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METABOLISM OF PLATINUM [^{14}C]ETHYLENEDIAMINE DICHLORIDE IN THE RAT

DAVID M. TAYLOR, JULIE D. JONES and A. BRIAN ROBINS
Division of Biophysics, Institute of Cancer Research, Belmont, Sutton, Surrey, England

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Abstract—The distribution of Pt [^{14}C]ethylenediamine dichloride (Pt [^{14}C]en Cl_2) and ^{195m}Pt [^{14}C]en Cl_2 has been studied for a period of up to 7 days in the tissues of normal and tumour-bearing rats. The half-life of retention of the drug in the tissues ranged from 2·5 to 7·6 days. The rate of clearance was most rapid in the serum and tumour and slowest in kidney, pancreas and liver. Studies of the subcellular distribution of Pt [^{14}C]en Cl_2 in liver, kidney and two transplanted tumours showed that the drug was localized mainly in the cytosol in the form of low molecular weight complexes. The concentration of the drug in different small areas of a tumour was found to vary by a factor of 2·7. The distribution of the ^{14}C and the ^{195m}Pt was identical following administration of the doubly labelled drug suggesting that the Pt ethylenediamine complex does not dissociate *in vivo*.

SINCE THE discovery of the cytostatic effects of certain platinum compounds by Rosenberg *et al.*[1] there has been considerable interest in the potential value of these compounds for cancer chemotherapy. The effects of platinum compounds on tumour growth[2] and on DNA synthesis in various tissues[3,4] have been described and a number of groups of workers have studied the interactions of these compounds with DNA and nucleic acid constituents.[5–7] However, the distribution and turnover of platinum compounds in animal tissues has been little studied. The ideal radioactive label for studies of the metabolism of platinum drugs would be a radionuclide of platinum. Several platinum radionuclides are known but most have short half-lives and are difficult to prepare at a suitable specific activity for tracer studies. The 3-day half-life isotope ^{191}Pt was used in studies of the distribution of *cis*-Pt $(NH_3)_2$ Cl_2 in bacteria[8] and recently the 4-day half-life ^{193m}Pt has been used to study the distribution of this compound in mice and rabbits.[9]

This paper describes studies of the tissue distribution and retention in normal and tumour-bearing rats of another cytotoxic platinum compound Pt (en) Cl_2 labelled with ^{14}C in the ethylenediamine (en) moiety and, for some experiments, also with ^{195m}Pt ($t_{\frac{1}{2}}$ 4·1 day γ 0·099 MeV). Some studies on the subcellular distribution of Pt [^{14}C]en Cl_2 in normal and tumour tissues, and the distribution of the drug within small areas of tumour, are also reported.

MATERIALS AND METHODS

The animals used were rats of the highly inbred August and Marshall strains. The rats which were 8–10 week old, 200 g body wt, at the start of each experiment were maintained on MRC Diet 41B, both food and water being allowed *ad lib*. For some experiments animals bearing subcutaneously implanted tumours were used.

The two tumour lines were BICR/A3, a radiation-induced osteogenic sarcoma with a tumour doubling time of 2–3 days and the BICR/M2, a slow-growing fibrosarcoma with a tumour doubling time of about 16 days. The growth rates of established tumours of both these lines were reduced significantly by a single dose of 4 mg Pt(en) Cl_2/kg).

The [^{14}C]-labelled and ^{195m}Pt-labelled Pt (en) Cl_2 were prepared by the methods described previously.[7] Both radionuclides were obtained from the Radiochemical Centre, Amersham. The radioactivity due to ^{14}C was determined by liquid scintillation counting of solutions of the tissues in 1 M NaOH using an emulsion scintillant comprising 2 vol. Tergitol TP9 (Union Carbide Ltd.) to 1 vol. 6 g/l. Butyl PBD in toluene. Gamma-ray emission from ^{195m}Pt was measured by counting in a NaI(Tl) well-type crystal automatic gamma-ray spectrometer.

