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Monkey feeding assay for testing emetic activity of staphylococcal enterotoxin

Keun Seok Seo

Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University

Summary

Staphylococcal enterotoxins (SEs) are unique bacterial toxins that cause gastrointestinal toxicity as well as superantigenic activity. Since systemic administration of SEs induces superantigenic activity leading to toxic shock syndrome that may mimic enterotoxic activity of SEs such as vomiting and diarrhea, oral administration of SEs in the monkey feeding assay is considered as a standard method to evaluate emetic activity of SEs. This chapter summarizes and discusses practical considerations of the monkey feeding assay used in studies characterizing classical and newly identified SEs.

Keywords

staphylococcal enterotoxin; emesis; monkey feeding assay

1. Introduction

Staphylococcal food poisoning (SFP) is usually a self-limiting disease with common symptoms including abdominal/muscle cramps, diarrhea, headaches, and emesis. Symptoms typically develop within 4–6 hours after ingestion of contaminated food and last for 1 to 88 hours. The first report that staphylococci produce an enterotoxin causing emesis and diarrhea was by Dock et al [1]. They isolated a large number of pigment-forming staphylococci from a Christmas cake responsible for illness with vomiting and diarrhea in several individuals, and demonstrated that a culture filtrate of the organism reproduced illness when ingested by human volunteers. To date, 23 different staphylococcal enterotoxins (SEs) and staphylococcal enterotoxin-like toxins (SElts) have been identified [2–7] and named as recommended by the International Nomenclature Committee for Staphylococcal Superantigens (INCSS) [8]. The INCSS recommends that to be classified as SEs, the toxins exhibit emetic activity in monkey feeding assay and SElts are defined as toxins that did not exhibit emetic activity in monkey feeding assay or have not been tested yet. According to this guideline, SEs include SEA-SEE, SEG-SEI, and SEK-SET that demonstrated emetic activity in the monkey feeding assay [9,4,10,5,6]. SElts include SEIU-SEIX that have not been tested yet. Results of monkey feeding assay were summarized in Table 1.

Although the specific cell types or the receptors in abdomen that interact with SEs and cause emesis have not been clearly identified, studies in primates demonstrated that SEs stimulate local neural receptors in abdomen which transmit impulses to the medullary emetic center at the fourth ventricle in the brain via vagus and sympathetic nerves [11]. Other studies suggested the involvement of inflammatory mediators and neural transmitters in the SE-induced emetic response. These studies demonstrated that oral administration of SEs induced elevated levels of vasoactive inflammatory mediators such as prostaglandin E₂, leukotriene B₄, 5-hydroxyeicosatetraenoic acid, and release of substance P by mast cells which may trigger the emetic center in the brain [12,11,13].

Toxic shock syndrome (TSS) is caused by superantigenic activity of SEs that induced massive activation T cells with uncontrolled production of cytokines, resulted in shock and fever. Some toxic shock syndrome (TSS) patients showed similar gastrointestinal symptoms in SFP patients including vomiting and diarrhea. This raises the question of whether superantigenicity of SEs might be related to emetic activity of SEs. However, several pieces of evidence suggest that superantigenicity of SEs is not directly responsible for emetic activity of SEs. Mutagenesis of several SEs revealed that a domain responsible for T cell activation is separated from a domain responsible for emetic activity of SEs. These studies demonstrated that SEA, SEB, SEC mutant toxins lacking superantigenicity still retain ability to induce emesis and vice versa [14–16]. Additionally, SFP patients rarely showed TSS like symptoms such as shock and fever. Considering that systemic exposures to SEs cause TSS which may mimic enterotoxic activity of SEs, oral administration of SEs is preferred method to evaluate enterotoxic activity of SEs. The monkey feeding assay developed by Bergdoll [9] is considered to be the standard method for determining the emetic activity of SEs following oral administration. Other animal models including dog, piglet, and house musk shrews have also been developed [17–19]. However, results with these animal models requiring systemic administration of SEs need to be carefully interpreted, since they may mimic TSS rather than SFP.

