A NEW TREATMENT FOR NEONATAL SCOURS[1]

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Summary

Scours account for significant losses to the US swine industry every year. A common treatment for scours is the administration of broad-spectrum antibiotics, a practice with increasing unpopularity in the eyes of consumers. Currently, no treatment is available to reduce or eliminate the fluid losses associated with scours that is both inexpensive and easy to use. In the present study, a variety of prospective drugs were used to determine if a single compound might inhibit the effects of bacterial toxins in a laboratory setting. The results indicate that a new class of drugs, which we call DASUs, likely will prove useful for the treatment of watery diarrhea. Additional studies are underway to validate this conclusion.

(Key Words: Scours, New Treatments.)

Introduction

Scours (watery diarrhea) accounts for at least $70 million in losses to the swine industry in the United States annually. Although several general mechanisms can produce diarrhea, the most frequently encountered causes can be grouped into two categories: 1) those that stimulate intestinal cells to secrete electrolytes and water (e.g., enterotoxins of *E. coli* or *V. cholera*) and 2) pathogens that destroy cells lining the intestine and result in 'bloody scours' or dysentery. Sickness from electrolyte and fluid losses is greatest in newborn and newly weaned animals, and mortality approaches 100% when it is untreated. Scours accounts for greater than 15% of all deaths in nursery age pigs in the US swine herd, with only respiratory infections accounting for greater mortality. Other economic losses occur from reduced piglet weight gain, increased susceptibility to opportunistic pathogens, and the additional labor and materials required to treat affected litters.

Once an outbreak of scours occurs, a common method of treatment includes the administration of antibiotics without regard to the causative agent. Broad spectrum antibiotics will not reduce or reverse most diarrheas, although they may be appropriate to preclude opportunistic infections. Furthermore, there is mounting public pressure to reduce or eliminate all uses of antibiotics in livestock production. Nonantibiotic drugs that limit the impact of scours are few and require labor-intensive application. A variety of management techniques can be used to reduce the likelihood of scours, but outbreaks will continue to occur, at least sporadically. Thus, an ongoing need exists to develop nonantibiotic interventions that will limit or reverse fluid loss, are inexpensive, and require minimal labor for administration.

Intestinal cells use distinct proteins as machinery to move electrolytes and water from the animal's body into the intestine. We currently do not know if the same cellu-

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lar proteins are used by all pathogens to result in scours or if each organism takes advantage of different cellular proteins. Thus, it is unclear if any single protein is required for scours to occur and could be 'targeted' for drug development to reduce the impact of scours. Numerous research drugs are available to assist in identifying which proteins are used by a given pathogens. Additionally, we have identified a drug (DASU-02) that may prove useful in the treatment of scours.

In the present study, we focused on bacteria and toxins that stimulate the cells lining the intestine to secrete electrolytes and water and attempted to answer two questions: 1) Do toxins that come from different strains of organisms take advantage of the same cellular proteins? and 2) Can we identify a long-acting nonantibiotic drug that will reduce the fluid loss during scours? We should emphasize that this is a study designed to identify drugs that have the potential to be developed for commercial use; such compounds are not yet available.

Procedures

All procedures were completed in the laboratory using tissues obtained from 7- to 11-day-old pigs recently slaughtered in accordance with protocols approved by the Institutional Animal Care and Use Committee. Because toxins are known to affect the intestine, we removed sections of the large intestine and kept them functioning in tissue chambers for up to 8 hours. Small electrical currents were used to measure the amount of electrolytes being secreted by each piece of tissue. In each experiment, a tissue was mounted in a chamber and exposed to a bacterial toxin (either the heat-labile or the heat-stable toxin of *E. coli* or cholera toxin). The toxins stimulated electrolyte secretion by the intestine. A variety of drugs that are known to block certain cellular proteins were then added to determine which might inhibit the effects of the bacterial toxins.

