bound to platinum they arrange stereospecifically in a propeller-like structure in which strong and spatially oriented hydrogen bonds between O(6) of guo and the NH_2 groups of the diamine stabilize the structure, but only with diamines of *S*-absolute configuration [6]. Deprotonation of guo at N(1), which occurs at a pH lower than in free guo ($pK_a = 7.9$ vs 9.25 [7]) and leads to the enol tautomer, is accompanied by profound changes in the conformation of these complexes. At least in one instance, [{(*R,R*)-chxn}Pt-{guo(-H)}$_2$], the propeller-like structure is completely lost.

In the case of the monoguanosine complexes $[(diam)Pt(guo)]^{2+}$ and $[(diam)Pt\{guo(-H)\}]^{+}$ neither the configuration of the diamine, nor deprotonation of guo have great influence on the chiroptical properties of the complexes. It has been proposed that in these complexes guo is chelated to Pt through N(7) and O(6) [8]. However, although some shifts in the frequencies of ν(C=O) and of ν(C(4)=C(5) + (C(5)–C(6)) in the IR spectra suggest a perturbation of O(6), we failed to detect the expected coupling of C(6) with ^{195}Pt in the ^{13}C NMR spectra. It could be, therefore, that chelation is achieved via an outer sphere coordination of O(6) through a bridging water molecule directly coordinated to the metal as found in some instances [9].

The absence of chiral discrimination observed in the interaction with DNA does not agree with the results obtained with our model compounds. It can be suggested that the cisplatin-DNA interaction modifies the essential conformational features of DNA, at least in the neighbourhood of the site of attack, in such a way that makes any chiral recognition irrelevant.

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V7

Chelates of Indium-111 as Agents for Labelling Human Granulocytes for Clinical Use

F. BRADY, H. J. DANPURE, S. OSMAN and D. J. SILVESTER

Medical Research Council, Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 0HS, U.K.

Chelates of the cyclotron produced radionuclide indium-111 ($t_{½}$ 2.8d) with 8-hydroxyquinoline [1] (oxine) and acetylacetone [2, 3] (acac) have been used for labelling human blood cells in diagnostic nuclear medicine [4, 5]. However, before labelling cells with oxine or acac complexes, the cells must be washed free from plasma before efficient labelling can be achieved. This may result in damage to the cells. Consequently we introduced the clinical use of tropolone (2-hydroxy-cycloheptatrien-1-one) with ^{111}In as an alternative cell labelling agent [6]. Indium tropolonate has the advantage of being able to label cells efficiently in the presence of plasma. Thus the time and degree of manipulation required for labelling is reduced and enhanced clinical results are also being obtained using granulocytes labelled with ^{111}In-tropolonate [7, 8].

Although tropolone offers considerable advantage over oxine and acac, all three still label blood cells non-selectively. It would be highly desirable clinically to find a ligand for ^{111}In which would selectively label only the cell type of interest. Labelling could then be carried out in whole blood, obviating the need for tedious cell separations and concomittant risk of cell damage. As part of a study to understand the mechanisms involved in cell-labelling and to produce more efficient and possibly more specific labelling agents, we have examined a series of indium-111 tris chelates with bidentate ligands for their ability to label granulocytes with ^{111}In.

Experimental

Materials. The following chemicals were used as supplied. Tropolone (2-hydroxy-cycloheptatrien-1-one); N,N′-dimethyldithiocarbamate; N,N′-diethyldithiocarbamate (Fluka). Acetylacetone (Sigma) was distilled before use. β-thujaplicin (4-iso-propyltropolone) (Roth) was converted to the more water soluble sodium salt [9] and recrystallised from water as the dihydrate. 8-hydroxyquinoline sulphate (Sigma) was recrystallised from water/ethanol. Purpurogallin (Aldrich) was recrystallised from anisole. The following coumpounds were synthesised according to reported methods; 7-carboxy-6-carboxymethyltropolone [10]; 6-methyltropolone [10]; ammonium 5-sulphonyltropolone [11].

TABLE I. Comparative Efficiency of Labelling Granulocytes with Various $^{111}InL_3$ Complexes in Plasma and Buffer Solutions.

Ligand L	Labelling Efficiency[a] (%)	
	Plasma[b]	Buffer[c]
Acetylacetone	[d]	86 ± 2 (4×10^{-2})
Oxine[e]	[f]	71 ± 1 (4×10^{-6})
Tropolone	17 ± 3 (4×10^{-4})	70 ± 1 (4×10^{-5})
6-methyltropolone	12 ± 3 (2×10^{-4})	64 ± 7 (4×10^{-5})
4-iso-propyltropolone[g]	14 ± 1 (4×10^{-4})	68 ± 1 (4×10^{-6})
N,N'-dimethyldithiocarbamate	29 ± 6 (1×10^{-4})	77 ± 3 (1×10^{-4})
N,N'-diethyldithiocarbamate	9 ± 1 (1×10^{-4})	66 ± 1 (1×10^{-5})

[a]Expressed as the ratio of activity remaining on the cells to that on the cells plus that in the supernatant. For cell concentration 5×10^6 cm^{-3} granulocytes. The optimum ligand concentration is shown in parentheses. (mol dm^{-3}). [b]Plasma (1.0 cm^3) + Hepes-saline buffer (0.1 cm^3). [c]Hepes-saline buffer. [d]Could not be determined for this cell concentration. At a granulocyte concentration of 2×10^7 cm^{-3} labelling efficiency was 28% in plasma with a ligand concentration of 5×10^{-2} mol dm^{-3}. [e]As 8-hydroxyquinoline sulphate. [f]Could not be determined for this cell concentration. At a granulocyte concentration of 2×10^7 cm^{-3} labelling efficiency was 9% in plasma and the optimum ligand concentration was 4×10^{-5} mol dm^{-3}. [g]As the sodium salt dihydrate.

Separation and Labelling of Granulocytes. Separation of granulocytes from fresh samples of venous blood and labelling with indium-111 complexes were carried out in a similar manner to that which we have previously described for labelling with indium-111-tropolonate [6].

Results and Discussion

The comparative efficiency of labelling granulocytes with a series of chelates of ^{111}In in plasma and buffer solution is shown in Table I, together with the optimum ligand concentrations for labelling. Indium-111 complexes with the seven ligands shown all label granulocytes very efficiently in buffer (64–86%). In plasma, however, the labelling efficiency is greatly reduced (9–29%). The largest reductions are for acac and oxine, for which labelling in plasma was negligible (for granulocyte concentrations of 5×10^6 cm^{-3}). Both 7-carboxy-6-carboxymethyltropolone and tropolone-5-sulphonate (neither shown in table) failed to label cells in either plasma or buffer.

The results support and extend our own previous observations of the existence of an optimum ligand concentration, which usually differs in plasma and buffer, and of the inhibition of labelling with ^{111}In-tropolonate by plasma proteins [6]. The sensitivity of labelling efficiency to gross changes in the ligand observed here is probably a consequence of differences in interaction with plasma proteins. Rao and Dewanjee propose that differences in labelling efficiencies of oxine, acac and tropolone complexes of ^{111}In with either platelets [12] or erythrocytes [13] are influenced largely by a combination of differences in partition coefficients and stability constants of the complexes. Our results with substituted tropolones do not support this view, but suggest a more complex model.

While all the ligands presented in Table I label cells efficiently in buffer solution, only N,N'-dimethyldithiocarbamate appears to offer any advantage over tropolone for labelling cells in plasma.

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