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Preparation, Characterization and Biological Evaluation of Copper(II) and Zinc(II) Complexes with Cephalexin

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Abstract

Copper(II) and zinc(II) complexes of cephalexin have been prepared and characterized by microanalysis and by thermogravimetric, magnetic and spectroscopic analysis.

The complexes were found to be five-coordinate, monohydrate, and ML_2 type. The electron paramagnetic resonance spectral lines revealed rhombic distortion from axial symmetry, with $g_{\parallel} > g_{\perp} > g_e$, in the elongated-tetragonal copper(II) complex. The geometry of the zinc(II) complex seems to be square-pyramidal. On complexation with copper and zinc the antimicrobial activity of cephalexin improved significantly. The copper complex was found to be active against kaolin paw oedema whereas the parent drug was inactive.

These results suggest that the metallic elements should be seriously considered during drug design, and that complexes already reported should be subjected to clinical evaluation. Their use could provide an easy way of improving the activity and reducing the toxicity of drug substances.

Although metallic elements are known to be extremely important in several biological processes, few medicinal products contain a metal atom as a part of the molecule. The use of cisplatin in cancer therapy (Prestayko et al 1980) and gold salts in the treatment of rheumatoid arthritis (Brown & Smith 1980) highlight the need to evaluate metallic elements for their therapeutic potential.

It has been demonstrated that complexation of metallic elements with inactive substances renders them active, and that complexation with active drugs make them more active and less toxic (Sorenson 1980); several other studies have confirmed this (Brown et al 1980; Jackson et al 1981; Sorenson 1982; Nagar & Mohan 1991; Oga et al 1991). In this paper we report the preparation, characterization and biological evaluation of copper(II) and zinc(II) complexes of cephalexin, a first-generation cephalosporin antibiotic active against Gram-positive cocci, and with moderate activity against some Gram-negative bacilli. The cephalexin molecule contains the $-NH_2$, $-COOH$ and $>CO$ functional groups and construction of molecular models indicates that its structure is suitable for chelate formation.

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Materials and Methods

Materials

Cephalexin sodium was obtained from Dobfer Italy and zinc acetate monohydrate, copper acetate monohydrate and solvents from E. Merck.

Preparation of copper(II)–cephalexin

Cephalexin sodium (37·04 g; 0·1 mol) was dissolved in a minimum quantity of distilled water (approx. 50 mL). Copper acetate monohydrate (9·8 g; 0·05 mol) was separately dissolved in distilled water (approx. 50 mL). The two solutions were mixed with stirring for 30 min (approx.) A green product precipitated and was isolated by filtration. The product was washed with isopropyl alcohol, acetone and ether, and dried by suction. The complex was soluble in *N*, *N*-dimethylformamide (DMF) and insoluble in other common organic solvents.

Preparation of zinc(II)–cephalexin

This complex was prepared from cephalexin sodium (37·04 g; 0·1 mol) and zinc acetate

monohydrate (10·95 g; 0·05 mol) by a method similar to that used for copper(II)–cephalexin. The compound was isolated as an off-white product. The complex was soluble in DMF and insoluble in other common organic solvents.

Characterization

Microanalysis was performed by the usual techniques. Copper and zinc were estimated by means of an Hitachi Z-8000 atomic absorption spectrophotometer. Molecular weights were determined mass-spectrometrically. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed with a Netzsch simultaneous thermal analyser. Conductivity measurements were conducted with a Wescan 212 conductivity meter, with DMF at room temperature. The magnetic moment of the copper complex was determined by Gouy's technique using Hg(II)-tetrathiocyanatocobaltate as calibrant; diamagnetic corrections were calculated from Pascal's constants (Earnshaw 1968). Infrared spectra were recorded with an FTIR (Midac) spectrophotometer using KBr and Nujol-mull techniques. Electronic absorption spectra were obtained with a Shimadzu UV-1601 spectrophotometer. Electron paramagnetic resonance (EPR) spectra were recorded as a powder and in solution (DMF), at room temperature, on a Jeol JES-FE 1XG instrument, in the X-band, operating at a microwave frequency of 9·44 GHz. The g values were determined by use of the Kneubühl approximation (Kneubühl 1960). The spectra were calibrated using the α,α-diphenyl-*p*-picrylhydrazyl radical ($g = 2{\cdot}0036$) as a field marker.