Results and Discussion

Figure 1A shows the results from a single experiment in which *E. coli* labile toxin (LT) was used to stimulate the intestine. After about 90 minutes of exposure to LT, the current slowly rose, which indicates that the toxin was causing the tissue to secrete electrolytes. After an additional 90 minutes, the tissue was exposed to $CdCl_2$, a compound that blocks some proteins that cause electrolyte secretion in tissues such as the stomach. It had no effect, which indicates that these proteins are not responsible for secretion in the pig large intestine. DNDS is a compound that blocks proteins that cause secretion in other tissues such as the trachea. Again, no effect occurred ,which indicates that these proteins are not responsible for LT-stimulated secretion in the pig intestine. Finally, the tissue was exposed to DASU-02, a compound that we previously discovered to block electrolyte secretion in cultured human intestinal cells. As the curve in Figure 1A shows, DASU-02 immediately and completely reversed the effects of LT on the intestine. Figure 1B shows the summarized results from 12 similar experiments. In each case, $CdCl_2$ and DNDS had no effect on LT-stimulated secretion regardless of the order in which they were applied, but DASU-02 completely reversed the effects of LT on intestinal electrolyte secretion.

Results presented in Figure 2 are similar to those presented in Figure 1, with the exception that *E. coli* stable toxin A (STa) was used as the stimulant of electrolyte secretion. STa behaves very differently from LT in that the stimulation of secretion is immediate, but not as great in magnitude. Nonetheless, results again show that neither $CdCl_2$ nor DNDS had any effect on STa-stimulated intestinal secretion. DASU-02 once again caused an immediate reversal of STa-stimulated electrolyte secretion. Results from a total of nine experiments are summarized in Figure 2B.

Results presented in Figure 3 demonstrate that inhibition by DASU-02 is extremely structure-specific. Again, *E. coli* labile toxin stimulated electrolyte secretion over a 3-hour period. Then DASU-H was applied to the tissue, but without effect even at the highest concentration employed. Subsequently, DASU-02 was applied to the tissue and produced the expected result of complete inhibition. We should note that DASU-H and DASU-02 are greater than 85% identical in structure; only one component of the molecule differs. Thus, the results presented in Figure 3 demonstrate that DASU-02 is interacting with a particular cellular target and that it is not merely causing a nonselective or toxic effect on the intestine. Furthermore, our data indicate that a significant inhibition can occur with a 10-fold lower concentration of DASU-02 (not shown).

Taken together, these data strongly suggest that pathogens that cause watery diarrhea take advantage of a single cellular protein that is inhibited by DASU-02, but is not affected by a closely related chemical, DASU-H, or by $CdCl_2$ or DNDS. Thus, the first criteria for drug development have been met, in that we have shown chemical selectivity for this target and reversal of toxin-induced effects in the pig intestine. Results that were not included in this report indicate that DASU-02 is selective for the protein that causes secretion in the intestine and, thus, will be selective for this tissue. Studies currently are underway to determine if the drug will be long-acting in pigs and if it will be effective in a clinical setting.

