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Zinc Sulphadiazines: Novel Topical Antimicrobial Agents for Burns

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Abstract

Two new zinc sulphadiazine ($Zn(SD)_2$)-amine complexes, zinc sulphadiazine-methylamine ($Zn(SD)_2(CH_3NH_2)_2$) and zinc sulphadiazine-ethylenediamine ($Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$), were prepared and compared with silver sulphadiazine (AgSD). The compounds were readily obtained by reaction of zinc nitrate hexahydrate with sulphadiazine or its salt in methylamine and ethylenediamine, respectively.

Structure was established by X-ray crystallography and ultraviolet-visible, infrared and nuclear magnetic resonance spectroscopy. The products were effective, in-vitro, against Gram-positive and Gram-negative bacteria as well as fungus. However, their activity is partially reversed by *p*-aminobenzoic acid. Further investigations in burned mice revealed that these compounds displayed a potential value in the prevention and treatment of wound healing, and diminution of mortality and weight loss. The toxicity of $Zn(SD)_2$ derivatives was much lower than that of AgSD. The better aqueous solubility and skin permeability may explain the reason for their superiority over AgSD in the efficacy for topical therapy.

$Zn(SD)_2(CH_3NH_2)_2$ was consistently more potent and was chosen for further development in clinical uses. The similarity in complexation between $Zn(SD)_2(CH_3NH_2)_2$ and AgSD may be significant to distinguish that from any other $Zn(SD)_2$ derivative in bioactivity.

Pseudomonas aeruginosa is one of the most virulent pathogens in man and has frequently been implicated as the causative bacteria in burn wound sepsis in hospitals (Yurt et al 1984). The current topical treatment of choice is silver sulphadiazine (AgSD) which was introduced by Fox (1968). AgSD is available as 1% (w/w) oil-in-water cream. The definite advantages of AgSD include an excellent antimicrobial spectrum of activity, low toxicity, ease of application and minimum tissue reaction (Wysor 1983; Nangia et al 1987; Monafo & West 1990).

Clinical trials have shown that AgSD is by no means free from adverse effects. Continual use of AgSD frequently causes leucopenia (Smith-Choban & Marshall 1987) and haemolytic anaemia in patients deficient in glucose-6-phosphate dehydrogenase (Harrison 1979). Long term use of AgSD may cause renal failure resulting from marked argyria, even when applied topically (Owens et al 1974). Furthermore, the emergence of resistant strains of bacteria to AgSD has been reported (Gayle et al 1978; Heggers & Robson 1978). These drawbacks prompted us to prepare zinc sulphadiazine ($Zn(SD)_2$), a hybridization of AgSD and zinc salts, with a good capability for management of infections without causing significant side-effects and acceleration in wound healing.

The idea to prepare $Zn(SD)_2$ is not new. Zinc, a micronutrient essential to man, is known for its potent catalysis in wound healing (Pories et al 1967; Henzel et al 1970). It has been suggested that this metal ion, like AgSD, interacts with bacterial DNA and $Zn(SD)_2$ should be an effective antiseptic agent. Fox et al (1976) introduced $Zn(SD)_2$ in 1976 but the preparation was criticized by Bult et al (1981). The zinc compounds prepared by Fox et al (1976) appeared to be a mixture of zinc hydroxide ($Zn(OH)_2$), sulphadiazine, and sodium nitrate. Fox later successfully obtained zinc sulphadiazine-ammonia complex ($Zn(SD)_2 \cdot 2NH_3$) by recrystallization of the crude products from ammonium hydroxide. The structure was unequivocally determined by X-ray diffraction patterns (Baenziger et al 1983; Brown et al 1985).

We have reported preliminary data on the facile synthesis and antimicrobial activity of $Zn(SD)_2 \cdot 2NH_3$ (Baenziger et al 1983; Lee et al 1989, 1990). The potent antibacterial activity of $Zn(SD)_2 \cdot 2NH_3$ represents the advent of a class of compounds that possess attractive potential as novel antibacterial agents in burn therapy. In this presentation we describe the preparation, structural features, physicochemical properties, and comparative efficacy studies of the two new derivatives of $Zn(SD)_2$, zinc sulphadiazine-methylamine ($Zn(SD)_2(CH_3NH_2)_2$) and zinc sulphadiazine-ethylenediamine ($Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$) (Fig. 1), systematically studied in our laboratories to evaluate the feasibility of their application to the control of burn wound sepsis in the military field.

Materials and Methods

Chemical syntheses: general procedure

All the chemicals and solvents were of analytical or reagent grade. AgSD was prepared by the patent method (Fox 1973). Commercial AgSD creams were obtained commercially.

