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COMMUNICATIONS

SOFRAMYCIN

ITS PENETRATION INTO THE EYE AND ITS EFFECT UPON EXPERIMENTALLY PRODUCED *STAPH. AUREUS* AND *PS. PYOCYANEA* CORNEAL INFECTIONS*

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THE topical application of antibiotics is useful in the treatment of superficial ocular infections, and systemic administration is often effective in overcoming cellulitis of the soft tissues which surround the eye; but it is only by subconjunctival injection that a high concentration of antibiotic can be obtained within the eye. Subconjunctival injection is essential if an attempt is to be made to overcome an intra-ocular infection. Antibiotics which can be administered subconjunctivally are therefore of particular interest to ophthalmologists. To be suitable for subconjunctival injection, an antibiotic must be readily soluble in water and the solution must not be unduly irritating to the tissues.

Only penicillin (Sorsby and Ungar, 1948), streptomycin (Sorsby, Ungar, and Bailey, 1952), and polymyxin (Ainslie and Smith, 1952) have hitherto fulfilled these conditions and all have certain limitations. Penicillin is only slightly irritant when injected, but its range of activity does not cover the Gram-negative bacilli. Many strains of staphylococci are also resistant. Polymyxin is highly effective against Gram-negative bacilli but has no effect upon the coccal organisms. Polymyxin is a moderate tissue irritant and while subconjunctival injection is perfectly safe the injections are very painful. Streptomycin is only slightly irritant and its range of activity covers both Gram-positive cocci and Gram-negative bacilli, but resistant strains of both types of organism develop with ease. Aureomycin, terramycin, chloramphenicol, and erythromycin are of no therapeutic value given subconjunctivally since their water solubility is very low and sufficient concentrations cannot be given. Achromycin is more soluble in water but the solution is irritating when injected.

The appearance, therefore, of soframycin, a new antibiotic with high water solubility and a wide range of activity, seemed to be of considerable interest.

Properties of Soframycin

Soframycin is a white powder highly soluble in water and is naturally produced from the mould *Streptomyces decaris*. It was found that 500 mg. would dissolve

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readily in 1 ml. water, and it was equally soluble in mydricaine and xylocaine with adrenaline. It was stable in solution.

Effect of Subconjunctival Injections

A subconjunctival injection of a solution of soframycin was given to six rabbits. The strength of the solution was 500 mg./ml. in every case. Two rabbits received 1·0 ml. (500 mg.), two received 0·50 ml. (250 mg.), and two 0·25 ml. (125 mg.).

Moderate chemosis was present 24 hours later in the case of the two receiving 1 ml. This subsided rapidly and the eyes appeared entirely normal after 4 days. There was no conjunctival necrosis and no corneal oedema. Of the two receiving 0·50 ml., one showed slight chemosis the following day and in the other the conjunctiva was merely reddened at the site of injection. Moderate conjunctival inflammation which rapidly subsided was the only effect noted in the two rabbits receiving 0·25 ml.

These initial observations appeared to suggest that soframycin was almost non-irritant when injected.

Penetration of Soframycin into the Eye after Subconjunctival Injection

Assay.—Since the volumes of aqueous fluids were very small, a capillary tube micromethod was used (Fleming and Smith, 1947; May, Voureka, and Fleming, 1947; Medical Research Council, 1948; Ainslie and Smith, 1952).

Estimates were carried out in duplicate, using the Oxford staphylococcus as the test organism. Unsealed capillaries were incubated in the horizontal position and the end-point was taken as being the last tube which showed no change of colour.

Experiment.—A subconjunctival injection of 250 mg. soframycin was given to one eye of eight rabbits. An aqueous solution of a strength 500 mg./ml. was used in every case. The aqueous humour was withdrawn from each eye 90 minutes later. Aqueous humour was also withdrawn from one eye of each of two rabbits which did not receive soframycin.

