NOTES

Chemoprophylaxis of \textit{Shigella flexneri} Keratoconjunctivitis in Rabbits

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Chloramphenicol, chlortetracycline, streptomycin, and sulfisoxazole prevented keratoconjunctivitis in rabbit eyes infected with \textit{Shigella flexneri}. The route of administration of the agent and the dose were important variables in controlling the infectious process. This assay system appears to be a useful technique for the in vivo evaluation of chemotherapeutic agents.

Bacterial conjunctivitis and keratoconjunctivitis are common diseases which are usually acute but may also become chronic (3). Although pyogenic organisms such as \textit{Diplococcus pneumoniae}, \textit{Neisseria gonorrhoeae}, and \textit{Staphylococcus aureus} account for the majority of the eye infections encountered by man (3), conjunctivitis and corneal infections in humans caused by \textit{Shigella flexneri} and \textit{Aerobacter aerogenes} have also been reported (1, 2). MacDonald et al. (2) reported that corneal ulcers in humans due to \textit{S. flexneri} were successfully treated with erythromycin. Eye infections in humans caused by \textit{A. aerogenes} have been treated with streptomycin (1). In our laboratory, we have used the rabbit eye infected with \textit{S. flexneri} as an experimental model for the study of mechanisms of pathogenicity (W. R. Cross and M. Nakamura, Bacteriol. Proc., p. 75, 1969; W. R. Cross and M. Nakamura, Bacteriol. Proc., p. 94, 1970).

In this study, we examined the efficacy of various antibiotics and sulfa drugs on \textit{S. flexneri}-induced keratoconjunctivitis in rabbits. Several routes of administration of these agents were compared.

\textit{S. flexneri} 2a, isolated from a human case of shigellosis, was obtained from Kern County General Hospital, Bakersfield, Calif. This strain consistently caused keratoconjunctivitis in New Zealand strain rabbits (1.5 to 2.5 kg) when 10^5 viable organisms were inoculated onto the surface of the conjunctiva.

Antibiotics were administered onto the eye by employing a syringe without a needle. Then the eyelids were held together for 1 min and the upper lid was gently massaged. In other experiments, the antibiotics were injected intraconconjunctivally (ic) with a 0.25-ml tuberculin syringe fitted with a 27 gauge needle. In addition, some animals were given the antibiotics by intramuscular (im) injections. Ointment preparations of the sulfa drugs were administered with an applicator stick.

In five experiments (15 animals), a single dose of the antibiotic was applied either 1 day before or on the day the eyes were infected with \textit{Shigella}. In seven experiments (21 animals), the antibiotic treatment was continued for several days.

The \textit{Shigella} was recovered from the infected eyes by irrigating the eyes with 0.5 to 1.0 ml of sterile 0.85\% NaCl. A 0.1-ml amount of the wash fluid collected was plated and spread onto Hektoen Enteric Agar (Pfizer Diagnostics, New York, N.Y.). The number of colonies was determined after 24 and 48 hr of incubation at 37 C.

\textit{Shigella} keratoconjunctivitis in rabbits responded to treatment with the antibiotics and sulfa drugs (Table 1). Keratoconjunctivitis failed to develop in rabbit eyes treated with chloramphenicol or chlortetracycline administered intravenously. However, this was possible only if the antibiotics were continued for several days.

After this administration of antibiotics, the number of viable \textit{Shigella} that could be recovered from the eyes decreased, and all treated eyes were free from \textit{S. flexneri} approximately 6 days after infection (Fig. 1). When 50 mg of streptomycin was administered ic, it was effective in preventing keratoconjunctivitis. However, im injection of 250 mg of this antibiotic did not prevent development of the disease. Topical applications of ophthalmic ointments containing sodium sulfacetamide or sulfisoxazole prevented...
Table 1. Treatment of Shigella flexneri keratoconjunctivitis in rabbits with several antibiotics and sulfonamide drugs

