic agents are teratogenic, and they also inhibit DNA synthesis. The relationship between the inhibition of DNA synthesis and the production of malformations is not clear (20). It may be that interference with cell proliferation and induction of cell death that often follows inhibition of DNA synthesis is responsible for the production of malformations (21). Interference with protein synthesis usually shows little or no teratogenic effect (20).

In summary, platinum thymine blue is both lethal and teratogenic to the developing rat embryo. Several criteria for teratogenic action are satisfied: a

dose response can be demonstrated, susceptibility changes with the stage of embryonic development, and a correlation can be made with the defect produced and the stage of embryonic development at the time of treatment. PTB is a powerful inhibitor of DNA synthesis, as are many other teratogens. The specific site and mechanism of teratogenic action of PTB in the induction of malformations, however, remains to be determined.

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