Antibacterial activity

Antibacterial study of the complexes under investigation was performed using standard strains of *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027) and *K. pneumoniae* (Glaxo 308E). Minimum inhibitory concentration (MIC) was determined by the standard dilution technique (Anhalt & Washington 1985) by use of tryptic soy broth (Difco), in which the complexes dissolved. The tubes were incubated at 37°C for 24 h.

Anti-inflammatory activity

Kaolin paw oedema was induced, by a method reported elsewhere (Lewis et al 1975), in male Wistar rats, 100–110 g, in groups of five. The complexes under investigation were administered orally in 5% Mulgophen (GAF, Manchester) in distilled water (0·2 mL per 100 g) 1 h before the kaolin. The rats were dosed on a weight of drug (mg) per body weight (kg) of animal basis. Oedema was evaluated 4 h after subplantar administration of kaolin in 0·9% w/v sodium chloride solution. Inhibition of oedema was evaluated by comparing the swelling obtained in treated animals with that in controls and was expressed as percentage inhibition. Statistical significance was evaluated by use of the Student *t*-test.

Toxicity study

Experiments were performed on albino Wistar male rats, 180–200 g. Animals were kept at constant temperature ($21 \pm 0{\cdot}5$°C) and humidity. Conventional laboratory diet and water were freely available. The complexes under investigation were administered orally in 0·15% agar suspension ($50\,mL\,kg^{-1}$) to four groups of ten rats. After treatment, the animals were monitored every hour for several hours and then every day for 14 days.

Results and Discussion

Characterization of complexes

Microanalytical data (Table 1) confirmed the ML_2 composition of the complexes, in which M is Cu(II) or Zn(II) and L the ligand (cephalexin). The complexes did not have sharp melting points and decomposed above 320°C. Thermal analysis indicated that the complexes were monohydrated—there was a weight loss equivalent to one water molecule at 120°C (approx.). This water molecule is coordinated to the metal ion. The molecular weights determined mass-spectrometrically also confirm the ML_2 composition. The infrared (IR) spectra of the complexes contained all the bands from the ligand and other bands indicative of coordination of the ligands with metal ions through N and O. Some important IR absorption bands and their assignments are listed in Table 2. The band at $1765\,cm^{-1}$ (approx.), $\nu(COO)_{asym}$, in the spectrum of cephalexin shifted to $1760\,cm^{-1}$ (approx.) on complexation with the metal ions. A new absorption band, $\nu(MO)$, appeared at $330\,cm^{-1}$ (approx.) in the spectra of complexes. This indicates that the carboxyl group is coordinated to the metal ion. There were also shifts of the $\nu(COO)_{asym}$ and $\delta(CO)$ bands towards lower wavenumber. A new band, $\nu(MN)$, appeared in the spectra of complexes, indicating coordination of the ligand through N.

Table 1. Microanalytical data of the complexes.

Complex	C	H	N	M
Copper(II)–cephalexin	49·60 (49·59)	4·37 (4·39)	10·88 (10·85)	8·25 (8·21)
Zinc(II)–cephalexin	49·59 (49·47)	4·35 (4·38)	10·84 (10·82)	8·47 (8·47)

The values in parentheses are the calculated values.

Table 2. Observed infrared bands (cm^{-1}) and assignments.

Complex	$\nu(COO)_{asym}$	$\nu(COO)_{sym}$	$\delta(CO)$	$\pi(CO)$	$\nu(MN)$	$\nu(MO)$
Copper(II)–cephalexin	1760	1350	720	532	410	330
Zinc(II)–cephalexin	1761	1352	725	531	400	325
Cephalexin sodium	1765	1355	750	530	–	–

In the electronic absorption spectra of the complexes (Figure 1) there is an intense band at 260 nm (approx.) which is assigned to a $\pi-\pi^*$ transition originating in the phenyl ring. The low-energy band at 678 nm (approx.) in the spectrum of the copper complex (Figure 1b) arises from a d–d transition. The position of this band is consistent with distorted square-planar geometry. Lower molar conductance values (14·12, 16·34 $ohm^{-1}\,mol^{-1}\,cm^2$ for zinc and copper complexes, respectively) indicate the non-electrolytic nature of the complexes.

