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Complexation of tetracylines with metal ions in relation to photosensitization

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It has been shown by spectroscopy and by a Photosensitive Index method, involving measurements of interfacial tension, that Cu^{2+} and Ni^{2+} form 2:1 complexes with oxytetracycline and 1:1 complexes with demethylchlortetracycline. Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+} form weaker 1:1 complexes with both drugs. The possibility is discussed of using metal ions to reduce photosensitization by tetracyclines in-vivo.

It is known that the absorption and therapeutic action of tetracyclines is reduced if the patient is taking milk, which contains Ca^{2+} (Scheinier & Altemeier 1962), or antacids which contain di or trivalent metals (Sweeney et al 1957), or is being prescribed iron salts for anaemia (Neuvonen et al 1970). Children receiving tetracyclines may develop brown discolouration of their teeth and exhibit unsatisfactory bone growth (Shulman & Sellers 1971). These effects have been ascribed to the formation of complexes between the drugs and various metal ions. The action of these drugs as antimicrobial agents (Skinner & Nalbandian 1975) and as agents for reducing the toxicity of Be^{2+} in rats (Lindenbaum et al 1954) appear to be due to the same cause.

In the present work spectroscopic and other techniques have been used to investigate the formation of complexes between two tetracyclines and various metal ions. The possibility is raised of employing complex formations for reducing the tendency of tetracyclines to cause photosensitization in patients when they are exposed to sunlight.

MATERIALS AND METHODS

Materials

Isopropylbenzene (99% Aldrich) was further purified by repeated filtration through freshly prepared beds of Fuller's earth, 2·5 cm thick.

The best available grades of oxytetracycline hydrochloride (Pfizer) and demethylchlortetracycline hydrochloride (Lederle) were used without further purification. The metal salts were AR grades from BDH.

Triple distilled water was obtained from an all glass still (surface tension 72·5 mNm^{-1} at 20 °C; specific conductivity $1·3 \times 10^{-6}$ ohm^{-1} cm^{-1} at 20 °C and pH 5·3).

Apparatus and procedure

15 cm^3 of isopropylbenzene was floated on 20 cm^3 of water containing between 0 and 10^{-4} M of the drug and between 0 and 5×10^{-4} M of Na^+ or of a divalent metal ion to form a layer at least 0·6 cm deep. Samples were irradiated with uv light for 240 min at 25 ± 2 °C using a 125 W Philips MB/U lamp fitted with a silica filter which transmitted in the range 290–360 nm with an intensity of $1·5 \times 10^{-9}$ einstein cm^{-2} s^{-1}. After the samples had been allowed to equilibrate for 10 min in the dark their interfacial tensions were measured with a du Nouy tensiometer (White Electrical Co.) using a platinum ring (circumference of 4 cm) and employing the equation of Zuidema & Waters (1941). The photosensitive indices (PI) of the samples were calculated from the equation of Riaz & Pilpel (1983a).

The strength and stoichiometry of the complexes formed between tetracyclines and the metal ions were investigated by making use of the PI values and by employing uv and fluorescence spectroscopy (Carl Zeiss spectrophotometer). The procedure was to measure the changes that occurred in PI, in the uv absorbance λ_{max} and ΔA and in the percentage fluorescence intensity $\Delta F(\%)$ of 10^{-5} to 10^{-4} M aqueous solutions of the two drugs when between 0 and 5×10^{-3} M of metal ions were added to them.

RESULTS AND DISCUSSION

The maxima in the uv absorption spectra (λ_{max}) of the aqueous solutions of the two tetracyclines shifted to longer wavelengths when various divalent metal ions were added to them. The values of these bathochromic shifts are in Table 1. It may be assumed that the shifts are caused by complex

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formation between the tetracyclines and the metal ions (Albert & Rees 1956; Conover 1956; Colaizzi et al 1965; Caswell & Hutchinson 1971). To determine the strength and stoichiometry of the complexes, graphs were plotted between the values of PI, ΔA and ΔF% and the ratios of the molar concentrations of metal to drug in solution (Meyer & Ayres 1957). Fig. 1(a, b) shows that there was a general decrease in the PI values as the above ratio was increased. Since the PI value is a measure of the extent to which uv radiation is decomposing the sample to form surface active products, it follows that a decrease in PI implies less decomposition. This could be due to the formation of complexes between tetracyclines and metals which appear to be more stable to uv than the drugs on their own (Albert & Rees 1956; Riaz & Pilpel 1983b). This shows that the metals were forming complexes with the drugs and thereby inhibiting their decomposition by ultraviolet light. Cu^{2+} was more effective than Ni^{2+} or Fe^{2+} and these were more effective than the other metals examined. The numerical values of the bathochromic shifts in Table 1 were in the same order as the decreases in PI produced by the different metals in Fig. 1 and both provide a measure of the strengths of the complexes formed.

