Protection from *Shigella sonnei* infection by immunisation of rabbits with *Plesiornonas shigelloides* (SVC O1)

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Summary. *Plesiornonas shigelloides*, an organism commonly found in water, is only rarely associated with diarrhoea in man. *P. shigelloides* serotype O:17 (SVC O1), which is antigenically similar to *Shigella sonnei*, was found to be neither virulent nor toxic for rabbits. Rabbits immunised by feeding with *P. shigelloides* (SVC O1) were completely protected against an oral challenge with $10^{10}$ cells of *S. sonnei* but non-immunised rabbits were not. *P. shigelloides* (SVC O1) may be a useful vaccine strain for shigellosis.

Introduction

*Plesiornonas shigelloides* is commonly found in surface water and has also been isolated sporadically from patients' faeces during episodes of diarrhoea. It is rarely found in the faeces of healthy individuals. Investigations of the enteropathogenicity of *P. shigelloides* by several workers have not demonstrated it to be a cause of diarrhoea. P. *shigelloides* has been reported as a causative agent of various extra-intestinal diseases, such as septicaemia, neonatal meningitis, cellulitis, septic arthritis, endophthalmitis and acute cholecystitis.

A few serotypes of *Plesiornonas* spp. have antigenic similarity with *Shigella sonnei* phase I O-antigen and this study was undertaken to find out if one such serotype, *P. shigelloides* O:17 (SVC O1), could give cross-protection to *S. sonnei* infection. As contaminated water is a frequent source of infection for man, this type of natural infection by *P. shigelloides* might provide immune protection against *S. sonnei* infection. This may be the reason for the relative absence of *S. sonnei* infection in developing countries compared to that in developed countries, where people usually drink boiled or chemically-treated drinking water.

Materials and methods

Bacterial strains

*P. shigelloides* (SVC O1) was obtained from Professor A. Lindberg, Department of Clinical Bacteriology, Huddinge University Hospital, Sweden. *S. sonnei* strain AB3998 was isolated from a patient with diarrhoea at the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh.

**Sereny test**

This test was carried out according to the method described by Sereny. The bacterial cultures were inoculated on to brain heart infusion agar (BHIA) and incubated at 37°C for 24 h. The cells were harvested and suspended in physiological saline to a density of $10^{10}$ cells/ml. An equal inoculum of the *S. sonnei* suspension was used as a positive control and sterile physiological saline as a negative control. The animals were inspected over a period of 72 h for keratoconjunctivitis.

**HeLa cell cytotoxicity tests**

Cytotoxicity tests with HeLa cells were done by the method of Herrington et al. P. *shigelloides* (SVC O1), and a strain of *S. dysenteriae* as a positive control, were grown on BHIA and incubated at 37°C for 24 h. The cells were suspended in brain heart infusion broth (BHIB) containing polymyxin B (0-2 mg/ml) and incubated for 30 min at 37°C. The suspensions were centrifuged at 6000 g for 10 min in a micro-centrifuge and the supernates were removed and filtered through Whatman filter paper (pore size 0.45 μm). A series of 10-fold dilutions of the filtrates in BHIB was made and 20 μl of each dilution was added in duplicate to HeLa cell monolayers in modified Eagles' minimum essential medium. Saline was used as a negative control. The cells were examined after incubation overnight at 37°C in a moist chamber. Toxin formation by the positive control caused the cells to shrink and detach from the well surface.

**Preparation of immunogen**

Tetracycline-sensitive *P. shigelloides* (SVC O1) and
S. sonnei strain AB3998 were cultured overnight on BHIA plates at 37°C. The cells were harvested and suspended to a density of 10^{10} cells/ml in physiological saline; 1 ml of the suspensions was added to 14 ml BHIB and fed to rabbits.

Animal challenge (oral immunisation and challenge)

The test group comprised 10 healthy albino rabbits each weighing 2.0-2.5 kg. They were starved for 36 h. During this period, 10 similar control rabbits were fed with tetracycline solution (25 mg/ml) to minimise the number of existing micro-organisms in the stomach. After 36 h, cimetidine (50 mg/kg body weight) was administered intravenously to rabbits of the test group followed by two doses of NaHCO₃ 5% solution by gastric tube at intervals of 15 min. Immediately afterwards, 10^{10} cells of P. shigelloides (SVC O1) in 15 ml of BHIB were given by gastric tube and followed by intraperitoneal injection of 2 ml of tincture of opium. A second dose of P. shigelloides (SVC O1) (10^{10} cells in BHIB) was given on the sixth day. The control group (non-immunised) was similarly treated but fed with sterile BHIB without any bacteria. On the sixteenth day, all the rabbits were challenged by feeding with 10^{10} cells of S. sonnei in BHIB. The fatality rate of the test and control groups was measured after 24 h. The experiment was performed twice.

Results

Sereny test

Guinea-pig eyes infected with P. shigelloides (SVC O1) did not develop keratoconjunctivitis but those infected with S. sonnei AB3998 did.

HeLa cell cytotoxicity tests

Culture filtrates of P. shigelloides (SVC O1) and the saline control did not cause any cytotoxic effect on HeLa cells. However, degenerative changes were seen after 24 h in HeLa cells treated with S. dysenteriae culture filtrates.

Animal challenge

No diarrhoea was observed in any of the test rabbits immunised with 10^{10} cells of P. shigelloides (SVC O1) and all remained healthy and symptomless after oral challenge with 10^{9} cells of S. sonnei. After 1 month they were killed. However, all of the rabbits of the non-immunised control group challenged with S. sonnei AB3998 died within 24 h.

Discussion

The toxicity and Sereny invasiveness tests were performed to ensure that the test strain of P. shigelloides (SVC O1) was safe for the immunisation programme. Although P. shigelloides may be isolated in higher numbers from faeces of patients with diarrhoea than from healthy controls, there is little evidence in the literature to support the idea that P. shigelloides is a causative agent of diarrhoea. It is possible that this organism may proliferate after some other infection has taken place and that it acts as an opportunist pathogen. There is evidence that P. shigelloides is neither able to cause diarrhoea in an animal model nor produce toxins. There is only one report of plesiomonas toxin accumulation in rabbit gut.

In this study, diarrhoeal symptoms were not induced in rabbits through oral administration of 10^{10} P. shigelloides (SVC O1) cells. As P. shigelloides (SVC O1) shares common antigens with S. sonnei, the immunological response to the former antigen may give protection against infection by S. sonnei. This study was carried out to investigate this relationship experimentally. All 10 rabbits immunised by feeding two doses of 10^{10} cells of P. shigelloides (SVC O1) at intervals of 5 days and challenged on the sixteenth day with 10^{10} cells of S. sonnei survived. However, all 10 rabbits not immunised with P. shigelloides (SVC O1) died within 24 h when challenged with S. sonnei. This indicated that oral immunisation with P. shigelloides (SVC O1) induced an immune response that protected rabbits against the virulent S. sonnei strain. The antigen responsible for the cross-protection is believed to be the lipopolysaccharide O-antigen. These results suggest that avirulent P. shigelloides strains that have an O-antigen identical to that of S. sonnei should be considered as potential new immunising agents against shigellosis caused by S. sonnei.

Since P. shigelloides (SVC O1) gave complete protection against S. sonnei infection in the animal model, further studies are now required to establish whether this organism is an acceptable vaccine candidate for man in protection against shigellosis caused by S. sonnei.

References

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