

2. Title: Hydroxyquinoline Drug in Cholera

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Objective To evaluate the use of hydroxyquinoline preparations as prophylactic and/or therapeutic agents against cholera.

#### General Information

Recent field studies show that the commonly used cholera vaccines offer limited degrees of protection for limited periods of time. Certain antibiotics reduce the period of excretion of cholera vibrios by cholera patients, and are effective adjuncts in treatment. However, their widescale use involves too many disadvantages. This limitation does not apply to chemosynthetic agents if they have negligible effects on the normal enteric flora and their use does not result in resistant vibrios. Provided they are economical, effective and unaccompanied by side reactions, these agents could be put to widescale use---preventing the spread of cholera in a community, and protecting travelers and troops in endemic/epidemic areas. One such agent, iodochlorohydroxyquinoline (Entero-Vioform<sup>R</sup>, Ciba), has been used widely for prophylaxis and treatment of amebiasis and has reduced the incidence of shigellosis in institutions. This agent has few side effects, is economical, and (to our knowledge) development of microbial resistance has not been reported. A study made in Egypt in 1947 (1) suggested it might reduce the period of vibrio excretion of cholera carriers.

#### Description

a. Sensitivity of enteric bacteria to iodochlorohydroxyquinoline. In vitro studies were initiated to determine the effects of Entero-Vioform<sup>R</sup> on cholera vibrios, other enteric pathogens and normal enteric bacterial microflora.

Preliminary tests were conducted on 1 V. cholerae, 2 Shigella, 1 Salmonella, 1 Proteus, 1 Bethesda, 1 Aerobacter and 1 Escherichia coli strains. Entero-Vioform<sup>R</sup> tablets were cut into 8 pieces each and placed on the surface of heavily streaked MEA (for vibrios) and trypticase soy agar (for other organisms) plates. After incubation at 37 C for 18 hours, zones of inhibition around the drug were measured. A comparison of two lots of Entero-Vioform, with and without Sapamine, a surface-active agent, was made with a battery of enteric bacteria. The effect of this drug on vibrios and Shigellae grown on blood agar was compared with that on MEA or TSA plates.

After these initial tests, tube dilution sensitivity tests were performed, using inocula of 10<sup>4</sup> organisms. Pure Vioform powder was dissolved in boiling absolute ethanol and diluted in tryptose

phosphate broth. Mixtures of organisms and dilutions of drug in test tubes were incubated at 37 C for 18 hours. The tubes were examined for turbidity and all non-turbid tubes were streaked on MEA or TSA plates. Effects of human intestinal juice on the anti-vibrio efficiency of Vioform were also tested. Resistant enteric bacteria such as E. coli and Paracolonobacterium aeruginoides were coupled with vibrio cultures to determine susceptibility of vibrios. The gradient plate technique<sup>(2)</sup> was used to test emergence of resistant isolates of V. cholerae to iodochlorohydroxyquinoline.

b. Effect of hydroxyquinoline preparation on experimental cholera. The chloro form (Quixaline, Squibb) produced larger inhibition zones than the iodochloro form (Entero-Vioform<sup>R</sup>, Ciba) of hydroxyquinoline. An in vivo study comparing these two preparations was carried out. Infant rabbits were infected by intra-intestinal inoculation of 10<sup>4</sup> organisms of V. cholerae 569 B. The two compounds were made into 10% suspensions and fed via stomach tube in three 10 mg doses at 4 hour intervals. Therapy was started 20 hours before vibrio inoculation in one group (prophylaxis), 6 hours before inoculation in the second group (early treatment, allowing 6-8 hours for the drug to reach the small intestine) and 1 hour after inoculation in the third group (treatment group). The animals were observed for diarrhea and death. They were examined for fluid accumulation in the intestines either after death or at sacrifice 46 hours after inoculation, at which time cultures were taken of intestinal contents.

c. Trial of Entero-Vioform<sup>R</sup> in cholera patients.

