TREATMENT OF PSEUDOMONAS LIFE THREATENING CHRONIC SUPPURATIVE OTITIS MEDIA BY NEW CONSERVATIVE THERAPY A PROSPECTIVE STUDY 1993-1994

FURAT T. EL-SAMERRAIE*
ABDULWAHAB R. MOHAMMED**
MOHAMMED A. AL-MOSAWI*
SAMI E. A. MATLOOB*

SUMMARY: A new phenazinomycin-related antibiotic was isolated as a cultural product of Ps. aeruginosa. This antibiotic possesses antibacterial activity against certain gram-positive and gram-negative bacteria including enteric bacteria and Ps. aeruginosa. This minimum inhibitory concentration (MIC) as determined by the both microdilution assay ranged between 25-65 μ G/mL.The product yield using a novel fermentation and purification technique was 100-605 μ G/mL, while by classical methods a yield of only 10-50 μ G/mL was obtained. The physico-chemical properties of the antibiotic such as melting point, ionization constant and spectrophotometric analysis were determined. Preliminary drug toxicological studies (LD50) were determined in mice but did not show toxic or important undesirable effects even at relatively high concentrations of 400 mG/kg using the intraperitoneal route of administration. Clinically the H1 antibiotic in the form of ear drops (500 μ G/mL) proved effective in the eradication of 82.6% (71/86) gentamicin-resistant. Ps. aeruginosa chronic middle ear infections with dramatic reduction in morbidity and negligible side effects.

Key Words: Chronic Otitis Media, Antibiotic, Phenazinomycin.

INTRODUCTION

Many antimicrobial agents were reported to be produced by strains of Ps. aeruginosa (2,9). One such antimicrobialis is pyocyanine, a phenazine-1-hydroxy-5-methyl hydroxide. Similar antibiotics from cultured mycelium of Streptomyces spp with 400 MW was investigated (3) and found to have antibacterial activities against gram-positive bacteria, direct experimental murine tumours *in vivo.* A pseudomonal fungal antibiotic, phenazine-1-carboxylic acid, was described by Jones *et al.* (3).

*From Department of Otorhinolaryngology, Saddam College of Medicine, P.O. Box 4083, Adamiah, Baghdad - Iraq.
**From Department of Medical Research Center, Saddam College of Medicine, Baghdad - Iraq.

Chronic suppurative otitis media (CSOM) is considered to be a major problem in the developing world with a relatively high morbidity and mortality. The overall prevalence of CSOM in these countries ranges from 5-10% (4). About 50% of brain abscesses are otogenic in origin (5) with a mortality rate of 50-75%. Several members of the aminoglycoside group were proved ineffective in the treatment of 70-80% of cases of SCOM (6,7). The bacterial pathogens involved are mainly E. Coli (40%), Pseudomonas aeruginosa (40%) and Proteus mirabilis (20%) (4). Surgery in the form of mastoidectomy (radical, modified radical or cortical) is now considered to be the ultimate treatment in resistant cases to avoid the above mentioned complications (7). The present study is concerned with testing of a long known

but not well exploited nor characterized group of antibacterial activity against both gram-positive and gram-negative bacteria. The introduction of these antibiotics to clinical use was proved by the limited resources and local non-availability of new effective antibiotics.

MATERIALS AND METHODS

Micro-organisms and strains

Bacterial isolates from patients with C.S.O.M. were preserved including 100 strains of Ps. aeruginosa and 20 of S. aureus. Pyocyanins were extracted from five selected strains of Ps. aeruginosa of animal origin.

Isolation and Growth Strains

Ear swabs were placed in transport medium containing 1% peptone and Kept at +4°C until processing with 24 hours. Identification of Ps. aeruginosa was carried out according to Shirinwas (1979). Culture media for isolation and characterization of pseudomonal strains included Kings A, Kings B and a new culture medium that enhances pigment production described thereafter.

Antibiotic Production and Purification

A liquid glycerol-peptone-phosphate medium was used for enhancing antibiotic production from Ps. aeruginosa. The above mentioned medium in Erlenmeyer flask was inoculated with 2 ml of starter culture and grown without agitation at 37°C. The antibiotic yield from the culture is estimated after a certain time of incubation. Crystallization of the purified antibiotic was carried out from ethanol after its delution with chloroform-ethanol mixture from a column of aluminium oxide mixture.

