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Effect of Platinum and Palladium Salts on Thymidine Incorporation into DNA of Rat Tissues

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The intraperitoneal administration of $PtCl_4$ or $Pd(NO_3)_2$ at levels of 28 or 56 μmole/kg body weight decreased the thymidine incorporation into DNA of spleen, liver, kidney, and testis. Spleen was most sensitive to both the platinum and the palladium salt. In liver, DNA syntheses in parenchymal cells and stromal cells were about equally sensitive to $PtCl_4$. In control rats, only 20–30% of the ^3H in the acid-soluble fraction of liver or spleen was in the form of thymidine and its phosphate esters 2 hr after the intraperitoneal injection of ^3H-thymidine; prior injection of $PtCl_4$ (56 μmole/kg body weight) did not change the pattern.

Introduction

Platinum and palladium compounds currently are being used as the active components in the catalytic converters of air pollution control devices on many motor vehicles. It is of interest, therefore, to determine the biological effects of the compounds of these metals. The present experiments were conducted to determine the acute effects of soluble salts of platinum and palladium on the incorporation of radioactive thymidine into DNA.

Materials and Methods

All experimental studies were conducted with male Sprague-Dawley rats (weighing 160–200 g) obtained from Zivic-Miller Laboratories. In each of these experiments, the metallic salt and the ^3H-labeled thymidine were injected intraperitoneally 4 hr and 2 hr, respectively, before the removal of the tissues. The ^3H-thymidine was injected at a level of 99 μCi/kg body weight, except that a level of 990 μCi/kg body weight was used when the distribution of ^3H in the acid-soluble fraction was under study (i.e., Table 5). (^3H-methyl) thymidine was purchased from New England Nuclear, $Pd(NO_3)_2$ aqueous solution from Research Organic/Inorganic Chemical, and $PtCl_4$ from the latter firm and from B.F. Goldsmith Chemical and Metal.

The administered doses of $PtCl_4$ and $Pd(NO_3)_2$ were 14, 28, and 56 μmole/kg body weight. For $PtCl_4$, these doses corresponded to 2.8, 5.5, and 11 mg Pt^{4+}/kg. For $Pd(NO_3)_2$, the doses by weight were equal to 1.5, 3, and 6 mg Pd^{2+}/kg body weight. The highest dose of Pt, namely 56 μmole/kg, was equal to one-half of the intraperitoneal LD_{50}. However, because of the steep slope of the lethal dose curve, the dose of 56 μmole $PtCl_4$/kg was appreciably less than the LD_5.

Thymidine incorporation was measured in spleen, liver, kidney, and testis. Tissue macromolecules were precipitated with cold 0.5*M* $HClO_4$ and, after centrifugation, the supernatant was collected as the acid-soluble fraction. The RNA in the pellet was hydrolyzed with 0.3*M* NaOH (37°C, 1 hr) and the intact macromolecules were precipitated with cold $HClO_4$ (final concentration of $HClO_4$ not neutralized, 0.5*M*). The DNA was hydrolyzed in hot $HClO_4$ (0.5*M*, 90°C, 20 min). After

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centrifugation, the supernatant (hydrolyzed DNA) was collected. The acid-soluble fraction and the hydrolyzed DNA were analyzed for nucleotide concentration by measuring the absorbance at 260 nm. Tritium was measured by scintillation counting by using a mixture containing toluene and Triton X-100 (*1*).

The radioactivity in the DNA is expressed as counts per minute (cpm)/μmole DNA-nucleotide. The radioactivity in the acid-soluble fraction is expressed as cpm/μmole total acid-soluble nucleotide. The radioactivity is also expressed as a ratio of the two values, i.e., as cpm/μmole DNA-nucleotide divided by cpm/μmole total acid-soluble nucleotide. This ratio takes into consideration two factors: (a) the total amount of radioactivity available for incorporation into tissue DNA, and (b) any variation in isotope concentration in the same tissue of different rats within an experimental group.

N-1 nuclei from hepatic parenchymal cells and N-2 nuclei from hepatic stromal cells were isolated according to the method of Potter and co-workers (*2, 3*). The method is similar in principle to the method used previously in this laboratory for the fractionation of nuclei from control and regenerating liver (*4*).

The absorption of nucleotides and related compounds by charcoal from acidic solution was conducted according to the method of Tsuboi and Price (*5*). The charcoal-absorbed compounds were eluted from the charcoal by two treatments with 1% ammonia in 60% ethanol (*5*). The eluted charcoal-absorbable compounds were chromatographed on thin layer sheets of polyethyleneimine—cellulose with 0.02*M* ammonium acetate–95% ethanol (1:1, v/v). Thymidine and thymine migrated to the solvent front; thymidine phosphates remained at or near the origin.

