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Acute Intramuscular Toxicity Study of Typhoid Vaccine in Rats and Beagle Dogs

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ABSTRACT *: Acute toxicity of typhoid vaccine was investigated using Sprague-Dawley (SD) rats and beagle dogs. SD rats and beagle dogs were administered intramuscularly with dosages of 0, 0.2, 0.1, 0.05 and 0.025 mg/kg, respectively. In animals administered with typhoid vaccine, there were neither dead animals nor significant changes of body weights. In addition, no differences were found between control and treated groups in clinical signs and autopsy findings. Therefore, LD_{50} of typhoid vaccine was considered to be higher than 0.2 mg/kg in SD rats and beagle dogs.*

Key Words *: Acute toxicity, Typhoid vaccine, Sprague-Dawley rats, Beagle dogs*

I. INTRODUCTION

Typhoid fever is an infection spread to travellers by food or water contaminated with the bacteria, *Salmonella typhimurium*. Early symptoms of typhoid fever are flu-like: body aches and pains, weakness, loss of appetite and continuous dull headache. Typhoid fever still remains a substantial public health problem in developing countries. Each year 33 million people become ill and over 500,000 people die of this infection (Institute of Medicine, 1986). Typhoid is rare in industrialized nations, though travellers to endemic countries may occasionally acquire the disease (Eric *et al.*, 1995). The interest in vaccines to prevent this typhoid disease is long standing. So far, several type-vaccines were developed for prevention typhoid vaccine, Whole cell vaccines consisting of relatively crude preparation of *Salmonella typhimurium* administered parenterally, were found to be effective but to have a high incidence of side effects (Ashcroft *et al.*, 1964). Two vaccines developed more recently, Ty21a (an attenuated strain *Salmonella typhimurium* administered orally) and Vi (the purified bacterial capsule, given parenterally), have seemed less toxic than the older whole cell vaccine and are thought to be equally effective (Bennish, 1995; Center for disease control and prevention, 1994). Whether any of the available vaccines would be useful in typhoid prevention in the developing world remains uncertain (Typhoid vaccination, 1992).

Recently Korea Green Cross Cooperation developed, for the convenience in practical immunization, vaccine for the Typhoid fever. The efficacy of the vaccine was confirmed. In this study, the acute toxicity of typhoid vaccines was investigated using the SD rats and beagle dogs.

II. MATERIALS AND METHODS

1. Test substance

The test substance, typhoid vaccine was produced and supplied by Korea Green Cross Corporation based in Korea. Sterile saline was used as the diluent for the test substance.

2. Animals

Fifty male and female Sprague Dawley rats, 4 weeks old, and sixteen male and female beagle dogs, 5 months old, were purchased at Samyuk

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laboratory animal research center (Osan, Korea) and Marshall farms (USA), respectively. All animals were acclimatized for 1 week prior to the administration of the test substance under the environmentally controlled rooms (temperature: 22±3°C, relative humidity: 55±5%, air circulation frequency: 10~12 times/hr, artificial light: 150~300 Lux from 7 am to 7 pm) in small or middle animal laboratory for Veterinary Medicine in Seoul National University. Rat and Beagle dogs were housed in polycarbonate cage (26×42×18 cm) and stainless-steel wire cages (64×42×53 cm), respectively. All animals fed with the mouse-rat pellets (Samyang Foods, Co., Seoul) and the dogs pellets (Science Hill, USA). All animals consumed tap water *ad libitum*.

3. Experimental design

Experiments were conducted according to the "Guidelines for Toxicity Testing of Pharmaceuticals" (KFDA, 1998). A total of 25 male and 25 female rats and 8 male and 8 female beagle dogs were randomly divided into 10 groups and 8 groups, respectively, according to the dosage levels. 0.2 mg/kg which was equivalent to 160 fold of a clinical dose given to a man weighing 60 kg was the high dose of the test substance and by serial dilution with sterile saline, the medium high dose, the medium low dose and the low dose were prepared to 0.1 mg/kg, 0.05 mg/kg and 0.025 mg/kg, respectively. The typhoid vaccine was administered into rats and beagle dogs intramuscularly at quadrate muscle of thigh.

All animals were observed for 1 week focusing on clinical signs and body weight changes. Clinical signs were observed for 6 hrs following treatment of the test substance on the day of administration and once everyday thereafter for 1 week. Body weight was measured immediately prior to dosing of the test substance and on the day 4, 7 after treatment. Following the observation period all animals were sacrificed by exsanguination. Autopsy was conducted on every animals and all major organs and tissues including brain, thymus, heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testis were examined for gross lesions. Samples with any abnormal finding were fixed in 10% buffered formalin for further examination.

For estimation of LD_{50}, Litchfield-Wilcoxon was used. Body weight changes were compared by using one-way ANOVA and two-tailed Dunnett's t-test. Chi-square test was used to analyze the incidence of abnormal findings at autopsy.

III. RESULTS AND DISCUSSION

No death and clinical abnormality among the all animal treated with the test substance was observed during the observation period of 1 week and all animals appeared to be healthy and normal during the this period (Tables 1, 2, 3 and 4). The body weight of SD rats and beagle dogs were measured at the days 0, 4, 7 after the administration of the typhoid vaccine. In body

Table 1. Mortality of male and female rats after single intramuscular administration of Typhoid vaccine

Sex	Dose (mg/kg)	Hours after treatment						Days after treatment							Final mortality
		1	2	3	4	5	6	1	2	3	4	5	6	7	
Male	0.2	0[a)]	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
Female	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5(0%)

[a)]Number of dead animals.

