Adsorption Effect of Activated Charcoal on Enterohemorrhagic Escherichia coli

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ABSTRACT. The adsorption property of activated charcoal on verotoxin (VT)-producing Escherichia coli (VTEC) was examined using E. coli O157:H7. In the present study, E. coli O157:H7 strains were effectively adsorbed by activated charcoal. Adsorption was dose-dependent, and the maximum adsorption occurred within 5 min. At 10 mg of activated charcoal, bacteria tested were completely adsorbed. Activated charcoal also had the capacity to adsorb toxin (verotoxin 2) activity from the bacterial extract. Furthermore, the adsorption efficiency of activated charcoal for the normal bacterial flora in the intestine was assessed using Enterococcus faecium, Bifidobacterium thermophilum, and Lactobacillus acidophilus. Activated charcoal showed lower binding capacity to the normal bacterial flora tested than that to E. coli O157:H7 strains. These results suggest that activated charcoal could be a good adsorbent system for the removal of VTEC and verotoxin.

KEY WORDS: activated charcoal, adsorption, enterohemorrhagic Escherichia coli, Escherichia coli O157:H7, verotoxin.

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Verotoxin (VT)-producing Escherichia coli (VTEC), especially serotype O157:H7, is the cause of diarrhea, hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome in humans [2, 15, 18, 21, 24, 25].

Microbiologic surveys indicate that up to 4.9% of clinically normal cattle shed VTEC in their feces [4, 7, 8, 12, 16, 26, 30]. There is an apparent age susceptibility to infection, such that weaned calves and heifers are more likely than adult cattle to shed VTEC and, after experimental inoculation, shed VTEC for longer periods of times [26, 30]. In addition, challenge with VTEC does not cause clinical disease in cattle [4, 7]. These observations strongly suggest that cattle are a principal reservoir of VTEC. Thus, methods for reducing or eliminating the carriage of VTEC in cattle are needed to reduce the level of exposure to the pathogen in food and the environment.

Activated charcoal is known as a universal adsorbent because it can bind with variety molecules [5]. It has been reported that activated charcoal is useful for the removal of bacteria and bacterial toxins, both in vitro and in vivo [9, 10, 13, 19, 22]. The effect of activated charcoal on VTEC, however, had not yet been investigated. Therefore, it seemed of interest to examine whether activated charcoal could effectively adsorb VTEC. In this paper, we have explored the effect of activated charcoal on VTEC by using E. coli O157:H7 as a model. In addition, the effect of activated charcoal on VT was also examined.

MATERIALS AND METHODS

Materials and bacteria: Activated charcoal (Ken-ei Pharmaceutical Co., Ltd., Osaka, Japan) was commercially obtained. E. coli O157:H7 strains NIHJ 96-1023 and CIP103571, which produce VT1 and VT2, were donated by Dr. F. Amano, Department of Biochemistry and Cell Biology, National Institute of Infectious Disease, Tokyo, Japan and Dr. N. Kimura, Fine Chemical Research Laboratory, Nissin Flour Milling Co., Ltd., Saitama, Japan, respectively. Enterococcus faecium (CL-5), Bifidobacterium thermophilum (BL-4), and Lactobacillus acidophilus (BL-2) were isolates from our culture collection at the Immunology Research Institute in Gifu. These strains were used for the adsorption test. E. coli O157:H7 (J-2) (a gift from Dr. Y. Takeda, Research Institute, International Medical Center of Japan, Tokyo, Japan), which produces only VT2, was used as the source of toxin. E. coli O157:H7 strains NIHJ 96-1023 and CIP103571 were cultured in nutrient broth (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 37°C overnight with shaking. On the other hand, E. coli O157:H7 (J-2) was cultured in Mueller Hinton broth (Sigma Chemical Co., St. Louis, MO, U.S.A.) containing 5 μg/ml of trimethoprim at 37°C overnight with shaking. E. faecium (CL-5), B. thermophilum (BL-4), and L. acidophilus (BL-2) were grown overnight in GAM broth (Nissui) at 37°C with shaking.