Table 1. Bathochromic shifts.

Drug (5×10^{-5} M)	Metal ion (5×10^{-3} M)	Bathochromic shift (nm)
Oxytetracycline	—	—
$\lambda_{max\ 356}$	Na^+	0
	Ca^{2+}	2
	Zn^{2+}	3
	Mg^{2+}	3
	Fe^{2+}	3
	Ni^{2+}	10
	Cu^{2+}	24
Demethylchlortetracycline	—	—
$\lambda_{max\ 368\ nm}$	Na^+	0
	Ca^{2+}	2
	Zn^{2+}	3
	Mg^{2+}	3
	Fe^{2+}	4
	Ni^{2+}	10
	Cu^{2+}	22

The stoichiometry of these complexes can be deduced from the positions at which kinks occurred in the graphs in Fig. 1(a, b) or in the slopes of the typical graphs in Fig. 2(a, b) (indicated by the dotted lines drawn tangentially to the rectilinear end-portions of the graphs) and Fig. 3(a, b) where ΔA and ΔF% respectively have been plotted against the ratio of metal to drug. The graphs of PI, ΔA and

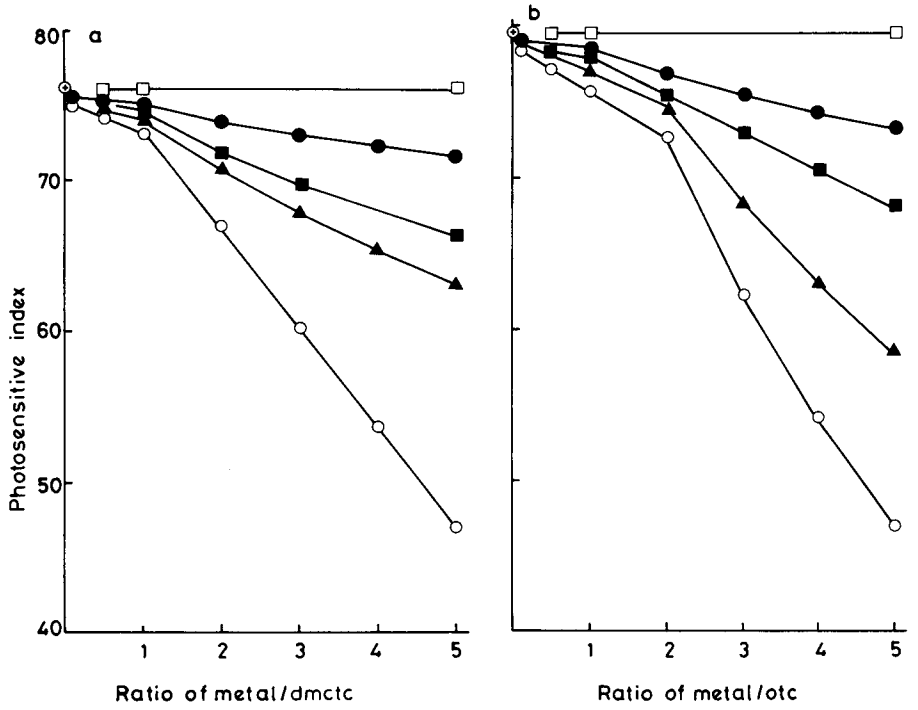

FIG. 1a. Plot of PI for 10^{-4} M demethylchlortetracycline versus ratio of metal/dmctc; b, Plot of PI for 10^{-4} M oxytetracycline versus ratio of metal/otc. ⊕ Drug alone; □ Na^+; ● Ca^{2+}; ■ Fe^{2+}; ▲ Ni^{2+} and ○ Cu^{2+}.

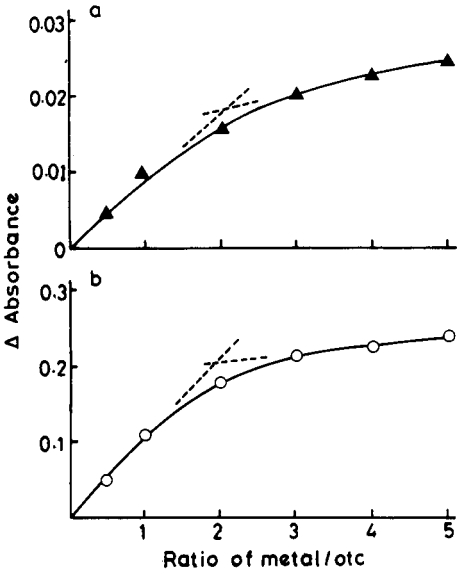

FIG. 2a, b. Plot of absorbance at 380 nm for aqueous solutions of oxytetracycline (5×10^{-5} M) versus ratio of metal/otc. ▲ Ni^{2+} and ○ Cu^{2+}.