Results

The effects of $PtCl_4$ on thymidine incorporation into DNA of rat tissues are presented in Table 1. In the tissues studied, the incorporation of thymidine into spleen DNA was the most sensitive to platinum. In spleen, thymidine incorporation was reduced by one-third at the lowest dose of $PtCl_4$. The two higher doses of $PtCl_4$ decreased thymidine incorporation by 50% or more. The ratios (DNA/acid-soluble fraction) gave the same pattern of inhibition. The incorporation of thymidine into liver DNA was not as sensitive to platinum as was the incorporation into spleen DNA. Although no inhibition was observed in liver at a dose of 14 μmole/kg, thymidine incorporation was inhibited by 40% and the ratio approached that observed in spleen at a dose of 56 μmole/kg. At the latter dose, the radioactivity in the acid-soluble fraction (ASF) was increased by approximately 50%. In kidney, thymidine incorporation into DNA was inhibited 40–50% at doses of 28 or 56 μmole/kg. At the two highest doses, the ratios in kidney approached those obtained in spleen. As in the case of liver, the radioactivity in the acid-soluble fraction was increased by approximately 50% at the highest dose of $PtCl_4$. In testis, the thymidine incorporation into

Table 1. Effect of $PtCl_4$ on incorporation of thymidine into DNA of rat tissues.[a]

Tissue	No. of samples	Dose of $PtCl_4$, μmole/kg	DNA		Acid-soluble fraction (ASF)		DNA/ASF	
			cpm/μmole	% of control	cpm/μmole	% of control	Ratio	% of control
Spleen	6	0	1560 ± 160	—	4950 ± 140	—	0.319 ± 0.039	—
	4	14		67 ± 6*		105 ± 4		63 ± 7*
	4	28		48 ± 2†		110 ± 4‡		43 ± 2†3
	6	56		42 ± 3†		105 ± 5		40 ± 5†
Liver	14	0	824 ± 106	—	2050 ± 180	—	0.428 ± 0.064	—
	4	14		100 ± 24		118 ± 19		100 ± 47
	10	28		87 ± 15		113 ± 10		78 ± 14
	8	56		60 ± 11*		147 ± 17*		45 ± 14*
Kidney	8	0	382 ± 58	—	5110 ± 170	—	0.075 ± 0.011	—
	4	14		99 ± 19		99 ± 10		107 ± 31
	4	28		58 ± 5*		118 ± 8‡		50 ± 7*
	8	56		50 ± 4†		151 ± 15†		35 ± 4†
Testis	8	0	402 ± 74	—	6880 ± 320	—	0.057 ± 0.009	—
	4	14		127 ± 8		113 ± 4‡		116 ± 11
	4	28		75 ± 10		114 ± 3*		67 ± 9
	8	56		74 ± 9		118 ± 3†		64 ± 7‡

[a]All values ± S. E. Statistical analysis (*t*-test): dagger (†) denotes $p < 0.01$; asterisk (*), $p < 0.05$; double dagger (‡), $0.05 < p < 0.10$. $PtCl_4$ and ^{3}H-thymidine were injected intraperitoneally at 4 hr and 2 hr, respectively, before removal of the tissues.

Table 2. Effect of $Pd(NO_3)_2$ on incorporation of thymidine into DNA of rat tissues.[a]

Tissue	No. of samples	Dose of $PtCl_4$, μmole/kg	DNA cpm/μmole	DNA % of control	Acid-soluble fraction (ASF) cpm/μmole	ASF % of control	DNA/ASF Ratio	DNA/ASF % of control
Spleen	6	0	1550 ± 200	—	5030 ± 300	—	0.316 ± 0.052	—
	4	14		78 ± 12		119 ± 6‡		65 ± 11
	6	28		52 ± 12*		111 ± 7		49 ± 13*
	7	56		44 ± 11†		107 ± 4		41 ± 11*
Liver	6	0	700 ± 129	—	2230 ± 520	—	0.342 ± 0.047	—
	4	14		73 ± 16		115 ± 27		60 ± 6*
	6	28		70 ± 19		138 ± 22		55 ± 21‡
	7	56		59 ± 15		105 ± 19		55 ± 10*
Kidney	6	0	263 ± 57	—	5590 ± 100	—	0.048 ± 0.011	—
	4	14		88 ± 14		108 ± 7		84 ± 20
	6	28		93 ± 31		104 ± 3		88 ± 29
	7	56		59 ± 14		105 ± 6		56 ± 13
Testis	6	0	380 ± 53	—	6310 ± 250	—	0.062 ± 0.012	—
	4	14		107 ± 7		105 ± 10		102 ± 14
	6	28		59 ± 17‡		108 ± 5		56 ± 18
	7	56		40 ± 9†		118 ± 4*		33 ± 7†