To a well stirred (25°C) mixture of sulphadiazine (5·02 g, 20·0 mmol) or equimolar amount of sodium sulphadiazine (NaSD) in 40% methylamine (20 mL) was added dropwise a

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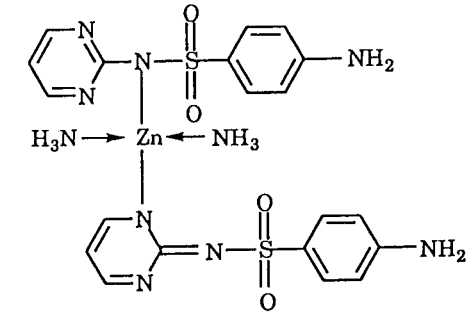
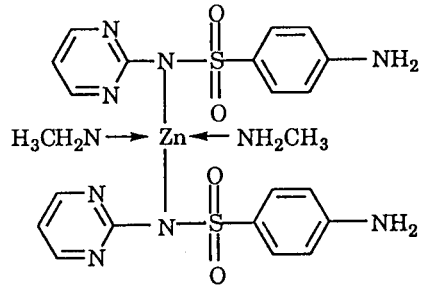
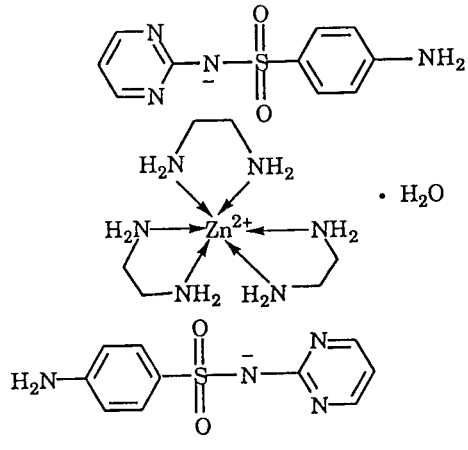


$Zn(SD)_2 \cdot 2NH_3$



$Zn(SD)_2(CH_3NH_2)_2$



$Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$

FIG. 1. The structure of $Zn(SD)_2 \cdot 2NH_3$, $Zn(SD)_2(CH_3NH_2)_2$ and $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$.

solution of $Zn(NO_3)_2 \cdot 6H_2O$ (3·96 g, 13·3 mmol) in 40% methylamine (10 mL). An endothermic reaction occurred and white solids precipitated immediately after mixing. Filtration and recrystallization from 40% methylamine gave $Zn(SD)_2(CH_3NH_2)_2$ (6·02 g, 74·1%) as white crystals. The filtrate and washings were combined and concentrated under reduced pressure to afford 1·12 g of the second crop mp 239–241°C. UV (0·005% NH_3) λmax: 241, 254 nm. IR (KBr): 3445, 3409, 3350, 3252 (νNH), 2951–2823 (νCH), 1631 (δNH), 1593, 1500 (νC = C), 1252, 1135 (νSO) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 100 MHz) δ: 8·16 (d, 4H), 7.54 (d, 4H), 7·54 (d, 4H), 6·57 (t, 2H), 6·43 (d, 4H), 5·52 (s, 4H), 2·34 (s, 6H). Anal. Calcd. for $ZnC_{22}H_{28}N_{10}O_4S_2$: C, 42·21; H, 4·51; N, 22·37. Found: C, 41·54; H, 4·45; N, 21·67.

$Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ was prepared by the method outlined above except that ethylenediamine was used instead of methylamine. The yield was 77·1% after recrystallization from ethylenediamine. mp 221–222°C. UV (0·005% NH_3) λmax: 241, 254 nm. IR (KBr): 3422, 3320, 3251 (νNH), 2953–2884 (νCH), 1640 (δNH), 1595, 1498 (νC = C), 1238, 1132 (νSO) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 100 MHz) δ: 8·11 (d, 4H), 7·43 (d, 4H), 6·43 (d, 4H), 6·39 (t, 2H), 5·38 (s, 4H), 2·61 (s, 12H). Anal. Calcd. for $ZnC_{26}H_{44}N_{14}O_5S_2$: C, 40·97; H, 5·82; N, 25·73. Found: C, 41·42; H, 6·00; N, 24·95.