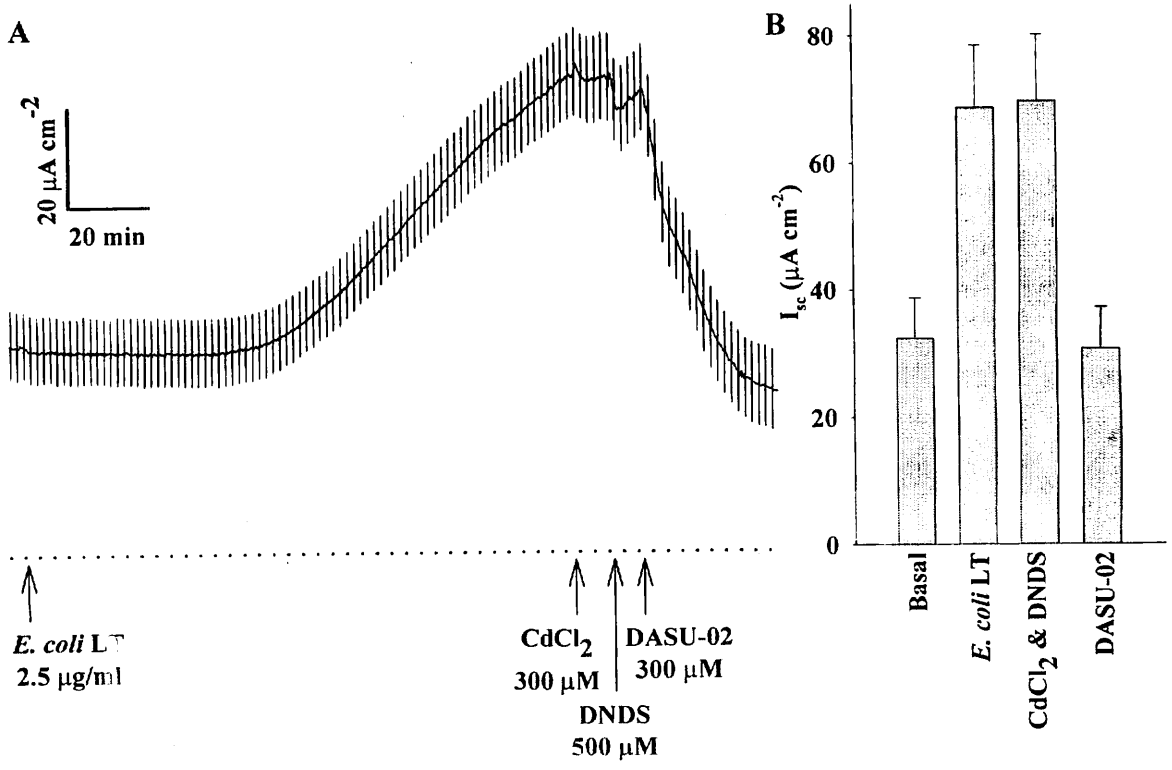

Figure 1 Inhibition of *E. coli* Labile Toxin-Stimulated Electrolyte Secretion.