[a]All values ± S.E. Statistical analysis (*t*-test): dagger (†) denotes $p < 0.01$; asterisk (*), $p < 0.05$; double dagger (‡), $0.05 < p < 0.10$. $PtCl_4$ and ^3H-thymidine injected intraperitoneally at 4 hr and 2 hr, respectively, before removal of the tissues.

DNA and the ratio were decreased by 25–35% by doses of $PtCl_4$ of 28 or 56 μmole/kg.

Thymidine incorporation into DNA of spleen was very sensitive to the administration of $Pd(NO_3)_2$, just as it was to $PtCl_4$ (Table 2). Moderate decreases were found in thymidine incorporation into DNA and in the ratio of spleen at the lowest dose of $Pd(NO_3)_2$. At the two highest doses, 50–60% inhibition was observed in the DNA and the ratio. $Pd(NO_3)_2$ decreased incorporation into DNA and the ratio of liver by 25–45%, depending on the dosage. The kidney was relatively resistant to treatment with $Pd(NO_3)_2$. The maximum inhibition in incorporation in kidney was approximately 40% with a corresponding decrease observed in the ratio. Thymidine incorporation into DNA of testis was markedly inhibited at the two higher doses of $Pd(NO_3)_2$ and the inhibition ranged from 40% to 60%; the decrease in the ratio was comparable at each dose.

The administration of Pt or Pd did not cause a decrease in radioactivity in the acid-soluble fraction in any of the tissues studied (Tables 1 and 2). Thus, the administration of either $PtCl_4$ or $Pd(NO_3)_2$ did not decrease the circulation of the radioactive thymidine from its site of injection and/or the entrance of the thymidine into the tissue. Increases in acid-soluble radioactivity were noted in some experiments. The highest dose of Pt caused this effect in liver, kidney and testis but not in spleen. In contrast, the highest dose of Pd caused an increase only in testis.

Potter et al. (*2, 3*) developed a method for the fractionation of liver nuclei into two classes: nuclei derived from parenchymal cells or hepatocytes (called N-1 nuclei) and nuclei derived from stromal

Table 3. Effect of $PtCl_4$ on incorporation of thymidine into nuclei of parenchymal (N-1) and stromal (N-2) cells of liver.[a]

	Experiment A: No $PtCl_4$	Experiment A: $PtCl_4$ = 28 μmole/kg		Experiment B: No $PtCl_4$	Experiment B: $PtCl_4$ = 56 μmole/kg	
DNA, cpm/μmole						
Total cellular	810 ±220	910 ± 260	(112%)[b]	660 ± 230	410 ± 70	(62%)[b]
N-1 nuclei	1590 ± 380	1670 ± 190	(105%)	730 ± 240	400 ± 160	(55%)
N-2 nuclei	700 ± 140	710 ± 130	(101%)	450 ± 60	300 ± 40‡	(65%)
Ratio: DNA/acid-soluble fraction						
Total cellular	0.51 ± 0.15	0.45 ± 0.12	(89%)	0.28 ± 0.04	0.11 ± 0.01*	(41%)
N-1 nuclei	0.99 ± 0.28	0.83 ± 0.14	(84%)	0.31 ± 0.05	0.11 ± 0.02†	(36%)
N-2 nuclei	0.43 ± 0.09	0.34 ± 0.06	(79%)	0.21 ± 0.04	0.09 ± 0.02*	(41%)
DNA ratio						
N-2 nuclei/N-1 nuclei of same sample	0.51 ± 0.15	0.42 ± 0.05		0.71 ± 0.10	0.82 ± 0.18	

[a]Number of samples was 4 at each level of $PtCl_4$ for both Experiments A and B. All values ± S.E. Statistical analysis (*t*-test): dagger (†) denotes $p < 0.01$; asterisk (*), $p < 0.05$; double dagger (‡), $0.05 < p < 0.10$. $PtCl_4$ and ^3H-thymidine were injected intraperitoneally at 4 hr and 2 hr, respectively, before removal of tissues.

[b]Values in parentheses are % of the control values in the same experiment.