The single-crystal diffraction patterns were obtained with a CAD4 Kappa Axis Single Crystal XRD equipped with an X-ray precession camera and a Microvax-III computer system and using monochromatic molybdenum $K\alpha$ radiation. White needle-shaped crystals of $Zn(SD)_2$ derivatives about 0·1 × 0·1 × 0·8 mm were used. The data were collected at room temperature (21°C), 2θ range: 2·5–50°, scan parameter: 2 (0·65 + 0·35 tan θ), scan speed: 20/15 – 20/2 deg min^{-1}. The structures were refined by the least-squares method on F using NRC programs (Ahmed et al 1970). $Zn(SD)_2(CH_3NH_2)_2$ was in the space group $P2_1/c$, with four molecules in a unit cell having the dimensions a = 10·518 (2) A, b = 8·769 (2) A, c = 29·909 (6) A, and $\alpha = \gamma = 90°$, β = 97·87 (2)°. Total number of independent reflections was 4805 ($3607 > 3\sigma$), R: 0·037, Rw: 0·033. $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ was in the space group $C2/c$ with four molecules in a unit cell having the dimensions a = 15·645 (2) A, b = 14·585 (2) A, c = 16·675 (6) A, and $\alpha = \gamma = 90°$, β = 115·20 (1)°. Total number of independent reflections was 3026 ($2508 > 3\sigma$), R: 0·042, Rw: 0·056.

The aqueous solubility and conductivity of $Zn(SD)_2$ derivatives were determined according to the methods described previously (Bult & Klasen 1978, 1980).

In-vitro assay of microbial inhibition

The strains, in this study, were provided by Professor M. Ding at the Institute of Microbiology and Immunology, National Defense Medical Center. For comparison, AgSD was used as a reference throughout the tests.

$Zn(SD)_2$ derivatives and AgSD were tested for their antimicrobial activity. Minimum inhibitory concentrations (MICs) were determined by a broth dilution method. A series of tubes containing 5·0 mL nutrient broth was prepared, with falling concentrations of the test compound. Each tube was inoculated with 0·2 mL of a 1–10 000 dilution of the test organism (approximately $3·8 \times 10^8$ cfu mL^{-1}) cultured at 25°C for 18 h. Bacterial growth was observed by turbidity measurement after incubation at 37°C for 18 h. The minimum concentration of test agents which prevented the development of turbidity was judged to be the MIC.

The minimum bactericidal concentration (MBC) was determined against *P. aeruginosa* in nutrient broth in a similar manner reported by Fox et al (1976, 1978) and Saffer et al (1980). The cultures were incubated at 37°C

after 48 h and then observed for bacterial growth. The MBC was a 99·9% kill as the endpoint.

The *p*-aminobenzoic acid antagonism test was determined in nutrient broth as described by Fox et al (1976).

Comparative experiments in mice

The preparation of various creams and the mice scalding experiments were as reported previously (Fox 1973). The creams were assayed by charring at 800°C for 2 h, HCl (1 mL) added and diluted with water to 10 mL. The zinc content was determined by atomic absorption spectrophotometry. The mice (12·22 ± 0·39 g) were carefully shaved one day before burning and received 40–45% burn area in the dorsum by contact with hot water (70 ± 0·5°C) for 5 s and then challenged by swabbing the dilutions of standardized 18-h broth culture (about $5·9 \times 10^7$ cfu mL^{-1}) over the burned dorsum. In each experiment, all the animals were anaesthetized, burned, pooled, infected and then grouped (eighteen of each), at random. First treatment with various topical agents was begun on test groups 4–6 h postburn. Subsequent treatments were carried out once daily and continued for 18 days. For comparison, 18 infected burns were treated with the cream base and 18 infected burns were untreated. During the treatment, the weight of mice were recorded at intervals. Animals that died were autopsied, and the cardiac blood was cultured to verify the presence of pseudomonas species (Gilardi 1985).

Toxicity

The toxicity of $Zn(SD)_2$ derivatives was measured in male rats (Sprague-Dawley) weighing 192 ± 15 g. Intraperitoneal injection of a volume not exceeding 1 mL (10% in dimethylsulphoxide for suspension or 10% in dimethylsulphoxide : acetic acid = 85 : 15 (pH 7·83) for solution) or vehicle was used throughout the experiments. The observation was continued up to three days.

Permeability

For studies of permeability, a Franz diffusion cell equipped with a stirring bar was employed (Chien & Huang 1983). A phosphate buffer solution (pH 7·4) was used as a receptor medium. The temperature was maintained at 37 ± 1°C and the stirring rate was kept at 700 rev min^{-1}. A sheet of nude mouse (ICR) skin was cut, defatted, and carefully fitted into the cell. The diffusion rate was determined by the zinc content measured by atomic absorption spectrophotometry at intervals. The observation was continued up to 24 h.