The subcellular fractions were prepared and characterized by the methods described by Worwood and Taylor.[10]

RESULTS

The concentrations of ^{14}C in the tissues of normal male August rats at 24 hr after intraperitoneal injection of 4 mg (20 μCi) Pt [^{14}C]en Cl_2/kg body wt are shown in Table 1. The highest concentration is found in the kidney, which contains about three-

TABLE 1. TISSUE DISTRIBUTION OF Pt [^{14}C]en Cl_2 AT 24 hr AFTER INTRAPERITONEAL INJECTION INTO MALE AUGUST RATS

Tissue	No. of animals	% Dose/g ± S.D.
Liver	10	0·34 ± 0·13
Kidney	21	0·93 ± 0·41
Spleen	10	0·30 ± 0·25
Pancreas	9	0·30 ± 0·15
Testis	3	0·066 ± 0·015
Lung	9	0·29 ± 0·22
Heart	4	0·18 ± 0·15
Muscle	1	0·27
Brain	1	0·12
Bone marrow	1	0·04
Plasma	3	0·16 ± 0·08
(% in total plasma volume)	3	(1·18 ± 0·45)

times as much radioactivity as the liver, spleen, pancreas or lung. The concentrations observed in testes and bone marrow are very low.

The retention of ^{14}C in the kidney, pancreas, blood serum, liver, ileum and tumour over a period of 7 days after intraperitoneal injection of 4 mg Pt [^{14}C]en Cl_2/kg into female August rats bearing subcutaneously implanted BICR/A3 tumour is illustrated in Fig. 1. The retention of ^{14}C in all these tissues between 1 and 7 days after injection can be described by exponential equations of the type

$$R = a\,e^{-bt}$$

when R is the percentage of the injected dose retained/gram tissue at time t and a and b are constants.

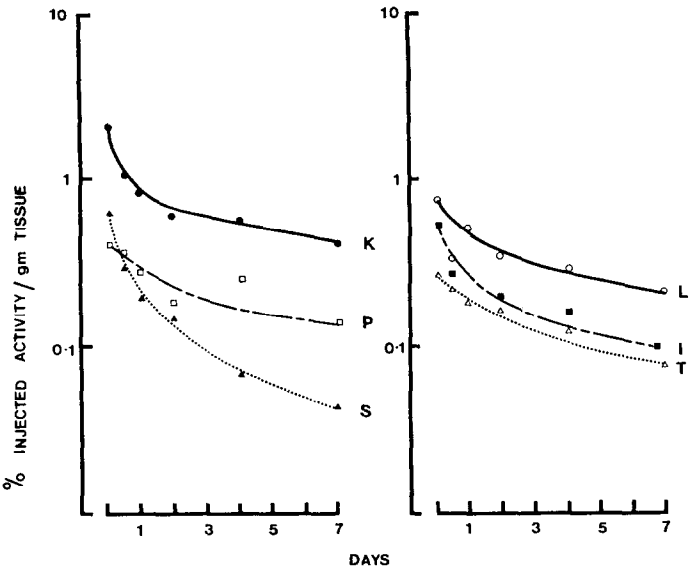

FIG. 1. The retention of radioactivity in kidney (K); pancreas (P); serum (S); liver (L); ileum (I) and BICR/A3 (T) tumour over a period of 7 days following intraperitoneal injection of 4 mg (20 μC) Pt [^{14}C]en Cl_2/kg body wt into female August rats.

The values for a and b and for the half-lives of retention of ^{14}C in the various tissues, as calculated by the method of least squares, are listed in Table 2. It is apparent that the rate of loss of the radioactive label, which is greatest for the serum and lowest for pancreas, liver and kidney, is relatively slow.

In order to determine if the distribution of ^{14}C in tissues was a true measure of the distribution of Pt (en) Cl_2 the tissue distribution of radioactivity was compared at 24 hr after intraperitoneal injection of [^{14}C]ethylenediamine or ^{195m}Pt [^{14}C]en Cl_2. The results illustrated in Fig. 2 show that there is close agreement between the distribution of ^{195m}Pt and ^{14}C in the tissues studied. The concentrations of ^{14}C are generally lower after administration of [^{14}C]en than after administration of Pt [^{14}C]en Cl_2. Previous studies[4] have shown that less than 0·5 per cent of the injected ^{14}C is lost as $^{14}CO_2$ during the first 4·5 hr after injection of Pt [^{14}C]en Cl_2.