Table 4. Effect of $PtCl_4$ on incorporation of thymidine into tissues of CCl_4-treated rats.[a]

Tissue	Group	Dose of CCl_4, ml/kg	Dose of $PtCl_4$, μmole/kg	No. of samples	DNA cpm/μmole	DNA % of group B	ASF cpm/μmole	ASF % of group B	DNA/ASF Ratio	DNA/ASF % of group B
Spleen	A	0	0	3	1800 ± 200	91	3790 ± 1120	102	0.57 ± 0.16	82
	B	1.0	14	3	1990 ± 530	—	3720 ± 850	—	0.70 ± 0.37	—
	C	1.0	28	4	1410 ± 210	71	4420 ± 490	119	0.35 ± 0.11	50
Liver	A	0	0	4	580 ± 130	10	1850 ± 90	58	0.32 ± 0.09	16
	B	1.0	14	5	6070 ± 1540	—	3180 ± 690	—	2.00 ± 0.50	—
	C	1.0	28	4	7610 ± 1940	125	2710 ± 1030	85	3.15 ± 0.46	157
Kidney	A	0	0	4	300 ± 60	100	6240 ± 1150	108	0.052 ± 0.012	100
	B	1.0	14	5	300 ± 50	—	5790 ± 290	—	0.052 ± 0.009	—
	C	1.0	28	4	200 ± 40	67	7090 ± 1530	122	0.032 ± 0.009	61
Testis	A	0	0	4	540 ± 80	114	7450 ± 120	106	0.073 ± 0.012	108
	B	1.0	14	5	470 ± 50	—	7050 ± 290	—	0.067 ± 0.005	—
	C	1.0	28	4	310 ± 40*	65	6860 ± 360	97	0.039 ± 0.011†	58

[a]All values ± S.E. Statistical analysis (t-test): asterisk (*) denotes $p < 0.05$; dagger (†), $0.05 < p < 0.10$. CCl_4, diluted in corn oil, was injected intraperitoneally at 42 hr, $PtCl_4$ at 4 hr, and ^{3}H-thymidine at 2 hr before removal of the tissues.

cells or non-hepatocytes (called N-2 nuclei). The effects of $PtCl_4$ on the incorporation of thymidine into DNA of N-1 and N-2 nuclei are presented in Table 3. In experiment A, no inhibition in thymidine incorporation occurred in either class of nuclei, and only a small decrease in the ratio was observed at a dose of $PtCl_4$ of 28 μmole/kg. However, in experiment B, a $PtCl_4$ dose of 56 μmole/kg decreased thymidine incorporation into DNA to 60% of control values. This dose of $PtCl_4$ also decreased the ratios to approximately 40% of control values. Thus, in experiment B, thymidine incorporation into both N-1 and N-2 nuclei was depressed equally when expressed either as DNA specific activities or as the ratios. Therefore, thymidine incorporation was inhibited to an equal extent in both parenchymal cells and stromal cells.

In control rats, the specific activities of DNA are in the following decreasing order: DNA of N-1 nuclei > total cellular DNA > DNA of N-2 nuclei; the specific activities of DNA from N-2 nuclei are approximately two-thirds those of DNA of N-1 nuclei. In Table 3 the specific activities of DNA from N-2 nuclei are expressed as a fraction of the specific activities of DNA from N-1 nuclei in the same experiment. Although the higher dose of $PtCl_4$ (Table 3) markedly inhibits thymidine incorporation in both cell types, the ratio of the N-2 to N-1 specific activities is not significantly altered by treatment with $PtCl_4$.

The administration of CCl_4 to rats results in the death of some hepatic cells and a rapid DNA synthesis and mitotic activity in surviving cells in order to replace the lost tissue. In data not shown, it was found that thymidine incorporation into DNA is approximately 8 to 10-fold greater in liver of the CCl_4-treated rats than in the control rats. Incorporation has been studied in four tissues of rats which received CCl_4 and $PtCl_4$ (Table 4). The data for each tissue are compared to the values of animals in group B, which received CCl_4 and a low dose of $PtCl_4$.