Results

Structures and physicochemical properties

The structures of the synthetic products were fully established by the spectral data (UV, IR, NMR), elemental analyses and X-ray crystallography. The two new $Zn(SD)_2$ derivatives were formulated as $Zn(C_{10}H_9N_4O_2S)_2(CH_3NH_2)_2$ and $Zn(C_{10}H_9N_4O_2)_2(C_2H_8N_2S)_3 \cdot H_2O$ according to the data of elemental analysis. The spatial arrangements of the molecules were determined by X-ray crystallographic patterns which are shown in Fig. 2. The values of ν^*SO, the weighted average value of $\nu_{as}SO$ and ν_sSO, for $Zn(SD)_2(CH_3NH_2)_2$ and

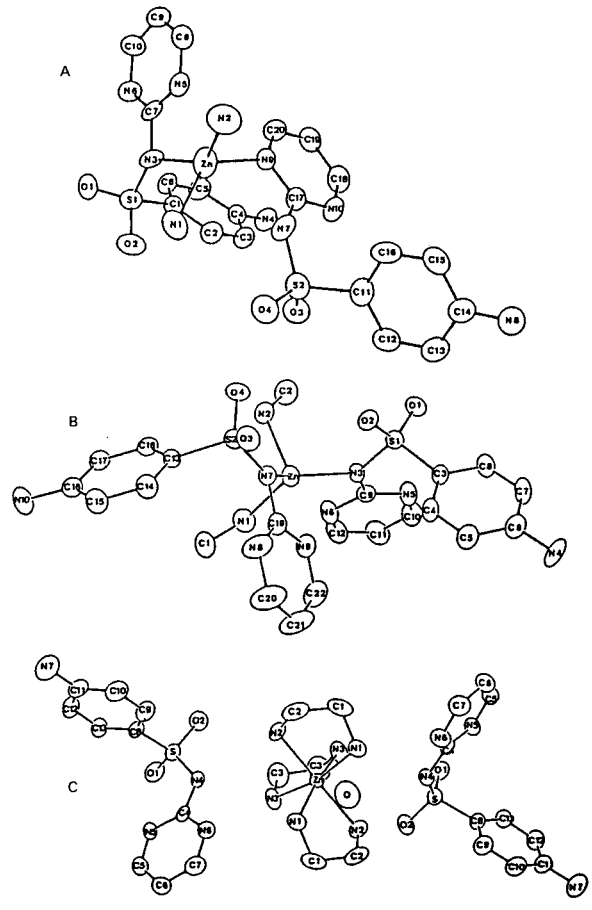


FIG. 2. X-ray crystallographic patterns of $Zn(SD)_2 \cdot 2NH_3$ (A), $Zn(SD)_2(CH_3NH_2)_2$ (B), and $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ (C) (Lee et al 1989).

$Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ were 1195 and 1186 cm^{-1}, respectively (Table 1). The solubility and conductivity are summarized in Table 1.

Assay in-vitro

Table 2 shows the MICs of different sulphadiazine derivatives against some Gram-positive, Gram-negative bacteria and fungus in nutrient broth. The two $Zn(SD)_2$ derivatives showed a broad spectrum of antimicrobial activity and were less active than AgSD but much more potent than NaSD or $Zn(NO_3)_2$. The rank order of potency of antimicrobial activity for $Zn(SD)_2$ derivatives was AgSD > $Zn(SD)_2(CH_3NH_2)_2$ > $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ > NaSD ≅ $Zn(NO_3)_2$.

The values of MBCs of $Zn(SD)_2$ derivatives against *P. aeruginosa* were found to be 0·1 mmol L^{-1} for $Zn(SD)_2(CH_3NH_2)_2$, 0·2 mmol L^{-1} for $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$, and 0·05 mmol L^{-1} for AgSD.

To determine the role of the sulphadiazine moiety in $Zn(SD)_2$ derivatives, the degree of antagonism of the growth inhibition by *p*-aminobenzoic acid was also measured. The results are indicated in Table 3. $Zn(SD)_2(CH_3NH_2)_2$ and $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ were partially blocked by *p*-aminobenzoic acid. However, when the concentration of $Zn(SD)_2(CH_3NH_2)_2$ was higher than 0·2 mmol L^{-1}, it was no longer reversed by *p*-aminobenzoic

Table 1. Physical data of sulphadiazine derivatives.