Cell: Vero cells were used for the assay of Vero cell cytotoxicity. Cells were maintained in Eagle’s minimal essential medium (MEM) (Nissui) supplemented with 10% fetal calf serum and antibiotics.

Preparation of crude toxin: Crude VT2 was prepared from E. coli O157:H7 (J-2) according to the method described previously [29]. The resulting toxin was filter-
sterilized through 0.22-µm filter unit (Millex-GV, Milli-pore, Bedford, MA, U.S.A.) and stored at –20°C until use.

**Adsorption test**: This study was designed to test the usefulness of activated charcoal for *E. coli* O157:H7 adsorption. Approximately 7.3–8.2 × 10⁶ *E. coli* O157:H7 (NIHJ 96–1023) or 5.4–5.7 × 10⁶ *E. coli* O157:H7 (CIP103571) in 1 ml of nutrient broth (Nissui) and activated charcoal (1, 3, 5, or 10 mg) were mixed together and incubated at 37°C with gentle agitation. Except when noted, incubation was for 1 hr. These mixtures were then centrifuged at 600 × g for 5 min to remove activated charcoal. After centrifugation, an aliquot of supernatant was diluted (1:400) and plated onto nutrient agar (Nissui) to determine the number of bacteria.

Activated charcoal was also tested for its capacity to bind normal bacterial flora in the intestines. We used *E. faecium* (CL-5), *B. thermophilum* (BL-4), and *L. acidophilus* (BL-2) as model bacteria. Adsorption test was carried out using approximately 2–3 × 10⁸ bacteria/ml in GAM broth (Nissui) as described above. After centrifugation for 5 min with 600 × g, an aliquot of upper phase, diluted 1:400, was streaked onto BL agar to calculate the number of bacteria.

**Adsorption of VT by activated charcoal**: This was performed to test the ability of activated charcoal to remove VT activity from *E. coli* O157:H7 (J-2) lysates. Briefly, 1 ml of crude toxin from the *E. coli* lysates (diluted geometrically from 1:200 to 1:1,600 with cell culture medium) was incubated with or without 5 mg of activated charcoal at 37°C for 30 min, and then the mixture was centrifuged at 16,000 × g for 10 min. After centrifugation, the supernatant (100 µl) was tested for cytotoxic activity on Vero cells.

**The Assay of Vero cell cytotoxicity**: Vero cells (2 × 10⁵) in a volume of 900 µl of culture medium per well were seeded into 24-well tissue culture plates (Sumitomo Bakelite Co., Ltd., Tokyo, Japan) and cultured for 24 hr at 37°C. After cultivation, samples (100 µl) of each adsorbed or non-adsorbed VT prepared as described above were added to the appropriate wells in 24-well tissue culture plates containing Vero cells and then cultured. After 2 days cultivation at 37°C, viable cells were counted. Vero cells non-treated with toxin served as controls. The results were expressed as percent survival of Vero cell compared to survival of control cells. Calculation formula is as follows: % survival = (the number of cells treated with adsorbed or non-adsorbed toxin/the number of control cells) × 100.

**Statistical analysis**: Student’s *t* test was performed for statistical evaluation of the results. Results are expressed as the arithmetic mean with the standard error of the mean (mean ± SE).