The eight aqueous fluids from the rabbits which received the subconjunctival injections all showed levels of soframycin. The amounts, as judged from the degree of inhibition in the tubes, varied from 10 to 40 μg./ml. Aqueous fluids from the two uninjected rabbits produced no inhibition in any tube.

The levels of soframycin found in the aqueous humour in this experiment compared very favourably with the levels of penicillin (Sorsby and Ungar, 1948), streptomycin (Sorsby and others, 1952), and polymyxin (Ainslie and Smith, 1952) in the aqueous humour after subconjunctival injection.

In vitro Sensitivity of Strains of *Pseudomonas pyocyanea*, *Staphylococcus pyogenes*, and "Coliform" Organisms

Ps. pyocyanea.—62 ocular strains of *Ps. pyocyanea*, the majority of which had been collected during a period of years by Dr. Charles Smith and maintained in Robertson's meat medium, were tested. For the tests, stock solutions of soframycin in sterile buffered distilled water (pH 6·8) and stored at 4°C. were used in concentrations of 1,000, 5,000, and 10,000 μg./ml. Strains were sown on nutrient agar plates and the sensitivity determined by the standard filter paper disk technique. The cultures were incubated for 72 hours and the tests were read after

24, 48, and 72 hours. When the plates were examined, strains growing right up to the edge of the disk were recorded as being insensitive. Those showing a definite zone of inhibition, but with a few scattered colonies growing within the zone but not reaching the edge of the test disk, were recorded as showing partial sensitivity. Those showing a completely clear zone were recorded as sensitive. The results of these tests showed that, at 24 hours, six strains of *Ps. pyocyanea* were resistant to 1,000 μg./ml. soframycin, and that after 72 hours the numbers had increased to eleven. Partial sensitivity at 24 hours was shown by fifteen strains and this number increased to 37 strains at 72 hours.

With concentrations of 5,000 μg./ml., there were no completely resistant strains at 24 hours. At 48 and 72 hours, only one strain was completely resistant. Two strains showed partial sensitivity at 24 hours, eight at 48 hours, and eleven at 72 hours.

With concentrations of 10,000 μg./ml., all strains were sensitive at 24 hours. At 48 hours two strains showed partial sensitivity only. At 72 hours three showed partial sensitivity, and two were completely insensitive.

Staphylococci.—250 strains of coagulase-positive staphylococci isolated from routine conjunctival cultures were also tested in a similar fashion. All strains were completely sensitive at 24, 48, and 72 hours with zones of inhibition which were wide and sharply defined. As far as could be judged by using a disk technique, the staphylococcal strains appeared more sensitive than any other organisms tested.

"Coliform" Organisms.—Sixty strains were tested. The results were similar to those obtained with staphylococci. All were completely sensitive to a concentration of 1,000 μg./ml. soframycin, and zones of inhibition were wide and clear cut.

Reports of other *in vitro* studies of soframycin have been few, but are in agreement with our findings. Massenat-Derouche (1954) noted the unusual sensitivity of staphylococci to soframycin, and that the streptococci were also sensitive though rather less so. Lutz and Hofferer (1955) observed that many strains of *B. proteus* were highly sensitive, and found that rather more than 80 per cent. of strains of *Ps. pyocyanea* were sensitive or partially sensitive to soframycin.

Treatment of Experimental Corneal Infections

The effect of soframycin, given subconjunctivally, upon the progress of experimentally-induced corneal infections was determined in a series of experiments.

(*a*) *Staphylococcal Infections.*—Infection was produced by the intracorneal injection of 0·03 ml. of an undiluted 24-hour broth culture of *Staph. aureus.* The right corneae of seventeen rabbits were injected; six were left as controls and eleven received a subconjunctival injection of soframycin within a few minutes of the infecting dose. In each case the strength of the soframycin solution injected was 500 mg./ml. Three rabbits received 500 mg., three 250 mg., and five 125 mg.