Compound	Solubility ($mg\,L^{-1}$)			Conductivity ($ohm^{-1}\,cm^2\,mol^{-1}$)	ν^*SO (cm^{-1})
	H_2O	Dimethylsulphoxide	25% NH_3	Dimethylsulphoxide (10^{-3} M)	KBr
$Zn(SD)_2(CH_3NH_2)_2$	138·8 ± 23·0	8550·7 ± 1530·0	$> 100 \times 10^3$	12	1195
$Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$	21 450 ± 8740	11 366·7 ± 373·1	$> 100 \times 10^3$	18	1186
AgSD	3·4	> 350	$> 50 \times 10^3$	2	1178

$$\nu^*SO = \sqrt{[(\nu_{as}SO)^2 + (\nu_sSO)^2]/2}$$

acid. In contrast, AgSD is not nullified by *p*-aminobenzoic acid at all (Fox et al 1976).

Experiments on animals

The results of experiments with burns in mice are summarized in Fig. 3. It was shown that survival after topical treatment with $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ was equal to or possibly slightly better than with AgSD. $Zn(SD)_2(CH_3NH_2)_2$, on the other hand, appeared to be far more effective and exhibited an effectiveness in reducing mortality in mice ($P < 0{\cdot}05$, Chi-square test). Mice exhibited oedema and fever on the first day after scalding. All the mice with burns died within 8 days if no treatment was given and died within 11 days if placebo was applied. The cumulative mortalities were 55·5% for commercial AgSD, 50·0% for AgSD, 50·0% for $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$, and 22·2% for $Zn(SD)_2(CH_3NH_2)_2$, respectively.

As revealed from Fig. 4, the weight gain during therapy was slightly higher in the $Zn(SD)_2(CH_3NH_2)_2$ group ($P < 0{\cdot}1$, posterior comparison, one-way analysis of variance, compared with placebo), although the difference was small.

Table 4 shows the total mortality and average viable tail lengths. Once again $Zn(SD)_2(CH_3NH_2)_2$ was far more effective than all the other compounds tested in this study ($P < 0{\cdot}05$, posterior comparison, one-way analysis of variance, compared with placebo). None of the burned and infected mice receiving either no treatment or placebo retained viable tails.

All the burned mice showed the pseudomonas colonies of $3{\cdot}60–43 \times 10^2$ cfu mL^{-1} in the blood culture after infection with pseudomonas for 12 h. Mice which died in the course of treatment showed those of $10^5–10^7$ cfu mL^{-1}.

The acute toxicity of $Zn(SD)_2$ derivatives was measured after intraperitoneal administration of drugs to mice. The LD50 values were 800 and 1000 mg kg^{-1} for the solutions of $Zn(SD)_2(CH_3NH_2)_2$ and $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$, respectively. The LD50 values were about 1200 mg kg^{-1} for both suspensions. No acute symptoms and no mortality were found up to doses of 600 mg kg^{-1} in either dosage form. In the case of AgSD suspension, the LD50 is 140 mg kg^{-1} (Fox et al 1978).

Permeability

Fig. 5 shows the results of the permeability study of $Zn(SD)_2$ derivatives on the skin of nude mice. The average fluxes of zinc ion were 0·539 and 0·313 $\mu g\,cm^{-2}\,h^{-1}$ for $Zn(SD)_2(CH_3NH_2)_2$ and $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$, respectively. The cumulative fluxes of zinc ion for 24 h were 7·084 ± 0·861 and 3·677 ± 0·149 $\mu g\,cm^{-2}$ for $Zn(SD)_2(CH_3NH_2)_2$ and $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$, respectively. Once again, $Zn(SD)_2(CH_3NH_2)_2$ demonstrated a better skin permeability than $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$. After linear regression analysis, the permeability rate of zinc ion exhibited a zero-order kinetic reaction in each compound tested.

Discussion

Chemical synthesis

Our facile procedures stated above represented the first general route suitable for large-scale preparation of $Zn(SD)_2$ derivatives. The reported $Zn(SD)_2$ (Fox et al 1976, 1978, 1990; Howell et al 1990a, b) as a topical antimicrobial agent is essentially a mixture of free sulphadiazine, $Zn(OH)_2$, and $NaNO_3$ which has a tendency to cause incidences of allergies to patients and, therefore, is not satisfactory for topical application. In the zinc sulphadrug complexes described by Narang & Gupta (1977), the sulphanilamide molecules are present as a neutral bidentate ligand. The zinc ion, therefore, interacts with anionic acetates to form the salts.

The first true structure of $Zn(SD)_2 \cdot 2NH_3$ was indepen-

Table 2. MICs of sulphadiazine derivatives.

