**Original Article**

*Shigella dysenteriae* Type 1-Induced Diarrhea in Rats

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**SUMMARY:** With the aim of setting up an animal model of *Shigella dysenteriae*-induced diarrhea, Wistar rats received per os increasing densities of *S. dysenteriae* type 1 (Sd1). Inoculum of $12 \times 10^8$ Sd1 provoked dysenteric diarrhea within 24 h. Feces of healthy rats were molded, brown to black and rough. Rats developing diarrhea presented blood at the anal orifice; stools were soft or liquid containing mucus, or molded, smooth and mucous-coated. At times, stools appeared longer, dark and shiny due to the presence of mucus and blood, or molded, lumpy and brittle. Diarrheal induction was associated with abdominal ailments, progressive increase in stool weight and frequency, and increase in bacterial population. Sixty-seven percent of the total number of deaths had occurred by day 6 after diarrheal induction. These results indicate that Sd1 induced in rats a model of shigellosis which might be helpful for physiopathological and pharmacological studies of this type of infectious diarrhea.

**INTRODUCTION**

*Shigella dysenteriae*-induced diarrhea is specific to mankind but can also occur in some simian species (1). The natural reservoir of this strain is the human gastrointestinal tract, and especially the colon (2). Since *S. dysenteriae* type 1 (*Sd1*) was first discovered as the cause of dysentery in Japan in 1893, there has been neither a licensed vaccine for this pathogen nor a consensus as to the mechanism of its pathology (3). The consequence has been an increase in shigellosis all over the world. The annual number of episodes of *Sd1* infection in developing countries has been estimated at 163.2 million, with 1.1 million deaths; children under 5 years represent 70% of all episodes and 60% of all deaths (4). The fact that mankind is the only or the main species developing *Sd1*-induced diarrhea hampers a deeper understanding of the pathological mechanism. One of the factors hampering the development of a vaccine against *Sd1* is the lack of a suitable animal model for studying the pathology and monitoring potential vaccines (3). Nonetheless, a few, limited animal models have been set up, such as mouse models using other strains of bacteria (5,6), or rabbit models for the study of verocytotoxins mainly on nervous system (7). The aim of this study was to establish an animal model that can develop *Sd1*-induced diarrhea, which could help to improve our understanding of the pathological mechanism and provide a tool for the development of pharmacological agents against this type of diarrhea.

**MATERIALS AND METHODS**

**Animals:** The animals used were laboratory Wistar rats bred in a laboratory. They were housed individually in metabolic cages and allowed food and water ad libitum.

**Bacterial strain:** The strain used in this study was *Sd1*, which was provided by the Centre Pasteur of Yaoundé, Cameroon and isolated from an epidemic of bloody diarrhea that occurred in Ngoela, east region of Cameroon (8).

**Induction of diarrhea:** The turbidity of different *Sd1* inocula was matched spectrophotometrically at 450 nm with a HACH DR2000 spectrophotometer to the 0.5, 1, 2, 3, 4, 5 and 6 McFarland standards. One of the different saline-diluted inocula was orally administered to each group of six rats.

**Evaluation of diarrheal parameters:** The frequency and weight of stools were evaluated daily during 3 consecutive days before diarrheal induction and during the 6 days following. Stools were collected using a white cloth fixed under the bars supporting the animals. Animals were observed for 7 days from the day of induction for lethality. *Sd1* were enumerated in stools before their oral administration and at 2, 26, 50 and 98 h after the appearance of diarrhea. To achieve this, 0.5 g of diarrheal feces was homogenized in 4.5 mL sterile saline, and further serial dilutions were made in saline. Five hundred microliters of each dilution tube were spread over the surface of a Salmonella-Shigella (SS) agar plate with a glass spreader. Plates were then incubated at 37°C for 24 h and the number of colonies was determined (9,10).

**Statistical analysis:** All the results are expressed as the mean ± SEM. Differences in the weight and frequency were analyzed using an analysis of variance followed by the paired student’s *t* test.

**RESULTS**

Before induction of diarrhea, animals presented normal feces: solid, molded, brown or dark and rough (Fig. 1a). Some primary diarrheal manifestations were observed in rats with inoculum $9 \times 10^8$ *Shigella* (the 3 McFarland standard). We then noticed few soft feces (unmolded) or liquid and blood...
appearing at the anal orifice. Real dysenteric diarrhea was obtained in rats with inoculum $12^{8}$ Sd1 (the 4 McFarland standard). Diarrheal stools appeared within 24 h after induction. Stools were either soft or liquid, containing mucus (Fig. 1b) or molded and smooth but mucus-coated, often with mucus-linked molded feces (Fig. 1c). At times, the feces also appeared longer, dark and shiny due to blood and mucus (Fig. 1d). These stools were molded and lumpy but brown and brittle (Fig. 1e) or presented blood marks (Fig. 1f).