In no case where soframycin was given was any sign of infection apparent after 24 hours and no infection developed during the subsequent week. After 24 hours all six controls showed heavy corneal infiltrates varying from 3·5 mm. to 6 mm. in diameter, and the infiltration increased markedly during the next 24 hours.

(*b*) *Ps. pyocyanea Infections.*—Infection was produced by the intracorneal injection of 0·03 ml. of 1:100 dilution of a 24-hour broth culture of *Ps. pyocyanea.* 23 corneae were infected; seven were left as controls and sixteen were given an immediate subconjunctival injection of an aqueous solution of soframycin (strength 500 mg./ml.), eight receiving 125 mg. and eight 250 mg.

Rapidly spreading corneal infection was apparent in all controls after 24 hours, but in nine of the treated cases the corneae were clear after 24 hours and remained so for the following week. In one instance there was a very slight corneal infiltrate apparent at 24 hours which became widespread by the following day. Two corneae remained clear until the third day when a small but spreading infiltrate occurred. In the remaining four, clinical infection was delayed until the fourth day. There was no significant difference between the cases receiving 250 mg. soframycin and those receiving 125 mg. In all cases the subconjunctival injections were well tolerated, there being scarcely any sign of irritation at the site of injection.

Clinical Trials

No extensive clinical trials have as yet been undertaken, but the experimental evidence suggests that soframycin is suitable for clinical use. One patient with a severe hypopyon ulcer has been treated, however. Clinically, the ulcer was of the type due to the pneumococcus, but no organism was grown on culture. This may have been due to the fact that previous treatment with antibacterial ointments had been applied. The infection subsided rapidly after two subconjunctival injections of soframycin 100 mg. dissolved in 0·25 ml. mydricaine given on two successive days.

Discussion

Soframycin is an antibiotic suitable for subconjunctival injection which is exceptionally active against staphylococci. In addition it is effective against many strains of streptococci and Gram-negative bacilli.

Both our *in vitro* and *in vivo* studies indicate the extreme activity of soframycin against the staphylococcus. The results of treatment of corneal infections due to *Ps. pyocyanea* were in accord with the results of the *in vitro* sensitivity tests. Thus, the absence of an absolutely clear zone of inhibition around the test disks, indicating a variable sensivity in the bacterial population of a particular strain, agrees well with the clinical observation that some corneal infections were entirely controlled by a single dose of soframycin, whereas in others the clearance of clinical infection was delayed for a few days.

Summary

(1) Soframycin, an antibiotic active against both Gram-positive cocci and Gram-negative bacilli, is described.

(2) Soframycin is highly soluble in water and was found to be suitable for subconjunctival injection.

(3) Therapeutic levels of the antibiotic were found in the aqueous humour of the rabbit after subconjunctival injection.

(4) All strains of staphylococci tested were highly sensitive to soframycin *in vitro*. Most strains of *Ps. pyocyanea* also showed sensitivity.

(5) Experimental corneal infections due to both *Staphylococcus aureus* and *Ps. pyocyanea* were successfully controlled.

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REFERENCES

AINSLIE, D. and SMITH, C. (1952). *Brit. J. Ophthal.*, **36**, 352.
FLEMING, A., and SMITH, C. (1947). *Lancet*, **1**, 401.
LUTZ, A., and HOFFERER, M. J. (1955). *Rev. immunol.*, **19**, 69.
MASSENAT-DEROUCHE, B. (1954). M.D. Thesis, Paris.
MAY, J. R., VOUREKA, A. E., and FLEMING, A. (1947). *Brit. med. J.*, **1**, 627.
MEDICAL RESEARCH COUNCIL (1948). "Report by the Pathological Subcommittee of the Streptomycin in Tuberculosis Trials Committee", *Lancet*, **2**, 862.
SORSBY, A., and UNGAR, J. (1948). *Brit. J. Ophthal.*, **32**, 864.
———, ———, and BAILEY, N. L. (1952). *Brit. med. J.*, **1**, 119.