Organism	MIC ($mmol\,L^{-1}$)		
	$Zn(SD)_2(CH_3NH_2)_2$	$Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$	AgSD
Pseudomonas aeruginosa	0·0625	0·0625	0·0313
Escherichia coli	0·125	1·0	0·0313
Klebsiella pneumonia	0·125	0·25	0·0313
Serratia marcescens	0·25	1·0	0·0625
Shigella dysenteriae	0·125	0·5	0·0156
Staphylococcus aureus	0·0625	0·25	0·0156
Micrococcus flavus	0·0156	0·0156	0·0156
Candida albicans	0·25	2·0	0·0625

Table 3. Inhibition of growth of *P. aeruginosa* by sulphadiazine derivatives in the presence of *p*-aminobenzoic acid after 18-h incubation.

p-Aminobenzoic acid (mmol L^{-1})	$Zn(SD)_2(CH_3NH_2)_2$ (mmol L^{-1})			$Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ (mmol L^{-1})			AgSD (mmol L^{-1})		
	0·2	0·1	0·05	0·2	0·1	0·05	0·2	0·1	0·05
0·2	+	−	−	−	−	−	+	+	+
0·1	+	−	−	−	−	−	+	+	+
0·04	+	−	−	−	−	−	+	+	+
0·02	+	−	−	−	−	−	+	+	+
0·01	+	−	−	−	−	−	+	+	+
0·004	+	−	−	+	−	−	+	+	+
0·002	+	+	−	+	+	−	+	+	+
0·001	+	+	−	+	+	−	+	+	+
0·0004	+	+	+	+	+	+	+	+	+

+, Inhibition; −, no inhibition.

dently demonstrated by Baenziger et al (1983) and Brown et al (1985). However, the extremely low yields in the preparation of $Zn(SD)_2 \cdot 2NH_3$ by the method reported therein makes it impractical in synthesis. A Chinese paper describes the preparation of $Zn(SD)_2 \cdot 2NH_3$ (Chou & Wang 1992). This method has a good yield but nevertheless needs high temperature (100°C) and takes a long time (3 days), compared with our mild (ambient temperature) and rapid (overnight) conditions.

Under our conditions, attempted synthesis of $Zn(SD)_2$ derivatives using $Zn(NO_3)_2$ and the bulkier amines such as ethylamine propylamine, isopropylamine, butylamine, dimethylamine, trimethylamine or aromatic amines such as aniline, pyridine and substituted pyridines failed. The attempted preparation of pure $Zn(SD)_2$, even in aprotic polar solvents such as dimethylsulphoxide or dimethylformamide was also fruitless. The products were mainly composed of $Zn(OH)_2$, sulphadiazine, and $NaNO_3$.

Structure and physicochemical properties

The IR absorption spectral data of $Zn(SD)_2$ derivatives are extremely informative for the structural determinations. The value of ν^*SO depends on the character of the groups attached to SO_2. In sulphadiazine where the sulphonamido NH is not substituted, the ν^*SO is at about 1241 cm^{-1} (Bult & Klasen 1978). The value of ν^*SO for AgSD shifts to 1178 cm^{-1}, which was attributed to the change in the electronic environment on sulphonamide N from the free form to the anionic amide form. The resemblance in lowering of ν^*SO in $Zn(SD)_2$ derivatives (Table 1) indicated that there existed an amide linkage between Zn^{2+} and an anionic N which was similar to that of AgSD.

The UV spectra of sulphadiazine, AgSD, and $Zn(SD)_2$ derivatives in 0·005% NH_3 were similar.

In NMR spectra, the peak for the fairly acidic proton of the sulphonamido groups of sulphadiazine occurred at about 11·3 ppm (exchangeable with D_2O) in DMSO-d_6. It disappeared after conversion to $Zn(SD)_2$ derivatives. The disappearance of the peak could be used for rapid identification. The remaining NMR signals were similar to AgSD.

X-ray diffraction patterns provide an unambiguous insight into the structures in the solid state. Fig. 2 shows the perspective view for the crystal structures of $Zn(SD)_2$ derivatives. In $Zn(SD)_2 \cdot 2NH_3$ the Zn atom was tetrahedrally (distorted) co-ordinated to two ammonia molecules (N(1), 2·005 A, N(2), 2·105 A) and to N atoms from two anionic sulphadiazine molecules. The N atoms in sulpha-

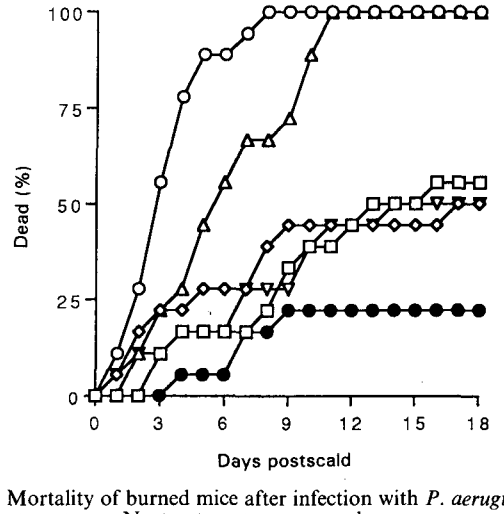


FIG. 3. Mortality of burned mice after infection with *P. aeruginosa* and treatments. ○, No treatment; △, cream base; □, commercial AgSD cream; ◇, AgSD cream; ▽, $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ cream; ●, $Zn(SD)_2(CH_3NH_2)_2$ cream.