**RESULTS**

*E. coli* O157:H7 adsorption by activated charcoal: The effect of activated charcoal on *E. coli* O157:H7 adsorption was examined. The results are shown in Fig. 1. The number of *E. coli* O157:H7 strains NIHJ96–1023 and CIP103571 decreased after the incubation with activated charcoal. The adsorption effect of activated charcoal on both *E. coli* O157:H7 strains was dose-dependent. That is to say, the number of bacteria, when *E. coli* O157:H7 strain NIHJ96-1023 was incubated with 1, 3, and 5 mg of activated charcoal at 37°C for 1 hr, was 1.47 ± 0.01 × 10⁷, 6.37 ± 0.21 × 10⁶, and 3.63 ± 0.36 × 10⁶ cells, respectively. The number of bacteria, on the other hand, when *E. coli* O157:H7 strain CIP103571 was incubated with 1, 3 and 5 mg of activated charcoal at 37°C for 1 hr, was 4.32 ± 0.29 × 10⁷, 1.20 ± 0.17 × 10⁷, and 0.80 ± 0.46 × 10⁶ cells, respectively. At 10 mg of activated charcoal, there were no detectable bacteria in both strains.

Additional experiment was also performed to elucidate the binding time of *E. coli* O157:H7 against activated charcoal. As shown in Fig. 2, adsorption of *E. coli* O157:H7 (NIHJ96–1023) to activated charcoal occurred within 5 min of mixing. Thereafter, the level of *E. coli* O157:H7-binding activity of activated charcoal did not change. Binding of *E. coli* O157:H7 (CIP103571) also occurred within 5 min of mixing the bacteria with activated charcoal (data not shown). These results suggest that activated charcoal could be effective as an adsorbent of *E. coli* O157:H7.

**Adsorption effect of activated charcoal on VT**: We also investigated whether activated charcoal inhibits the effect of VT by adsorption. The results of this experiment are shown in Fig. 3. Non-adsorbed VT2 was cytotoxic for Vero cells, but VT2 remaining after treatment with activated charcoal showed no demonstrable cytotoxic activity. This suggests
that activated charcoal may also be beneficial to eliminate free VT.

**Effect of activated charcoal on normal microbial flora of intestine:** In order to elucidate the effect of activated charcoal on normal bacterial flora in the intestinal tract, experiment was carried out using *E. faecium* (CL-5), *B. thermophilum* (BL-4), and *L. acidophilus* (BL-2) which are intestinal bacteria. As shown in Fig. 4, activated charcoal caused a dose-dependent adsorption against *E. faecium* (CL-5), *B. thermophilum* (BL-4), and *L. acidophilus* (BL-2). That is to say, when *E. faecium* strain CL-5, *B. thermophilum* strain BL-4, and *L. acidophilus* strain BL-2 were treated with activated charcoal at 37°C for 1 hr, bacterial concentrations were 5.91 ± 0.17 × 10⁵, 1.78 ± 0.11 × 10⁵, and 5.23 ± 0.23 × 10⁵ cells at 1 mg of activated charcoal, 1.41 ± 0.14 × 10⁵, 1.43 ± 0.05 × 10⁵, and 4.19 ± 0.22 × 10⁵ cells at 3 mg of activated charcoal, 7.60 ± 0.42 × 10⁴, 6.76 ± 0.57 × 10⁴, and 3.15 ± 0.28 × 10⁴ cells at 5 mg of activated charcoal, and 5.07 ± 0.48 × 10³, 2.99 ± 0.11 × 10³, and 2.13 ± 0.16 × 10³ cells at 10 mg of activated charcoal, respectively. In this experiment, however, the important point to note is that *E. faecium* (CL-5), *B. thermophilum* (BL-4), and *L. acidophilus* (BL-2) were not completely adsorbed by activated charcoal at a dose of 10 mg. This observation suggests that the binding capacity of activated charcoal to *E. faecium* (CL-5), *B. thermophilum* (BL-4), and *L. acidophilus* (BL-2) may be lower than that to *E. coli* O157:H7 strains.