| Agent              | Time of administration of agent<sup>a</sup> | Amt of agent administered<sup>b</sup> | Route of administration | Severity of keratoconjunctivitis<sup>b</sup> |
|--------------------|---------------------------------------------|--------------------------------------|--------------------------|-----------------------------------------------|
| Chloramphenicol    | days                                        | mg/kg                                |                          |                                               |
|                    | −1, 0, +1, +2                              | 50                                   | Intravenous              | +++                                           |
| Chlortetracycline  | −1, 0, +1, +2                              | 50                                   | Intravenous              | +++                                           |
| Streptomycin       | 0, +1, +2, +3                              | 50                                   | Topical                  | 0                                             |
| Sulfacetamide<sup>d</sup> | 0, +1, +2, +3                              | 250<sup>e</sup>                      | Intraconjunctival         | 0                                             |
| Sulfisoxazole<sup>f</sup> | 0, +1, +2, +3                              |                                      | Intramuscular             | +++                                           |

<sup>a</sup> Symbols: −1, agent administered 1 day before infection; 0, agent administered on day of infection; +1, agent administered 1 day after infection; +2, agent administered 2 days after infection; +3, agent administered 3 days after infection.

<sup>b</sup> Symbols: 0, absence of keratoconjunctivitis; +, slight keratoconjunctivitis; +++, mild keratoconjunctivitis; ++++, moderate keratoconjunctivitis; +++++, severe keratoconjunctivitis.

<sup>c</sup> Expressed as milligrams.

<sup>d</sup> Sodium sulamyd, an ophthalmic ointment containing 10% sodium sulfacetamide.

<sup>e</sup> Gantrisin, an ophthalmic ointment containing 4% sulfisoxazole diethanolamine.

<sup>f</sup> Ointment preparations were administered with an applicator stick four times a day.

Fig. 1. Recovery of Shigella flexneri from the eyes of antibiotic-treated rabbits infected on day 0. Symbols: ●, rabbits injected intravenously with chloramphenicol (50 mg/kg) only on day 0; ○, rabbits injected intravenously with chlortetracycline (50 mg/kg) only on day 0; □, rabbits injected intravenously with chlortetracycline (50 mg/kg) only on days 0, 1, and 2; Δ, rabbits injected intravenously with chloramphenicol (50 mg/kg) on days 0, 1, and 2; ○, rabbits treated with sulfisoxazole ointment on days 0, 1, 2, and 3 after infection.

The appearance of keratoconjunctivitis. Nevertheless, Shigella organisms were discharged from the eyes for 6 days or more (Fig. 1).

Chemotherapy initiated with chloramphenicol, chlortetracycline, and sulfacetamide (two animals per drug) on the fifth day after infection did not alter the course of the development of keratoconjunctivitis in the rabbits, in spite of the fact that the antibiotics were effective in eliminating the S. flexneri from the infected eyes.

Several factors appear important in controlling S. flexneri keratoconjunctivitis by chemotherapeutic agents. First, the antibiotics must be applied early and continuously during the initial stages of the infection. Second, bactericidal concentrations of the antibiotics must be sufficiently high at the site of infection. This concept is supported by the fact that streptomycin administered im was not effective in preventing keratoconjunctivitis, whereas the agent administered ic prevented the clinical syndrome.

Early treatment of the infected eyes may have prevented the multiplication of the pathogen. This, in turn, may have prevented the elaboration and accumulation of endotoxin. Purified endotoxin has been shown to produce keratoconjunctivitis (W. R. Cross and M. Nakamura, Bacteriol. Proc., p. 94, 1970).

We think that this model, namely, the chemical treatment of rabbit eyes infected with Shigella, may be a useful assay system for the in vivo evaluation of potential chemotherapeutic agents for shigellosis.

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LITERATURE CITED
1. Clark, G., and D. Locatcher-Khorazo. 1951. Corneal ulcer produced by Aerobacter aerogenes. Arch. Ophthalmol. 45:165-167.
2. MacDonald, R., Jr., M. Blatt, and W. C. Edwards. 1965. Shigella corneal ulcer. Amer. J. Ophthalmol. 60:136-139.
3. Vaughan, D., R. Cook, and T. Asbury. 1968. General ophthalmology. Lange Medical Publications, Los Altos, Calif.