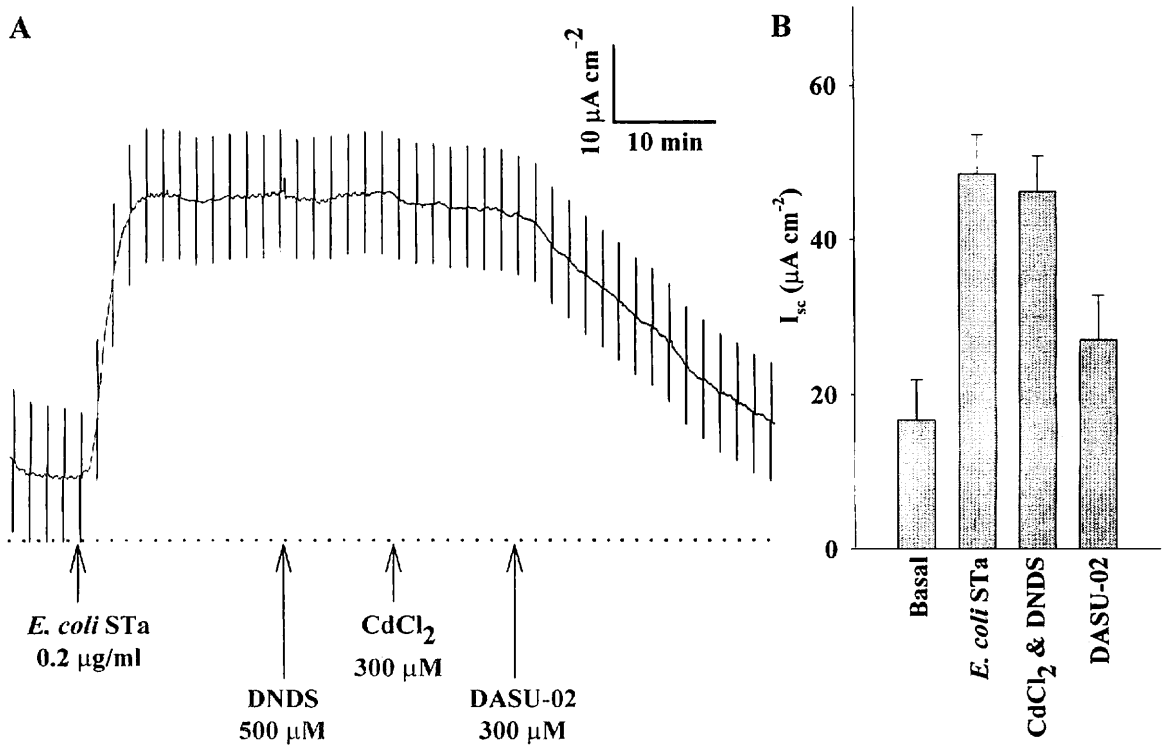

Figure 2. Inhibition of *E. coli* Stable Toxin-Stimulated Electrolyte Secretion.

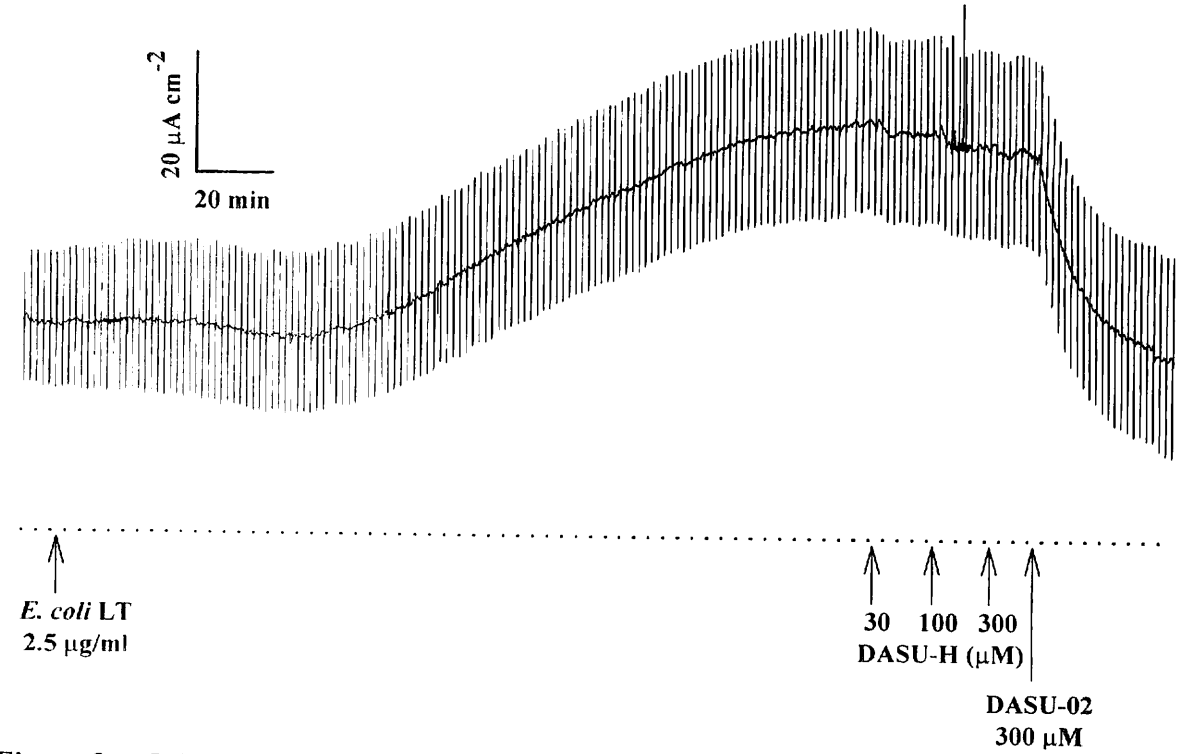

Figure 3. Inhibition of *E. coli* Labile Toxin-Stimulated Electrolyte Secretion by DASU-02 but Not by a Closely Related Compound, DASU-H.