The copper complex had a normal value of the magnetic moment, i.e. 1·91 BM, indicating its mononuclear nature. The polycrystalline and solution EPR spectra of copper(II)–cephalexin are shown in Figure 2. There was a general correspondence between the powder (Figure 2a) and solution (Figure 2b) spectra, however, the $g_{\|}$ region

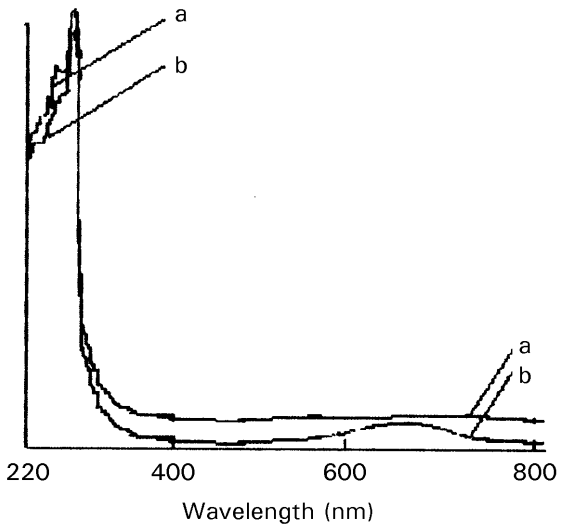

Figure 1. Electronic absorption spectra of zinc(II)–cephalexin (a) and copper(II)–cephalexin (b) in *N,N*-dimethylformamide.

Table 3. Electron paramagnetic resonance parameters of copper(II)–cephalexin.

Solid		Solution		
$g_{\|}{}^a$	$g_{\perp}{}^a$	$g_{\|}{}^b$	$g_{\perp}{}^b$	$\|A_{\|(Cu)}\|^c$
2·21	2·06	2·285	2·019 2·077	16·00 (150 G)

$^a \pm 0{\cdot}02$, $^b \pm 0{\cdot}002$, $^c 10^3\,cm^{-1} \pm 0{\cdot}07$.

was well resolved in solution. The spectra were indicative of rhombic distortion from axial symmetry owing to coordination of two different kinds of atom (O and N) with the metal ion. The spectra were characteristic of magnetically dilute systems with Cu(II) ions in the $d_{x^2-y^2}$ ground state ($g_{\|} > g_{\perp} > g_e$ (Table 3)). Hyperfines as a result of nitrogen ($I = 1$) are visible on the main absorption line, $g_{\perp}$ (Figure 2b), confirming the coordination through N. Thus the complexes were characterized as five-coordinate with the fifth position occupied by one water molecule. Unsuccessful attempts to isolate crystals suitable for X-ray analysis prevented further structure elucidation. The structures of the complexes under investigation, proposed on the basis of the above experimental evidence, are shown in Figure 3.

Antibacterial activity

The results of antibacterial study are given in Table 4. Copper(II)–cephalexin was four times more active against *S. aureus* (minimum inhibitory concentration (MIC) 2·5 $\mu g\,mL^{-1}$) than cephalexin sodium (MIC 10·0 $\mu g\,mL^{-1}$) and about fourteen times more active against *E. coli* (MIC values copper(II)–cephalexin, 7 $\mu g\,mL^{-1}$; cephalexin sodium, 100 ($\mu g\,mL^{-1}$). The copper complex was