Physico-Chemical Data

Melting point was determined according to the method of Von Saltze (10). Absorption spectra of the antibiotic were determined using spectrophotometer. The ionization constants were determined spectrophotometerically using 0.54 mM phenazine-1-carboxylic acid in 0.09 M sodium format. The pH and A373.3 nm were measured after each of 30 discrete changes in pH range 5-2.8. The absorption maximum was at 365 nm.

Biological Properties

Three antibiotic diffusion methods including spot dilution, filter paper disc and agar block were used for the preliminary estimation of the drug potency against microbial isolates. The minimal inhibitory concentration (M.I.C.) of the antibiotic was determined by the microbroth dilution method using microtiter plates (11).

Patients

One-hundred patients with CSOM were selected from ENT Department consulting room in the period September 1993-March 1994 according to clinical suspicion, as having pseudomonal infection, confirmed by laboratory isolation of the microorganisma.

Management and Treatment Protocol

The protocol followed can be summarized in the following steps:

* Ear swabs of culture and sensitivity (C and S) tests are taken to start with for every patient.
* The prepared antibiotic eardrops (500uG/MI) are administered as three drops, three times daily for two weeks.
* Another ear swab(s) is taken and submitted to C and S at the end of two weeks.
* Maintenance treatment, three eardrops once daily at bedtime, is given for another two weeks.
* Patients are re-evaluated at the end of one month by both clinical examination and laboratory C and S of ear swabs.
* All patients are followed up for another period of three months, reassessing the patient monthly by clinical examination and culture of swabs.
* Any side effects, even trivial, arising during the treatment were carefully recorded.
* Any patient who failed to show up during the follow up was excluded from the study.

Table 1: Comparison between different Pyocyanin extraction procedures.

Extraction method	Procedure	Pyocyanin Yield (μG/ml)
Lee and Walden	Chloroform-Ethyl Alcohol-Aluminium Oxide	0 - 8
Langley *et al.*	King's Medium-Chloroform	20 - 405
Fanaki *et al.*	Cuanomycin Extraction Procedure	100
Local Extraction	Pseudomonas Agar-Aluminium	340 - 500
Method (Al-Shibib *et al.*)	Oxide (Agar Extraction)	

Table 2: Antibacterial activity and absorption spectra of pyocyanin groups.

Ps. Aeruginos Strain	Origin	Properties of Pyocyanin groups	
		Absorption Peak	M.I.C. (μG/ml) for Ps. Aeruginosa
3C, 8P (I)	Animal	356.314.329 + Shoulder at 363 nm	25 - 55
82 (II)	Human	363	35 - 65
3P (III)	Food	256.314.329	45 - 65

RESULTS

Clinical outcome

Of the one-hundred patients originally participated in the study, only 86 continued on the protocol, the others dropped out during the follow-up period.

The clinical response of the 86 patients who continued on the protocol can be summarized as follows:

*The foul-smelling ear discharge disappeared in 71 (82.6%) patients with residual mucoid discharge in the remaining 15 (17.4%) patients, two weeks after start of treatment.

*The hearing improved by 10 dB or more in each ear of 58 (67.4%) cases one month after treatment using conventional pure tone audiogram.

*Seven patients (8.1%) who presented with moist mastoid cavities, all developed dry cavities at the end of one month but with recurrence in four cases at four months.

*The tympanic membrane returned to normal (i.e. healing) in one or both ears in 9 (10.5%) patients exploration of the mastoid bone was scheduled before the advent of this treatment.

The side effects

Developed during the treatment were minor and included mild to moderate itching of the external meatus developing in 13 (15.3%) patients but responded to local 1% hydrocortisone ointment for two days. Another complication is mild to moderate otorrhoea that developed during the first two weeks of treatment in 8 (9.3%) patients and then resolved spontaneously.

Antibacterial activity of pyocyanin group

Pyocyanins were categorized into three groups according to the origin of producer strains and absorption spectra of the pigments (Table 2). The groups showed different ranges of antibacterial activity against clinical isolates of Ps. aeruginosa as determined by their M.I.Cs. Moreover, the antibacterial spectra also differed among the groups (Table 3). Group 1 shows a wider spectral activity against both gram (+) and gram (-) bacterial isolates of human origin.

Preliminary acute toxicity studies

In cell cultures - preliminary in vitro toxicity studies

Table 3: Antibacterial Spectra of Pyocyanin Group.

Pyocyanin	Antibacterial spectra				
GROUP	E. Coll	Meisseria spp.	S. aureus	Sal. Typhi	Pr. mirabilis
Group I	+	+	+	+	+
Group II	+	-	+	-	+
Group III	+	+	-	-	+

using primary Balb/c mouse embryo fibroblast cell cultures and simple neutral red and trypan blue staining (not DU50) showed toxic effects at doses more than 10 mG/mL.

