Detection of Cholera Enterotoxin Activity in Suckling Hamsters

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For detection of cholera enterotoxin activity, suckling hamster, mouse, and rat models were compared. It was found that not only suckling mice but also suckling rats and hamsters were sensitive to cholera enterotoxin. Among these models, suckling hamsters were the most sensitive and gave positive results with about 1/100 the cholera toxin seen in suckling mice and rats.

The biological activity of cholera enterotoxin has been studied in several animal systems, such as infant rabbits (6), dogs (15), ligated rabbit ileal loops (3), and rabbit skin (2). Methods to measure morphological changes in Chinese hamster ovary cells (10) and Y-1 adrenal cells (5) have also been established. Gill (9) developed a method to measure a stimulation of adenylate cyclase activity by cholera enterotoxin by using pigeon erythrocytes. Among these models, infant rabbits and dogs reflect directly the pathogenesis of cholera enterotoxin, which causes fluid secretion into intestinal lumen (7).

Ujiiye and Kobari (18) reported that suckling mice were sensitive to Vibrio cholerae infection, and the animals were killed after oral administration of the organisms. This work was extended by the recent work of Baselski et al. (1), in which it was reported that cholera enterotoxin caused fluid accumulation in intestinal lumen. However, Giannella (8) reported that suckling mice were insensitive to the action of cholera enterotoxin. The suckling mouse model has been used to detect a heat-stable enterotoxin of Escherichia coli and has been considered to be a convenient, reproducible, and inexpensive model (4, 8, 12). If the suckling mouse model could be an acceptable assay system for cholera enterotoxin, it would be a more convenient and inexpensive whole animal system than the others.

The present work was initiated to examine whether the suckling mouse model can be applied to detect cholera enterotoxin activity, and during the course of the study, it was found that not only suckling mice but also suckling rats and hamsters could be used for the same purpose. Moreover, it was found that suckling hamsters were about 100 times more sensitive than suckling mice and rats.

Breeding colonies of white mice (Nippon Clea Co., Osaka), Sprague-Dawley rats (Breeding Station for Laboratory Animals, Osaka University), and Syrian hamsters (Nippon Clea Co.) were established in our laboratory. Suckling animals, 2 to 4 days old, were used throughout this study. Cholera enterotoxin was purified as previously reported (14). Purified cholera enterotoxin in 0.1 ml of a buffer containing 50 mM tris(hydroxymethyl)aminomethane-hydrochloric acid (pH 7.5), 50 mM NaF, 1 mM disodium ethylenediaminetetraacetate, and 200 mM NaCl was administered into the stomach of each animal by using an appropriate gastric tube. About 0.08% Evans blue dye was added to each inoculum as a marker. At the desired time interval after administration, the suckling animals were sacrificed by inhalation of chloroform. After confirming the existence of the dye in intestinal lumen, the entire intestine was removed. The ratio of (weight of entire intestine)/(total body weight − weight of entire intestine) of each animal was calculated. Ratios more than 0.05% were considered to be positive results.

All suckling animal models used in this study responded to cholera enterotoxin when the animals were sacrificed at 17 h or more after the toxin administration (Table 1). The sensitivity of suckling hamsters was about 100 times higher than that of suckling mice and rats. As low as 0.05 μg of the cholera enterotoxin gave positive results in suckling hamsters, whereas 5 μg of the toxin was required for positive results in suckling mice and rats. Sensitivity of suckling mice and rats was almost the same. Moreover, suckling hamsters showed positive results with a shorter incubation time than that observed in suckling mice and rats.

Baselski et al. (1) reported that a dose as low as 0.5 μg of cholera enterotoxin gave positive results in the suckling mouse model. On the other hand, our results showed that at least 5 μg of the toxin was necessary to give positive...
This discrepancy may either be due to differences in toxin preparation or differences in stability of the toxin in solution. Differences of calculation of the ratio may also reflect the effective dose. The fluid accumulation ratio as reported by Baselski et al. (1) is rather low. They considered a ratio of less than 0.065 as negative and more than 0.075 as positive. On the other hand, we considered a ratio of more than 0.095 as positive, since in some cases, especially in suckling hamsters, a high ratio of around 0.085 was seen at an early incubation time. This is probably due to differences in weighing the guts. Baselski et al. (1) weighed both the stomach and entire intestine, whereas we weighed only the entire intestine, as has been applied for E. coli heat-stable enterotoxin (4). Also, we noticed that in suckling hamsters, absorption of the administered solution was slower than that in suckling mice and rats, which was shown when a control solution (a buffer) was administered (Table 1).

Although oral administration was applied in this study, direct administration of the toxin into the stomach of the animals by use of a needle and syringe was also applicable. Oral administration, however, was more precise, since in some cases direct injection of the toxin solution would cause leakage. Weighing animals individually, rather than in groups of three or four, was quite reproducible as reported by Baselski et al. (1), and statistically reliable results were obtained with five animals.

Recently, Lepot and Banwell (13) reported that infant hamsters were useful for detecting an activity of cholera enterotoxin. They used the animals weighing about 90 to 110 g and inoculated more than 75 μg of the cholera enterotoxin in 1.5 ml of a buffer solution to get positive results. Suckling hamsters gave positive results with about 1/2,500 the toxin required by infant hamsters (Table 1).

The suckling hamster model reported in this paper is sensitive, reproducible, and convenient for detecting cholera enterotoxin activity, and thus may be applicable for studying the pathogenesis of cholera enterotoxin and V. cholerae infection. Also, an application of this model to other enterotoxins, such as E. coli heat-labile enterotoxin, V. parahaemolyticus toxin (11), Salmonella typhimurium toxin (16), and Shigella dysenteriae toxin (17) may give interesting results.

### LITERATURE CITED