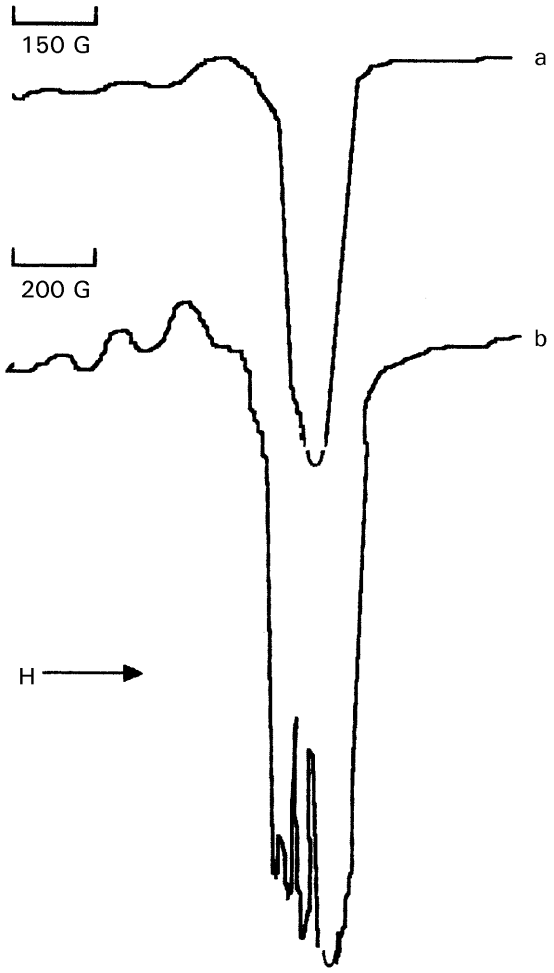

Figure 2. Electron paramagnetic resonance spectrum of copper(II)–cephalexin as a powder (a) and as a frozen solution in *N*,*N*-dimethylformamide (b).

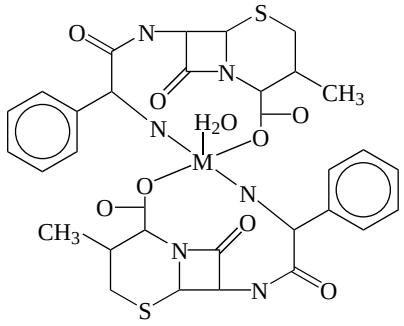

Figure 3. Proposed structure of the metal (M) complexes of cephalexin zinc(II)–cephalexin and copper(II)–cephalexin.

active against *K. pneumoniae* (MIC 150 $\mu g\,mL^{-1}$) whereas cephalexin sodium was not. Cephalexin sodium and copper(II)–cephalexin up to 10 $mg\,mL^{-1}$ were not active against *P. aeruginosa*.

Zinc(II)–cephalexin seemed to be three times more active against *S. aureus* (MIC 3·5 $\mu g\,mL^{-1}$) than cephalexin sodium (MIC 10·0 $\mu g\,mL^{-1}$) and ten times more active against *E. coli* (MIC 10 $\mu g\,mL^{-1}$) than cephalexin sodium (MIC 100 $\mu g\,mL^{-1}$). The zinc complex was found to be active at 10 $mg\,mL^{-1}$ against *K. pneumoniae* whereas cephalexin sodium had no effect. At 10 $mg\,mL^{-1}$ the zinc complex and cephalexin sodium were not active against *P. aeruginosa*.

Copper(II)–cephalexin was 40% more active than zinc(II)–cephalexin against *S. aureus* and *E. coli* (Table 4). Similarly copper(II)–cephalexin was 66% more active than zinc(II)–cephalexin against *K. pneumoniae*.

Anti-inflammatory activity

The results of the paw oedema test are summarized in Table 5. The copper complex was found to be active whereas the zinc complex and cephalexin sodium had no significant activity. This confirms the previously reported role of copper in inflammation (Brown et al 1979; Iqbal 1982).

Toxicity study

The LD50 values (quantities resulting in the death of half the rats) are given in Table 5. Toxicity was reduced (lower LD50 values) by complexation.

Conclusion

These results show that complexation with copper and zinc improved the anti-bacterial activity and reduced the toxicity of cephalexin. The drug acquired anti-inflammatory activity on complexation with copper. These observations, in line with other studies, suggest that the metallic elements should be seriously considered in drug design, and that the complexes already reported should be subjected to clinical evaluation. Their use could provide an easy way of improving the activity and reducing the toxicity of several drug substances.

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Table 4. Minimum inhibitory concentrations ($\mu g\,mL^{-1}$) of the complexes against bacteria.

Complex	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
Copper(II)–cephalexin	2·5	7·0	Resistant	>150
Zinc(II)–cephalexin	3·5	10·0	Resistant	>10 000
Cephalexin sodium	10·0	100·0	Resistant	Resistant

Table 5. Anti-inflammatory effect of the complexes in kaolin paw oedema and LD50 values.

Complex	Dose ($mg\,kg^{-1}$)	Inhibition of oedema (%)	LD50[a] ($g\,kg^{-1}$)
Copper(II)–cephalexin	50	35*	8·12
Zinc(II)–cephalexin	50	5*	9·07
Cephalexin sodium	50	2*	5·25

[a]Quantity resulting in the death of half the rats. *$P < 0{\cdot}05$ compared with control.

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