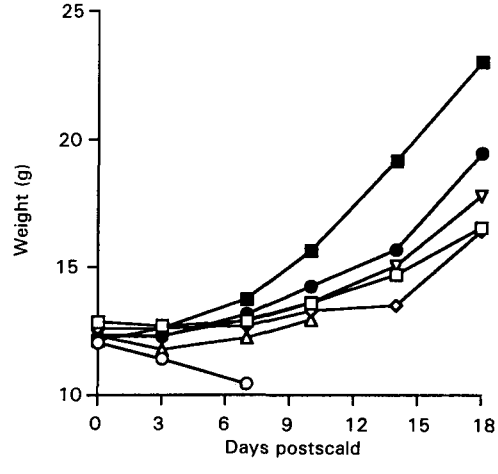


FIG. 4. Body weight gain of burned mice after infection with *P. aeruginosa* and treatments. ○, No treatment; △, cream base; □, commercial AgSD cream; ◇, AgSD cream; ▽, $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ cream; ●, $Zn(SD)_2(CH_3NH_2)_2$ cream; ■, normal.

Table 4. Total mortalities and average viable tail lengths of burned and infected mice at 18-days postburn.

Therapy	Total mortality (%)	Average viable length (cm)
$Zn(SD)_2(CH_3NH_2)_2$ cream	22·2	3·35 ± 1·00
$Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ cream	50·0	1·73 ± 0·47
AgSD cream	50·0	1·79 ● 0·60
Commercial AgSD cream	55·5	2·18 ± 0·66
Control	0	8·27 ± 0·43

diazines involved in the co-ordination were different. One was from a sulphonamido N atom (N(3), 2·142 A) and the other was from one of the pyrimidine ring N atoms (N(9), 2·099 A). The atoms (N(5) and N(7)) were located much farther away. The arrangement of co-ordination of $Zn(SD)_2 \cdot 2NH_3$ is in good agreement with that reported by Baenziger et al (1983). In $Zn(SD)_2(CH_3NH_2)_2$ the Zn atom was also tetrahedrally (undistorted) co-ordinated to N(1) (2·067 A) and N(2) (2·084 A) in two methylamine molecules and to N(3) (2·026 A) and N(4) (2·097 A) of two sulphonamido N atoms in anionic sulphadiazines. This arrangement of co-ordination was reminiscent of AgSD found by Cook & Turner (1975) and Baenziger & Struss (1976). $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ shows a unique diffraction pattern. The Zn atom was symmetrically surrounded by three molecules of ethylenediamine, forming an octahedral co-ordination which was in turn co-crystallized with two anionic sulphadiazines and a water molecule. The symmetry of the $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ molecule is clearly shown in Fig. 2.

The molar conductivity of $Zn(SD)_2$ derivatives in 10^{-3} M dimethylsulphoxide ranged from 12 to 18 $ohm^{-1} cm^2 mol^{-1}$ (Table 1). Compared with that of AgSD (2 $ohm^{-1} cm^2 mol^{-1}$), $Zn(SD)_2$ derivatives exhibited a better dissociation in the hydrophilic solvent.

The aqueous solubility of $Zn(SD)_2$ derivatives were higher than that of AgSD and they were soluble in 25% NH_3.

Assay in-vitro and in-vivo

Both the $Zn(SD)_2$ derivatives exerted significant activity, in-vitro, against both Gram-positive and Gram-negative bacteria in addition to fungus (Table 2). $Zn(SD)_2(CH_3NH_2)_2$ was the more potent of the two and its antimicrobial activity was comparable with AgSD. We reasoned that the excellent activity of $Zn(SD)_2(CH_3NH_2)_2$ was attributed, at least partly, to the similar complexation mode between $Zn(SD)_2(CH_3NH_2)_2$ and AgSD as shown in Fig. 2. An important feature for the bioactivity of AgSD is that the silver ion, which is responsible for antimicrobial actions, is co-ordinated to the 2-(sulphonamido)pyrimidine part of the sulphadiazine molecule (Cook & Turner 1975; Baenziger & Struss 1976; Bult & Klasen 1978). In $Zn(SD)_2$ derivatives, only $Zn(SD)_2(CH_3NH_2)_2$ maintained the right co-ordination and this similarity in co-ordination was reflected in the in-vitro antimicrobial activity. In addition, the better aqueous solubility and ready dissociation of the molecule may significantly facilitate the inhibition of micro-organisms. Thus, the effectiveness of the burn-treatment compounds probably not only depends on the nature of Ag^+ or Zn^{2+}, but also depends strongly on the co-ordination between the metal ions and ligands.