TABLE 2. HALF-LIVES OF RETENTION OF Pt [^{14}C]en Cl_2 IN FEMALE AUGUST RAT TISSUES BETWEEN 1 AND 7 DAYS

Tissue	a	$b \pm$ S.E.	Half-life (days)
Kidney	0·80	−0·092 ± 0·015	7·6 (6·5–9·1)
Liver	0·43	−0·106 ± 0·022	6·5 (5·4–8·2)
Pancreas	0·28	−0·099 ± 0·046	7·0 (4·7–13·2)
Ileum	0·25	−0·191 ± 0·041	4·9 (3·8–6·9)
BICR/A3 tumour	0·21	−0·146 ± 0·020	4·7 (4·2–5·5)
Serum	0·30	−0·276 ± 0·024	2·5 (2·3–2·7)

($R = a\,e^{-bt}$ % Dose/g).

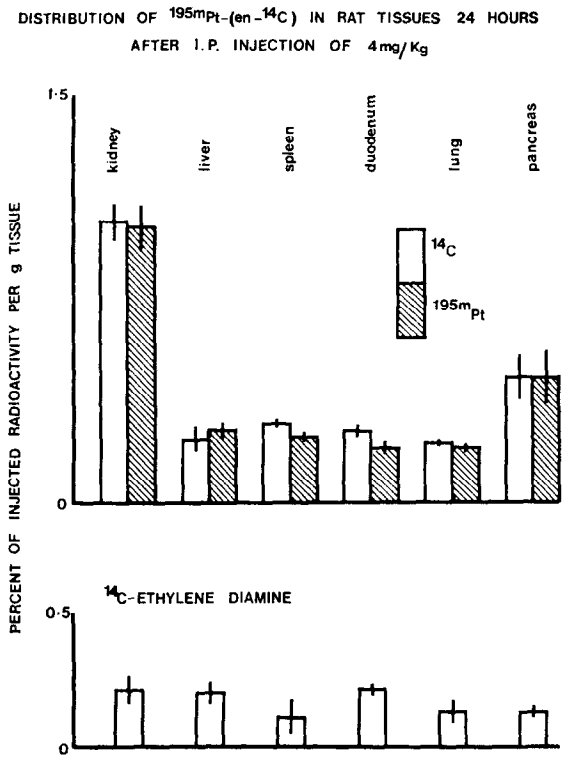

FIG. 2. The distribution of ^{195m}Pt and ^{14}C in rat tissues at 24 hr after intraperitoneal injection of 4 mg (20 μCi ^{14}C + 20 μCi ^{195m}Pt) ^{195m}Pt [^{14}C]en Cl_2/kg into male August rats. The lower picture shows the tissue distribution of ^{14}C at 24 hr after i.p. injection of an equimolar amount of [^{14}C]ethylenediamine.

These results all suggest that the Pt–ethylenediamine complex does not dissociate to any marked extent *in vivo* and that following administration of Pt [^{14}C]en Cl_2 the distribution of ^{14}C provides a good measure of the distribution of the drug.

The subcellular distribution of Pt [^{14}C]en Cl_2 was studied in liver and kidney and in two different transplanted tumours at 24 hr after intraperitoneal injection of 4 mg drug/kg body wt. The distribution of radioactivity in five fractions, nuclei-cell debris, mitochondria, lysosomes, microsomes and cytosol, is shown for each of these tissues in Fig. 3. Although the fractionation procedures used were relatively crude it is apparent that in the liver and tumour samples there is no evidence of a specific association of the drug with any organelle and that a very large proportion of the ^{14}C is located in the cytosol. In the kidney, however, although most of the radioactivity was recovered in the cytosol there is evidence of some specific association of the drug with the microsomal fraction. The two tumours contained much fibrous material and were homogenized only with difficulty. Both tumours showed a high proportion of the ^{14}C in the nuclei cell debris fraction but this fraction also contained a similarly high proportion of the total cytochrome oxidase activity of the homogenate; thus the high proportion of the ^{14}C in the nuclei-cell debris fractions of the tumours is probably accounted for largely by that contained in unbroken cells. Similar considerations