Animal Studies

Trials to determine the L.D.50 in adult Balb/c mice failed to show acute toxic effects at doses up to 400 mg/KG given by the intraperitoneal route. Higher doses and other animal species were not tried.

DISCUSSION

The pyocyanin group antibiotic, extracted and purified from certain strains of Ps. aeruginosa possessed physico-chemical properties different from that described for pyocyanins by other workers (Table 2). The new antibiotic has a melting point of 232 C and a single absorption spectral peak at 363 nm. Gurusidaiat (1986), described a methyl ester N, N-diacetyl dihydrephenazine 1-carboxylic acid which exhibit a single absorption maximum near 260-265 nm.

The biological properties of this antibiotic as reported here differ from those of phenazine-1-carboxylic acid pigment extracted by Elliot in 1958 from cultures of Ps. ovalis. The latter pigment is a brown amorphous powder with a melting point extending over a temperature of 230-250 PoC, whereas this new antibiotic crystallized as bluish-green crystals with 132C and found to be homogenous by both paper chromatography and paper electrophoresis. In addition the Elliott pigment gave a brownish precipitate when treated with 5% (w/v) silver nitrate solution, a reaction not encountered with this new product. The differences in the properties between the two pigments probably reflect differences in chemical structure due to differing producing bacterial strains and fermentation and extraction procedures (Table 1).

The clinical outcome of the treated CSOM, patients as evaluated at the end of the follow-up period can be categorized into four groups.

Group I (10.5%) include patients with a dramatic response, having dry ear and healed tympanic membrane that is maintained 3 months after cessation of treatment.

Group II (37%) are patients showing marked response with the development of dry ears.

Group III (35%) are patients with a moderate response having persistant scanty mucoid discharge with negative culture or culture of Staph. epidermidis.

Group IV (17.4%) are patients with minor response as evidenced by persistance of discharge but with change of isolated microbial pathogen to Staph. aureus. The side effects were mild and self limiting in most cases with an incidence of 24%.

The high response rate accompanied by mild and self-limiting side effects encourages the use of this antibiotic in topical treatment of patients with CSOM caused by Ps. aeruginosa or probably other multidrug-resistant gram-negative bacteria especially in situation where other effective antibacterial drugs are not easily available.

REFERENCES

1. Al-Shibib AS and RK Al-Huseini : Phage typing of Pseudomonas aeruginosa. Iraqi Med J, 35:8-23, 1987.

2. Omura S, S Eda, SK Funayama, Y Takahashi, HB Woodruff : J Antibiotic-Tokyo, 42:1073-1042, 1989.

3. Jones PL-DG ME, MR Tate Snow, ER Tiekink : Structure of the Pseudomonas Fungal antibiotic phenazine-1-Carboxylic acid. 44:2220-2222, 1988.

4. Manni JJ, PN Lema : Otitis media in Dar es Salam, Tanzania. J Laryngol-Otol, 101:222-228, 1987.

5. Sulla I, JM Fugul'a, M Santa : Treatment in patient with multiple brain abscesses, Rozhi Chir, 68:637-639, 1989.

6. Brook I, P Yocum : Quantitative bacterial cultures and beta-lactamase activity in chronic suppurative otitis media. Ann. Oto Rhino Laryngol, 98:293-297, 1989.

7. Papastavros T, H Tiamaxvellou, S Varlejides : Pre-operative therapeutic considerations in chronic suppurative otitis media. PTO, Laryngoscope, 99:655-659.

8. Brisbane PG, LJ Janik, ME Tate, FO Warrren : Revised Structure for the phenazine antibiotic from Ps. Fluorescence Antimicrobial Agents and Chemotherapy. 79:1967-1971, 1987.

9. Cox C, P Adams : Siderophore activity of pyoverdin for Ps. aerugions. Infection and Immunity, 48:130-138, 1985.

10. VonSalze MH, JA Last, PG Stapleton, ML Rathnum and SL Neidleman : Its identity with pyocyanine J Antibiotics. XXII:49-54, 1969.

11. Gavan TL and AL Barry : Microdilution test procedures in Manual of clinical Microbiology. 3rd ed, American Society of Microbiology, pp 459-462.

Correspondence:
Furat T. El-Samerraie
Saddam College of Medicine,
P.O. Box 4083-Adamiah,
Baghdad, IRAQ.