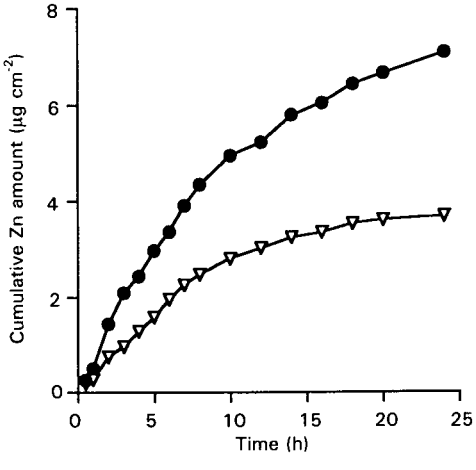


FIG. 5. Permeability of $Zn(SD)_2$ derivatives on the skin of nude mice. ●, $Zn(SD)_2(CH_3NH_2)_2$; ▽, $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$.

The arrangement of co-ordination of $Zn(SD)_2 \cdot 2NH_3$ was highly distorted and deviated from that of AgSD to such an extent that it showed an inferiority in the antimicrobial potencies. In $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$, the zinc ion was well stabilized through ligation, with three neutral ethylenediamines. This stabilization might result in the prevention of binding of Zn^{2+} to DNA, and thus was detrimental to the biological activity.

Both the two $Zn(SD)_2$ derivatives were more soluble than AgSD but were still considerably less soluble than other topical agents. Because of the relatively low solubility, these compounds remain active at the wound area for the appreciable period which is necessary for microbial inhibition.

Although $Zn(SD)_2$ derivatives demonstrated potent antimicrobial actions both in-vitro and in-vivo, their activity was indeed partially reversed by *p*-aminobenzoic acid (Table 3). In this connection, the twice molar amount of sulphadiazine in $Zn(SD)_2$ derivatives and their greater solubility in water may bring about a compensatory effect as demonstrated in the animal tests.

The favourable effects of zinc ion in burned animals have been well documented (Pories et al 1967; Henzel et al 1970). The reduced weight loss and slight improvement in food consumption with $Zn(SD)_2$ derivatives (Fig. 4) have significant implications for the later stage of burn therapy in patients when nutrition becomes a major problem, particularly in children.

After the burn and treatment with $Zn(SD)_2$ derivatives, the eschar separated in about two weeks and pink, healthy-

looking epithelium appeared. The tissues and fur of the mice were normal in looks, while those treated with AgSD darkened. None of the burned and challenged mice receiving either no treatment or placebo survived after 11 days postburn.

$Zn(SD)_2$ derivatives were, in general, quite safe in animals. The high therapeutic indices mean that these compounds provide potential advantages of AgSD and zinc salts, as evidenced from Fig. 3 and Table 4, without their attendant disadvantages and a method of treating burns in man and animals.

Permeability

Both $Zn(SD)_2$ derivatives showed a good permeability of zinc ion on the skin of nude mice. The permeability exhibited a zero-order kinetic reaction and reached a plateau in 8 h (Fig. 5). The rapid skin permeation as well as the favourable concentrations of zinc ion might facilitate continuous replacement of the zinc lost after thermal trauma and therefore lead to a promotion of wound healing and gain of body weight in mice once infection is under control.

X-ray crystallography has established that the $Zn(SD)_2$ derivatives were monomers (Fig. 2), whereas AgSD polymerizes into a double chain that extends through the crystal (Cook & Turner 1975; Baenziger & Struss 1976). This structural difference together with the better aqueous solubility may significantly contribute to rapid permeation of $Zn(SD)_2$ derivatives. Accordingly, $Zn(SD)_2$ derivatives may have the capability of inhibition of the growth of bacteria colonized under the skin.

In summary, $Zn(SD)_2(CH_3NH_2)_2$ consistently demonstrated a very potent antimicrobial activity and was superior to AgSD, $Zn(SD)_2 \cdot 2NH_3$, and $Zn(SD)_2(C_2H_8N_2)_3 \cdot H_2O$ considering overall survival, wound-healing, high LD50 value in animals and without staining of tissues.

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