**DISCUSSION**

During the past decade, VTEC, in particular serotype O157:H7, an important human pathogen causing HC and HUS, has been reported as a cause of human illness with increased frequency [2, 15, 18, 21, 24, 25]. Cattle have been implicated as reservoir of VTEC [6, 11, 23, 26, 27, 30]. Thus, eliminating or reducing VTEC carriage in cattle would be beneficial to humans. It has been shown that activated charcoal can adsorb bacteria, viruses, bacterial toxins, and various other biochemicals, both in vitro and in vivo [5, 9, 10, 13, 19, 22]. In this paper, experiments were carried out to investigate the effectiveness of activated charcoal as an adsorbent of VTEC using *E. coli* O157:H7 and VT.

In the present study, *E. coli* O157:H7 strains NIHJ 96-1023 and CIP103571 were adsorbed depending on the dose of activated charcoal. At 10 mg of activated charcoal, both *E. coli* O157:H7 strains were not detected (Fig. 1). In addition, it was also shown that activated charcoal has adsorption activity to VT (Fig. 3). These findings suggest the usefulness of activated charcoal for removal of VTEC and VT. This adsorption effect of activated charcoal on *E. coli* O157:H7 and VT may be mainly attributed to the enormous
An activated charcoal has a high adsorptive capacity although it tends to be nonselective [5, 28]. However, adsorptive capacity of activated charcoal depends on the pore size of activated charcoal. For removal of relatively large materials, an activated charcoal having larger pores is needed, while small substances need small pore [5]. In this study, activated charcoal showed differing effectiveness for removal of E. coli O157:H7 and the resident bacteria of the intestinal tract, such as E. faecium, B. thermophilum, and L. acidophilus. That is to say, no bacteria were detected when both E. coli O157:H7 strains NIHJ 96–1023 and CIP103571 were incubated with 10 mg of activated charcoal (Fig. 1). In E. faecium strain CL-5, B. thermophilum strain BL-4, and L. acidophilus strain BL-2, on the other hand, bacteria were detected after treatment with the same dose of activated charcoal (Fig. 4). The probable reason why activated charcoal has low binding capacity to E. faecium, B. thermophilum, and L. acidophilus may be that activated charcoal used in this study does not possess pores of sufficient diameter for these bacteria. However, this low binding capacity of activated charcoal with E. faecium, B. thermophilum, and L. acidophilus has important implication for the clinical application of activated charcoal, because this suggests that activated charcoal given orally could be able to minimize the removal of normal bacterial flora in the intestinal tract.

In this study, although we did not assess the effect of the activated charcoal on the removing other gram-negative enterobacteria, especially non-pathogenic E. coli, and different serotype of VTEC strains, it is likely that the binding capacity of activated charcoal to non-pathogenic E. coli and other serotype of VTEC strains is almost the same as that to E. coli O157:H7 because activated charcoal is a non-specific adsorbent [5, 23]. If this were so, there is the possibility that the decrease of non-pathogenic E. coli results in the development of pathogenic bacteria and various intestinal infections and diarrhea are induced. It has been shown that lactic acid-producing bacteria are useful in treating and preventing various intestinal infections and diarrhea caused by pathogenic bacteria [1, 3, 14, 17, 20]. Thus, the incidence of various intestinal infections or diarrhea by administration of activated charcoal would be decreased by use of lactic acid-producing bacteria. For use of activated charcoal in clinical practice, however, further studies are required to fully clarify its binding capacity to other gram-negative enterobacteria, especially non-pathogenic E. coli and also to reveal the adsorptive effect of activated charcoal on different serotype of VTEC strains.

Cattle have been implicated as a principal reservoir of VTEC [4, 7, 8, 12, 16, 26, 30]. Thus, reducing VTEC carriage in cattle should decrease the likelihood of meat contamination. In order to find out whether activated charcoal is an excellent system for removing VTEC, further studies need to be done to examine its in vivo efficacy in removing VTEC from gastrointestinal tract of cattle and also the influence of activated charcoal on the removing various species of normal bacterial flora in the intestine.

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REFERENCES
2. Bell, B.P., Goldoft, M., Griffin, P.M., Davis, M.A., Gordon,