2. Materials

2.1. Preparation of SEs

In the literature, SEs used in the monkey feeding assay have mainly been prepared by two different methods. Early studies have used SEs expressed in non-toxigenic host, *Staphylococcus aureus* RN4220 strain harboring a single SE gene cloned into a plasmid. Most recent studies have used SEs expressed in *Escherichia coli* harboring a single SE gene cloned into a plasmid with inducible promoter and tagging system. The use of SEs expressed in *S. aureus* is preferred since it warrants physicochemical properties of SEs as their native forms. However, it is relatively difficult to purify SEs in high purity from *S. aureus*, as compared to other methods, primarily due to contamination by other toxins such as hemolysins and leukotoxins which affect host responses to SEs [20,21]. The use of SEs expressed in *E. coli* excludes the possibility of contamination of other toxins and is preferred due to the simplicity of purification and the large amount of protein that can be produced. However, contamination by endotoxin may become problematic since SEs are able to enhance endotoxin-mediated shock responses including vomiting and diarrhea [22].

Therefore, it is essential to ensure the removal of endotoxin in the SE preparation. Detailed SEs preparation methods are described in Chapter X.

2.2. Animals

Male and female animals ranging 2–5 kg in weight have been used in the monkey feeding assay. Animals are individually housed at 23–27 °C and 50–70 % humidity and a 12h/12h light/dark cycle. The rhesus monkey (*Macaca mulatta*) have been used in the method developed by Bergdoll to characterize emetic activity of classical SEs including SEA-SEI [9,4,6]. The cynomolgus monkey (*Macaca fascicularis*) was used in the study to characterize emetic activity of newly discovered SEs including SEK-SET [10].

3. Methods

The animals are fasted for 24 hours before administering SEs. While one animal handler holds the animal from the back between the knees with one hand holding the head and the other hand holding the mouth open, the other animal handler insert a nasogastric tube (outside diameter 5 mm, bore 3 mm) lubricated with glycerol directly to the esophagus until it reaches to the stomach. Purified SE dissolved in 50 ml of NZ-Amine NAK nutrient broth or 10 ml of distilled water is delivered through a nasogastric tube using a syringe.

For the safety of animal handler, animals may be sedated with ketamine-HCl. A study found that the rhesus monkey (*Macaca mulatta*) did not show emetic response to intramuscular injection of ketamine-HCl at doses of 7.5–15 mg/kg, and sedation did not inhibit emetic activity of subsequent oral administration of SEB [23]. However, another other study demonstrated that 10 of 41 cynomolgus monkey (*Macaca fascicularis*) showed emetic response to 2.5–20 mg/kg of ketamine-HCl intramuscular injection [24].

After administering SE, animals are monitored for emesis up to 5 hours. The number of vomiting events, the time to the vomiting event (latency period), and behavioral changes are recorded. Occasionally, an animal may vomit due to the intubation procedures and other provocation. It may require at least two vomiting events to be positive reaction.

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Table 1.

Emetic activity of SEs in monkey feeding assay

Toxin	Animal	Administration	Toxin preparation	Dose	No. of Tested	No. of vomiting	Reference
SEA	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	5 µg/animal	10	4	[9]
	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	10 µg/animal	30	16	[9]
	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	20 µg/animal	12	9	[9]
	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	10 µg/kg	10	5	[10]
	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	7	6	[10]
SEB	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	12 µg/animal	16	9	[9]
SEC	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	3 µg/animal	6	5	[9]
SED	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	10–20 µg/animal ^a	NA ^b	NA	[9]
SEE	NT ^c						
SEG	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	80 µg/kg	6	4	[4]
SEH	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	30 µg/kg	1	1	[6]
SEI	<i>Macaca mulatta</i>	Nasogastric tube	Purified toxin expressed in <i>S. aureus</i>	150 µg/kg	4	1	[4]
SEIJ	NT						
SEK	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	6	2	[10]
SEL	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	6	1	[10]
SEM	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	7	1	[10]
SEN	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	6	2	[10]
SEO	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	8	1	[10]
SEP	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	6	3	[10]
SEQ	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	6	2	[10]
SER	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	4	2	[5]
SES	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	4	2	[5]
SET	<i>Macaca fascicularis</i>	Nasogastric tube	Purified toxin expressed in <i>E. coli</i>	100 µg/kg	4	3	[5]
SEIU	NT						
SEIV	NT						
SEIX	NT						

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The number shown are ED50 (a dose induced 50% animals were emetic) of SED.

Not available
 N_q

Not tested
 N_c