Table 2. Mortality of male and female beagle dogs after single intramuscular administration of Typhoid vaccine

Sex	Dose (mg/kg)	Hours after treatment						Days after treatment							Final mortality
		1	2	3	4	5	6	1	2	3	4	5	6	7	
Male	0.2	0[a]	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)
	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)
	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)
	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)
Female	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)
	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)
	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)
	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2(0%)

[a]Number of dead animals.

Table 3. Abnormal clinical findings of rats after single intramuscular administration of Typhoid vaccine

Sex	Dose (mg/kg)	Number of rats	Hours after treatment						Days after treatment						
			1	2	3	4	5	6	1	2	3	4	5	6	7
Male	0.2	5	0[a]	0	0	0	0	0	0	0	0	0	0	0	0
	0.1	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.05	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.025	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Female	0.2	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.1	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.05	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.025	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Number of animals with abnormal clinical signs.

Table 4. Abnormal clinical findings of beagle dogs after single intramuscular administration of Typhoid vaccine

Sex	Dose (mg/kg)	Number of beagle dogs	Hours after treatment						Days after treatment						
			1	2	3	4	5	6	1	2	3	4	5	6	7
Male	0.2	2	0[a]	0	0	0	0	0	0	0	0	0	0	0	0
	0.1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.05	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.025	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Female	0.2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.05	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.025	2	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Number of animals with abnormal clinical signs.

Table 5. Body weights of male and female rats after single intramuscular administration of Typhoid vaccine (unit: g)

Sex	Days after treatment	Dose (mg/kg)				
		0	0.025	0.05	0.1	0.2
Male	0	173.00±12.04[a]	174.00±7.42	158.00±9.75	167.00±10.95	170.00±7.91
	4	193.10±15.67	189.46±10.83	173.68±12.72	172.60±19.04	187.98±11.21
	7	205.18±16.66	203.16±12.79	188.62±16.47	172.70±44.54	192.94±15.32
Female	0	174.00±13.87	165.00±10.00	167.00±10.37	178.00±8.37	165.00±10.61
	4	173.24±23.81	172.48±10.45	176.42±9.23	181.88±8.64	168.56±11.59
	7	181.98±24.24	183.36±14.53	185.72±12.43	191.46±8.04	176.06±12.43

[a]Values are expressed as mean±S.D. of 5 rats.

Table 6. Body weights of male and female beagle dogs after single intramuscular administration of Typhoid vaccine

(unit: kg)

Sex	Days after treatment	Dose (mg/kg)			
		0.025	0.05	0.1	0.2
Male	0	6.80±0.42	6.75±0.35	6.60±0.14	6.95±0.07[a)]
	4	6.95±0.21	7.00±0.42	6.75±0.07	7.05±0.07
	7	6.95±0.21	7.05±0.35	6.85±0.07	7.10±0.00
Female	0	7.15±0.35	6.25±0.49	6.20±0.85	6.50±0.42
	4	7.20±0.42	6.30±0.42	6.40±0.85	6.65±0.78
	7	7.35±0.35	6.35±0.64	6.25±0.64	6.60±0.85

[a)]Values are expressed as mean±S.D. of 2 beagle dogs.

Table 7. Autopsy findings of male and female rats after single intramuscular administration of Typhoid vaccine

		Dose (mg/kg)									
		0		0.025		0.05		0.1		0.2	
Fate		tk	fd	tk	fd	tk	fd	tk	fd	tk	fd
No. of animals	Male	5	0	5	0	5	0	5	0	5	0
	Female	5	0	5	0	5	0	5	0	5	0
Male	NAD	5		5		5		5		5	
Female	NAD	5		5		5		5		5	

tk: Terminal kill; fd: Found dead; NAD: No. of abnormality detected.

Table 8. Autopsy findings of male and female beagle dogs after single intramuscular administration of Typhoid vaccine

		Dose (mg/kg B.W.)							
		0.025		0.05		0.1		0.2	
Fate		tk	fd	tk	fd	tk	fd	tk	fd
No. of animals	Male	2	0	2	0	2	0	2	0
	Female	2	0	2	0	2	0	2	0
Male	NAD	2		2		2		2	
Female	NAD	2		2		2		2	

tk: Terminal kill; fd: Found dead; NAD: No. of abnormality detected.

weight changes of all the animals, there were no statistically significant differences observed among the treated groups (Tables 5 and 6). At the termination of the observation period, all animals were sacrificed and autopsied. All major organs including brain, thymus, heart, lung, liver, spleen, stomach, intestine, kidney, adrenal gland, and ovary or testis were examined for gross lesions. In all animals, both SD rats and Beagle dogs, there were no abnormal lesions regardless of treated dose (Tables 7 and 8). In conclusion, the typhoid vaccine showed no signs of the acute toxicity when given at a dose as large as 0.2 mg/kg and is considered not to have the acute toxicity in SD rats and Beagle dogs.

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