Trial of hydroxyquinoline as a treatment agent was initiated when cholera broke out in Samut-prakarn (a province 25 kms from Bangkok) in mid-December. All patients admitted to the cholera ward of Samut-prakarn Provincial Hospital were studied. On admission suspected cholera patients received the usual prompt fluid replacement but no antimicrobials. Patients given odd admission numbers received drug A (Entero-Vioform<sup>R</sup>), while those given even numbers received drug B (placebo). The dosage of drug A or placebo was 250 mg every 4 hours for 72 hours in the form of tablets for adults and suspension for children 1-10 years of age. Rectal swabs were taken prior to the first dose of drug and at 0600 daily thereafter. The swabs were incubated in alkaline peptone broth at 37 C for 6 hours before streaking on polymyxin MEA<sup>(3)</sup> and modified tellurite<sup>(4)</sup> plates and subcultured into fresh alkaline peptone broth for 5-20 hours before restreaking on the same media. Plates were incubated at 37 C for 16-20 hours. Suspected colonies on MEA<sup>(5)</sup> and tellurite plates were tested for agglutination with V. cholerae "O", type specific antisera, and, if positive, with chicken red cells<sup>(6)</sup>. These colonies were picked to KIA tubes, and later tested for motility and fermentation of mannitol, sucrose, arabinose and mannose; production of indol, acetyl methyl carbinol (Voges-Proskauer test), liquefaction of gelatin; and resistance to 50 U of Polymyxin B. Routine procedures were used to detect other enteric pathogens. Patients who did not have cholera and those who had received antimicrobials were deleted from the study. After three successive negative cultures each study patient received a sodium sulphate purge and a stool culture was taken. The assessment of results was by (1) comparison of duration of vibrio excretion and (2) relapse rate after purge.

### Progress

a. Sensitivity of enteric bacteria to iodochlorohydroxyquinoline (Entero-Vioform<sup>R</sup>).

Initial tests indicated that V. cholerae and two Shigella strains were sensitive to Entero-Vioform<sup>R</sup> whereas the others were resistant. Zones of inhibition obtained with Entero-Vioform<sup>R</sup> with or without Sapamine were virtually identical with few exceptions (Table 5). Of the enteric pathogens tested, all strains of V. cholerae, classical and El Tor, and all the Shigellae were found to be sensitive but all Salmonellae were resistant. The non-pathogens were generally resistant or had only faint hazy zones, found in some cases surrounding the tablet with Sapamine. This suggests they might be resistant to Entero-Vioform<sup>R</sup> but sensitive to Sapamine. In the tests where blood agar plates were used the zones of inhibition were much smaller suggesting interference of activity of drug by blood.

Tube dilution sensitivity tests of 35 strains of cholera vibrios and 1 NAG vibrio strain revealed that 23 strains were inhibited by 1.95 mcg of pure Vioform/ml; 12 strains by 3.90 and 1 strain by 7.80 mcg. No differences in sensitivity between classical and El Tor vibrios were noted. The bactericidal concentration usually doubled the inhibitory level. Intestinal juice decreased sensitivity of vibrios 4 or 8 fold. *E. coli* and paracolons mixed with vibrios had no effect on drug resistance. Drug sensitivities of vibrios were not significantly affected after 6 consecutive passages by the gradient plate technique.

b. Effect of hydroxyquinoline preparations on experimental cholera.

Results of the experiments are summarized in Table 6. Post-mortem examinations revealed that almost all rabbits had 4+ fluid accumulation in their intestines. Cultures taken from all animals were positive for *V. cholerae*. Both preparations delayed onset of diarrhea and death. This was more pronounced in the prophylaxis than in the treatment groups. The chloro form sustained life longer in both groups. The large dose of the drug (200–300 mg/kg body wt.) administered over a period of 8 hours did not have bactericidal action on the organisms in vivo. However, the protective effect demonstrated suggests that better results might be obtained if the drug was administered longer.

c. Trial of Entero-Vioform<sup>R</sup> in cholera patients.

Of 93 patients admitted with diarrhea 29 were positive for El Tor Inaba, 3 for *Shigella* spp and 10 for *Salmonella* spp. Twenty-three of the 29 patients positive for El Tor vibrios were studied. Although the patients were distributed equally into both treatment groups on admission there were 15 in the drug A group and 8 in the drug B group. All of the 4 children received drug A. Results are summarized in Table 7. The range of duration of vibrio excretion in drug A group was from 1 to 8 days. Seven of 15 receiving drug A were free of vibrios on day 5 while all patients in the placebo group were still positive at that time. Children did not respond as well—3 remained vibrio positive for 5 days and 1 for 8 days—although they received the same dosage of drug as adults. The one relapse after purge was a man receiving drug A. No untoward side effects of the drug were noted. No decreased sensitivity to pure Vioform of strains isolated after treatment with Entero-Vioform<sup>R</sup> was demonstrated by the tube and plate dilution sensitivity tests.

Summary

In vitro studies show that hydroxyquinoline was active against vibrios as well as *Shigellae* while it had no effect on *Salmonellae* and non-pathogens. When administered to infant rabbits experimentally infected with *V. cholerae* it delayed onset of diarrhea and sustained life longer although it did not have bactericidal action in vivo. In a clinical trial in a small number of El Tor cholera patients the period of vibrio excretion was shortened. No untoward side effects were noted and no resistance of the vibrios to the drug was demonstrated by in vitro sensitivity tests.