Fig. 1. Rat stool characteristics before and after Shigella dysenteriae type 1 ($12^{8}$ CFU) administration.

From 4 h following Sd1 administration, behavioral changes were observed: the animals were weak, not as mobile, curled up and presented stronger aggressiveness.

The frequency of diarrheal feaces increased gradually from the 1st to the 5th day after induction, whereas the frequency of total feces decreased significantly the 1st day after induction ($-57.42\%, P<0.05$), and increased on each of the following days, including a significant ($P<0.05$) increase on the 3rd day (Fig. 3). Beginning on the 2nd day following induction, the Sd1 number in feces was higher than the quantity of Sd1 orally administered: $3.8^{8} 10^{9}$ versus $1.2^{8} 10^{9}$ Sd1. Sixty-seven percent of the total number of deaths had occurred by day 6 (Table 1).

Table 1. Shigella dysenteriae type 1 number in stools and death rate after diarrheal induction ($n=6$)

<table>
<thead>
<tr>
<th>Day after induction</th>
<th>Time (h) after diarrhea appearance</th>
<th>Sd1 number/g of feces ($\times 10^{6}$)</th>
<th>Death rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td></td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>$12.8 \pm 0.6$</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>$3886.6 \pm 115.8$</td>
<td>16.7</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>$2947.5 \pm 881.6$</td>
<td>33.33</td>
</tr>
<tr>
<td>5</td>
<td>98</td>
<td>$2500 \pm 672.1$</td>
<td>33.33</td>
</tr>
<tr>
<td>6</td>
<td>120</td>
<td>-</td>
<td>67</td>
</tr>
</tbody>
</table>

Fig. 2. Stool frequency (number of feces per day: Nbr/day) before and after diarrheal induction in rats with Sd1 ($12^{8}$ CFU). DS: diarrheal stool; TS: total stool; -1: mean of feces frequency the day before diarrheal induction; 1-5: days after diarrheal induction. Significant difference: $*P<0.05$. n = 6.

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DISCUSSION

In humans, 10-100 Shigella can cause dysenteric diarrhea (11). In rats, diarrhea has been induced with $9 \times 10^{8}$ organisms, while true dysenteriae was obtained with $12 \times 10^{8}$ Sd1. This could mean that other animal species are highly resistant to Sd1 infection, and thus that basic susceptibility accounts for Sd1 infection being specific to mankind. Human Sd1-induced diarrhea is characterized by an approximately 400% increase in stool frequency per day (2,8,12,13). In our animal model, the increase was about +130% per day after diarrheal induction.

Human dysenteric diarrhea presents two phases: a first watery diarrhea phase and a second dysenteric phase with stools containing mucus and/or blood followed by abdominal pain, cramps and fever (3,8); mucus and blood are observed in the stools from the 3rd day of infection (8). Infected rats presented either watery diarrhea at the beginning with blood appearing at the anal orifice or soft to liquid (unmolded) feces. This step could be compared to the first phase of human dysenteric diarrhea. This phase was followed by mucus-coated to liquid feces with or without blood; the stools were generally mixed with mucus and/or blood. This phase could correspond to the second phase of human dysentery. The animal curling up after the 1st day of Sd1 administration could be an expression of abdominal ailment and
cramps. A decrease in stool frequency and weight after the infection induction has also been observed in rabbits challenged intravenously with Shiga-like toxins (7). In the present study, the decrease in the stools on the 1st day may have been due either to the importance of the metabolic activity of bacteria in the intestinal tract, since there was an exponential growth of bacteria at that time, or of the Shiga toxin (STX) resulting from tract pollution. Since STX, by its toxicity, leads to limb weakness, it may have been responsible for the paralysis, pain, aggressiveness and animal death observed in the present study (7). The behavior of our animals would seem to corroborate this hypothesis. The delay of the appearance of diarrhea (within 24 h) is consistent with previous findings (16 - 48 h) in rabbits (7).

A unique feature of our model was that we used Sd1, which presents a greater virulence (due to STX) and multidrug resistance (8,12); in addition, we used a natural, oral contamination route, while other models have used rabbits, mice, or rats, but with other bacterial strains and different administration routes (5-7,14). The model of shigellosis presented herein could be useful for clarifying the physiopathology of shigellosis induced by Sd1, as well as for the study of the pharmacological activity of drugs or medicinal plants possessing toxicity to bacterial strains that induce diarrhea.

ACKNOWLEDGMENTS

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REFERENCES