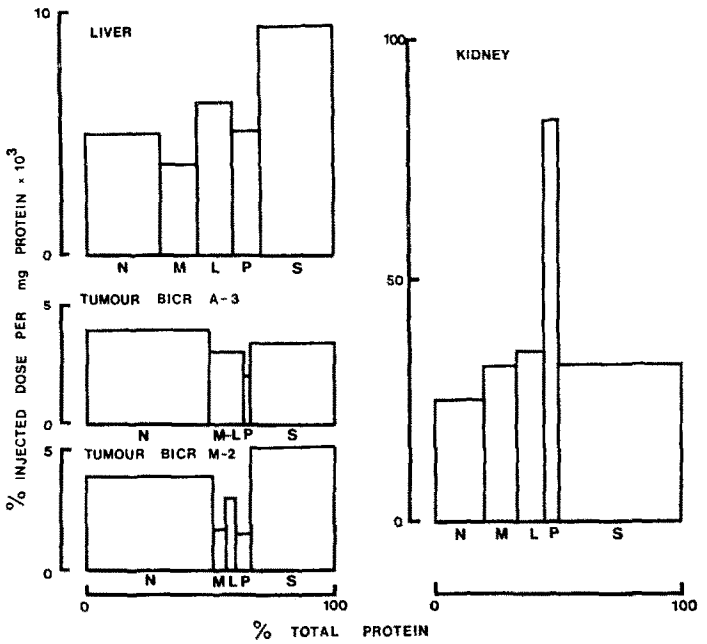

FIG. 3. The subcellular distribution of Pt [^{14}C]en Cl_2 in liver and kidney of August rats and in two transplanted rat tumours. N, Nuclei-cell debris fraction (400 *g* × 10 min); M, Mitochondria (3300 *g* × 10 min); L, lysosomal fraction (25,000 *g* × 10 min); P, microsomal fraction (105,000 *g* × 65 min); S, cytosol (105,000 *g* supernatant).

probably apply to this fraction in the liver and kidney since after nuclear purification, by centrifugation through 2·2 M sucrose + 0·003 M $CaCl_2$ for 60 min at 45,000 *g*, only a very small proportion of the [^{14}C]-labelled drug was found to be associated with the nuclei.

The radioactivity contained in the cytosol of the BICR/M2 tumour was not precipitated by 0·2 M perchloric acid. Filtration through a 25 nm pore diameter membrane filter showed that almost all the radioactivity passed into the filtrate. Fractionation of the cytosol by gel filtration on Sephadex, G-25, G-100 or G-200 also showed that most of the radioactivity from tumour, liver or kidney cytosols was associated with components, of molecular weight less than 10,000 daltons, whose chromatographic behaviour was different from that of the free drug.

In tumour chemotherapy one of the important factors is the amount of the cytotoxic agent which reaches the tumour and its distribution with different areas of the tumour. The results in Fig. 1 and Table 2 show that the overall concentration and retention of drug in the BICR/A3 tumour is similar to those observed in tissues such as pancreas and ileum. The uptake in the BICR/M2 tumour was about 0·2 per cent injected dose/g tumour which is very similar to that found for the BICR/A3 tumour. In order to study the distribution of drug within a tumour, a BICR/A3 tumour, measuring 2·1 × 1·6 cm was removed from a rat 1 hr after intraperitoneal injection of 4 mg Pt [^{14}C]en Cl_2/kg. The tumour was cut longitudinally and a 3 mm thick slice was removed and cut into a series of approx. 2 mm^2 pieces. The pieces were weighed and