In liver, thymidine incorporation into DNA of group B rats was 10-fold greater than that of rats receiving no CCl_4. Moreover, increasing the dose of $PtCl_4$ to 28 μmole/kg did not inhibit thymidine incorporation into liver DNA of CCl_4-treated rats. For spleen, kidney, and testis, thymidine incorporation into DNA of each tissue of groups A and B was essentially equal. In these three tissues, in contrast to the pattern seen in liver, thymidine incorporation in rats of group C (CCl_4 and 28 μmole $PtCl_4$/kg) was decreased approximately 30% in comparison with group B animals. The same pattern of results was obtained for all three tissues if the ratios of DNA to acid-soluble fraction were examined. The ratio in liver of group C animals was apparently (but not statistically) greater than in liver of group B rats; in contrast, the ratios in spleen, kidney, and testis were approximately 40% less in group C animals. Thus, $PtCl_4$ (at 28 μmole/kg) apparently did not inhibit the stimulated synthesis of DNA of liver in CCl_4-treated rats but did inhibit thymidine incorporation into DNA of the other three tissues, similar to the pattern seen above (Table 1).

In these experiments, the radioactivity in the acid-soluble fraction has been used as a reference for the total availability of radioactive precursor in individual tissue samples. It was of interest, therefore, to examine the distribution of radioactivity in the acid-soluble fraction. The acid-soluble

fractions of liver and spleen were examined in control animals and in rats treated with 56 μmole $PtCl_4$/kg, the highest dose used in the prior incorporation studies.

Charcoal adsorption was used to separate intact pyrimidine compounds from their open-ring metabolites. Only those compounds which had the pyrimidine ring intact were adsorbed by charcoal from an acidic solution. At the end of the 2-hr incorporation interval, the majority of the radioactivity in the acid-soluble fraction was in the form of the open-chain metabolites and other nonadsorbed metabolites in liver and spleen of control rats (Table 5). In liver, 30% of the radioactivity in the acid-soluble fraction was in the form of compounds

Table 5. Distribution of ^{3}H-labeled compounds in the acid-soluble fraction.[a]

	Liver		Spleen	
	No $PtCl_4$	$PtCl_4$ = 56 μmole/kg	No $PtCl_4$	$PtCl_4$ = 56 μmole/kg
Charcoal adsorption, % of ^{3}H in acid-soluble fraction				
Intact pyrimidine compounds (adsorbed)	30 (28, 31)	28 (22, 34)	22 (20, 24)	23 (19, 26)
Pyrimidine catabolites (not adsorbed)	70 (69, 72)	72 (66, 78)	78 (76, 80)	77 (74, 81)
Thin layer chromatography, % of ^{3}H of charcoal-adsorbable fraction				
Thymidine phosphates	48 (47, 48)	44 (37, 51)	54 (54, 55)	59 (54, 64)
Thymidine and thymine mixture	43 (43, 44)	47 (42, 52)	41 (41, 42)	36 (33, 40)

[a]Means of values of two rats; percentage values of each rat are given in parentheses. $PtCl_4$ and ^{3}H-labeled thymidine were injected intraperitoneally 4 hr and 2 hr, respectively, before removal of the tissues.

having the pyrimidine ring intact. Furthermore, the administration of the highest dose of $PtCl_4$ used in these experiments did not alter this distribution. In spleen the situation was similar. Approximately 22% of the radioactivity in the acid-soluble fraction of spleen of control rats was in the form of intact pyrimidine compounds, and the administration of $PtCl_4$ did not alter this value.

The charcoal-adsorbable compounds were separated by thin-layer chromatography on polyethyleneimine-cellulose. Two classes of compounds were separated: (a) the thymidine phosphates, and (b) a mixture of thymidine and thymine. In liver and spleen of control animals, one-half of the total charcoal-adsorbable radioactivity was in the form of thymidine phosphates (Table 5). Administration of $PtCl_4$ did not appreciably alter the values in these two tissues.

Discussion

The present study indicates that $PtCl_4$ and $Pd(NO_3)_2$ inhibit the synthesis of DNA as measured by the incorporation of radioactive thymidine. Waters et al. (*6*) report that the incorporation of thymidine into DNA is more sensitive to inhibition by $PtCl_4$ than the incorporation of uridine into RNA or of leucine into protein in cultured cells. The inhibition by $PtCl_4$ may be analogous to the effect of Pt-containing antitumor compounds (*7–9*). The inhibition of thymidine incorporation into DNA is consistent with an inhibition of DNA polymerase due to the interaction of the metallic cations with the template DNA. The interaction *in vitro* of the Pt-containing antitumor compounds and of Pd^{2+} ions with DNA has been demonstrated (*10–12*).

The structural features of the active antitumor, Pt-containing compounds have a major role in the activities of these compounds (*8*). It is unresolved what modifications in biological effects are made by the selection of the salt $PtCl_4$ for these studies. It is unknown whether the rates of ionization or hydration, and resultant biological effects, may be significantly different if an alternate soluble Pt^{4+} salt such as $Pt(SO_4)_2$ had been selected for these studies.

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