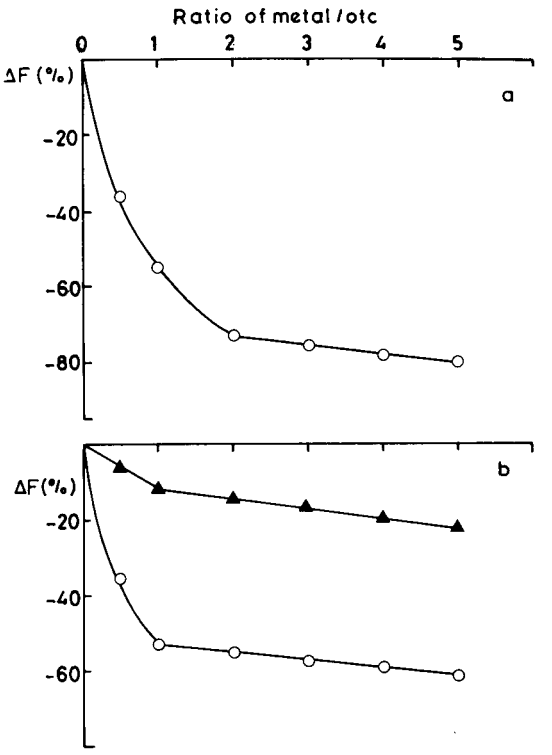

FIG. 3a. Plot of fluorescence intensity % for 10^{-5} M aqueous solutions of oxytetracycline at wavelengths of λ_{Ex} = 400 nm, λ_F = 505 nm, against ratio of metal/otc. b, Plot of fluorescence intensity % for 10^{-4} M aqueous solutions of demethylchlortetracycline at wavelengths of λ_{Ex} = 395 nm, λ_F = 530 nm against ratio of metal/dmctc. ○ Cu^{2+} and ▲ Ni^{2+}.

ΔF% for all the metals employed show that Cu^{2+} and Ni^{2+} form well defined, strong 2 : 1 complexes with oxytetracycline and strong 1 : 1 complexes with demethylchlortetracycline. Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+} form weaker 1 : 1 complexes with both drugs: Na^+ does not form complexes. These findings are in agreement with those of Isben & Urist (1962) and can be assumed to arise from differences in the binding between the different metals and the drugs.

The binding probably occurs at two sites on the tetracyclines (Fig. 4a) where the oxygen atoms can act as electron donors to the metal atom M (Baker & Brown 1966; Williamson & Everett 1975). These sites are shown in Fig. 4b, c, the positions (i) and (ii) in the latter being equivalent. Binding at both sites produces a 2 : 1 complex; at one site a 1 : 1 complex.

Relevance to photosensitization in-vivo

If it is assumed that the simple oil/water interface employed in the present work provides a model for a lipid/water interface in a cell membrane (Sanniez & Pilpel 1980) and that the bathochromic shift or PI value is a measure of the potential photosensitizing action of a drug for a patient (Felmeister & Schaubman 1969) then one might expect that taking Ca^{2+} in the form of milk or milk products or other divalent metal ions e.g. Mg^{2+}, Zn^{2+}, or Fe^{2+} in the form of antacids or other pharmaceutical preparations might reduce photosensitization in patients being treated with tetracyclines.

The potentialities of this hypothesis will need to be more fully investigated by carrying out experiments on biological systems. For example one might expect that the addition of metal ions might alter the rate of haemolysis of red blood cells (Michelson & Durosay 1977) and the inactivation of micro-organisms (Houba-Herin et al 1982) caused by combining treatment with tetracyclines and irradiation with uv light. These experiments are now being planned. If further work is undertaken on animals or man in-vivo it will be necessary to ensure that ingestion of heavy metal ions does not impair the therapeutic efficiency of the tetracyclines or produce other adverse side effects in recipients.

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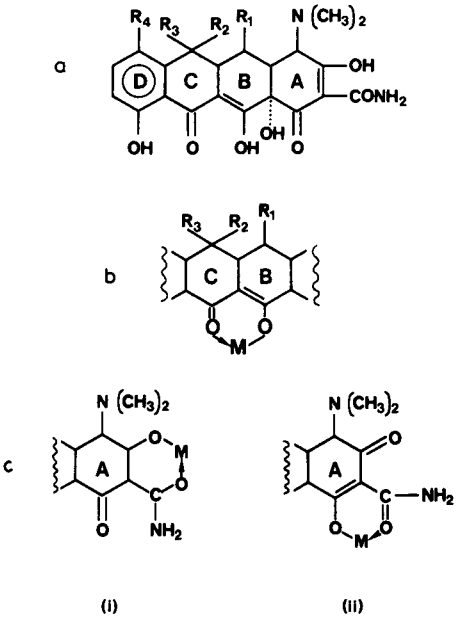

FIG. 4a. Tetracyclines. b, c Possible structures of tetracycline-metal complexes.

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