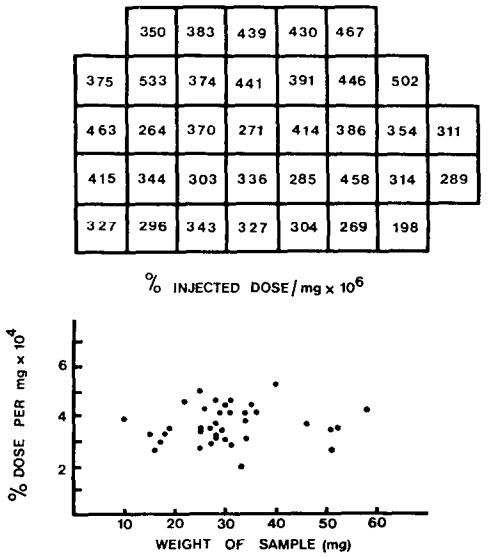

FIG. 4. The concentration of ^{14}C in approx. 3 × 2 × 2 mm fragments of a BICR/A3 tumour taken from an August rat 1 hr after injection of 4 mg (20 μCi) Pt [^{14}C]en Cl_2/kg body wt. The lower picture represents the individual concentrations plotted against the mass of the tumour sample measured.

assayed for ^{14}C. The results are shown in Fig. 4, the data show that the activity/milligram of tumour did not vary systematically with the weight of sample taken. However, there were differences in the concentration of ^{14}C observed in different areas of the tumour, the ratio between the lowest and the highest concentration was 2·7:1. There was no macroscopic evidence of a correlation between areas of high or low drug uptake and regions of necrosis. More detailed studies are required to determine the relationships between drug uptake and biochemical activity in tumours.

DISCUSSION

The data presented in Table 1 and Figs. 1 and 2 suggest that the distribution of Pt (en) Cl_2 in rat tissues has a similar pattern to those observed by Lange *et al.*[9] in rabbit and mouse tissues following injection of ^{193m}Pt $(NH_3)_2$ Cl_2.

The retention data, Fig. 1 and Table 2, indicate that the Pt (en) Cl_2 is only slowly cleared from tissues. This prolonged retention of the drug appears to correlate with the long-term effects of several platinum drugs on DNA synthesis which have been observed in the kidney and intestine.*[4] However, the cell fractionation studies suggest that the drug is retained in tissues in bound form rather than as the free drug and this raises the question as to whether, in bound form, the drug retains cytotoxic activity. Studies with mouse lymphoma cells (L5178Y) in culture suggest that Pt (en) Cl_2 rapidly loses its cytotoxic activity when incubated in the presence of horse serum.† Only a small proportion of the Pt (en) Cl_2 appears to be bound to the cell nuclei but, in view of the known reactions of the platinum drugs with DNA and nucleotides,[5,7] it

* J. D. Jones and D. M. Taylor, unpublished observations.
† A. B. Robins, unpublished observations.

is conceivable that the small amount retained in the nuclei may be sufficient to account for the long-term effects on DNA synthesis.

The studies reported here confirm our earlier suggestion[4] that a [^{14}C]-label on the ethylenediamine moiety of Pt [^{14}C]en Cl_2 provides a satisfactory tracer for the platinum complex. In all the tissues studied the retention of this drug is prolonged and this may account for the long-term effects of the drug on DNA synthesis.

REFERENCES

1. B. Rosenberg, L. van Camp and T. Krigas, *Nature, Lond.* **205,** 688 (1965).
2. B. Rosenberg, L. van Camp, J. E. Trosko and V. H. Mansour, *Nature, Lond.* **222,** 385 (1967).
3. R. J. Kociba and S. D. Sleight, *Cancer Chemother.* **54,** 325 (1970).
4. J. D. Jones, A. B. Robins and D. M. Taylor, *Proc. Seventh Internat. Cong. Chemother.*, in press.
5. J. J. Roberts and J. M. Pascoe, *Nature, Lond.* **235,** 282 (1972).
6. J. A. Howle, G. R. Gale and A. B. Smith, *Biochem. Pharmac.* **21,** 1465 (1972).
7. A. B. Robins, *Chem. Biol. Int.* **6,** 35 (1973).
8. E. Renshaw and A. J. Thomson, *J. Bact.* **94,** 1915 (1967).
9. R. C. Lange, R. P. Spencer and H. C. Harder, *J. Nuc. Med.* **13,** 328 (1972).
10. M. Worwood and D. M. Taylor, *Biochem. Med.* **3,** 105 (1969).