

## Evaluation of Subcutaneous and Intramuscular Irritation of the Typhoid Vaccine in Rabbits

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**ABSTRACT:** A newly developed typhoid vaccine was tested for subcutaneous and intramuscular irritation in male New Zealand White rabbits. In subcutaneous and intramuscular irritation tests, there were no observed clinical signs, body weight changes and gross pathologic findings at doses of 1 mg/ml and 0.0125 mg/ml during experimental period. However, in positive control (0.75% acetic acid), we could find various lesions that had hemorrhage, necrosis and infiltration of inflammation cells in both subcutaneous and muscular tissues. From these results, we suggest that typhoid vaccine is not irritant in subcutaneous and muscular tissue of rabbits.

**Key Words:** Typhoid Vaccine, Subcutaneous, Intramuscular, Rabbits

### I. INTRODUCTION

A bacterial infection characterized by diarrhea, systemic disease and rash most commonly caused by *Salmonella typhi*. Typhoid is spread by contaminated foods and water. Following ingestion, the bacteria spread from intestine to the intestinal lymph nodes, liver and spleen via the blood stream. Early symptoms are very general such as acute fever, malaise and abdominal pain. As the disease progresses, fever becomes higher and diarrhea becomes prominent and other symptoms like weakness, profound fatigue, delirium, obtundation and an acutely ill appearance develop (Pang *et al.*, 1998). "Rose spots", a rash characteristic in typhoid appears in most cases of typhoid. Rose spots are small dark and red flat spots that appear most often on the abdomen and chest. Typically, children have milder symptoms of disease and fewer complications than adults (Klotz *et al.*, 1984). Therefore for the prevention of the outbreak, vaccine is recommended for travel outside of the U.S, Canada, northern European, Australia and New Zealand during epidemic

outbreak (Reynes and Bertrand, 1990; Engels *et al.*, 1995).

In this study, before trying clinical test of new typhoid vaccine made by Green-Cross Co., subcutaneous and muscular irritation tests in rabbits were performed.

### II. MATERIALS AND METHODS

#### 1. Material

Typhoid vaccine was obtained from Green-Cross Co. Ltd.. This test material was used after dilution in sterilized saline.

#### 2. Animals

Male New Zealand White rabbits (1.5~2.1 kg) were purchased from Sam-Yuk Experimental Animal Breeding Center (O-San, kyunggi-Do, Korea), quarantined and acclimatized for 1 week. The animals were housed in stainless-steel cages (420×500×310 mm), fed pellet diet for rabbits (Purina Korea Co.) ad libitum. The animals were maintained an a constant 12 hr light/dark cycle with temperature and humidity kept at 22±3°C

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and  $55 \pm 10\%$ , respectively.

### 3. Subcutaneous and intramuscular irritation tests

For subcutaneous irritation test, twelve healthy rabbits were used for the test. rabbits were separated into group I (0.0125 mg/ml) and group II (1 mg/ml) and 0.2 ml/rabbit was injected subcutaneously to the right of median-dorsal back, clipped free of hair. The left side of each rabbit was injected subcutaneously with 0.75% acetic acid as a positive control.

For muscular irritation test, twelve healthy rabbits were used for the test. rabbits were separated into group III (0.0125 mg/ml) and group IV (1 mg/ml) and 0.2 ml/rabbit injected intramuscularly to the right of muscle, vastus lateralis of hip, clipped free of hair. The left vastus lateralis muscle of each

rabbit's hip was injected intramuscularly with 0.75% acetic acid as a positive control.

Food and water consumption, clinical signs were checked daily. Body-weight was recorded on day 0, 3 and 6 after treatment. Half of each group necropsied on day 3 after treatment, The other of each group necropsied on day 6 after treatment, and then we observed gross and histo-pathological findings.

### III. RESULTS AND DISCUSSION

No remarkable changes in clinical signs and body weight changes were observed. Also, no mortality was seen in all animals (Tables 1 and 2). Also there were no treatment-related changes in gross finding and histo-pathological findings in all test group (Tables 3, 4, 5 and 6). But in positive control we could find apparent lesions that had subcutaneous

**Table 1.** Mortality and Clinical findings in New Zealand White rabbits treated with Typhoid vaccine

Group	No. of animals	Substance	Route of injection	Clinical signs	Mortality
I	6	Typhoid vaccine (0.0125 mg/ml)	s.c. <sup>2</sup>	NAD <sup>3</sup>	0/6
		A.A. <sup>1</sup>		NAD	0/6
II	6	Typhoid vaccine (1 mg/ml)	s.c.	NAD	0/6
		A.A.		NAD	0/6
III	6	Typhoid vaccine (0.0125 mg/ml)	i.m. <sup>3</sup>	NAD	0/6
		A.A.		NAD	0/6
IV	6	Typhoid vaccine (1 mg/ml)	i.m.	NAD	0/6
		A.A.		NAD	0/6

A.A.<sup>1</sup>: acetic acid (0.75%). s.c.<sup>2</sup>: subcutaneous injection. i.m.<sup>3</sup>: intramuscular. NAD<sup>3</sup>: No abnormalities detected.

**Table 2.** The changes of individual body weights in New Zealand White rabbits treated with Typhoid vaccine (unit: g)

Group		B.W. (g) on day of			
		Injection	1st Necropsy	2nd Necropsy	
I	SCL <sup>a)</sup>	Mean	1831.6	1906.6	1900.0
		S.D.	212.3	211.8	300.0
		N <sup>e)</sup>	6	6	3
II	SCH <sup>b)</sup>	Mean	1845.8	1883.3	1981.6
		S.D.	167.2	247.0	214.6
		N	6	6	3
III	IML <sup>c)</sup>	Mean	1824.1	1842.5	1783.3
		S.D.	162.8	217.0	145.0
		N	6	6	3
IV	IMH <sup>d)</sup>	Mean	1803.3	1828.3	1670.0
		S.D.	159.4	253.0	466.0
		N	6	6	3

<sup>a)</sup>SCL: subcutaneous low dose (0.0125 mg/ml). <sup>b)</sup>SCH: subcutaneous high dose (1 mg/ml). <sup>c)</sup>IML: intramuscular low dose (0.0125 mg/ml). <sup>d)</sup>IMH: intramuscular high dose (1 mg/ml). <sup>e)</sup>N: number of animals.

**Table 3.** Individual pathological findings of New Zealand White rabbits treated subcutaneously with Typhoid vaccine (1 mg/ml)

Necropsy day	3day						6day					
	A.A.*			TV**			A.A.			TV		
	1	2	3	1	2	3	4	5	6	4	5	6
Substance specimen No.												
Gross findings												
hemorrhage foci	+	+	+	-	-	-	+	+	+	-	-	-
necrotic foci	+	-	-	-	-	-	-	+	-	-	-	-
Histological findings												
subcutaneous tissue												
cell infiltration	+	+	+	-	-	-	+	+	+	-	-	-
focal hemorrhage	+	-	+	-	-	-	+	+	-	-	-	-
cutaneous muscle												
cell accumulation	+	+	+	-	-	-	+	+	+	-	-	-
dermis												
increased histiocytes	+	+	+	-	-	-	+	+	+	-	-	-
focal hemorrhage	+	-	+	-	-	-	+	-	+	-	-	-

\*Acetic acid (0.75%). \*\*Typhoid vaccine.

**Table 4.** Individual pathological findings of New Zealand White rabbits treated subcutaneously with Typhoid vaccine (0.0125 mg/ml)

Necropsy day	3day						6day					
	A.A.*			TV**			A.A.			TV		
	1	2	3	1	2	3	4	5	6	4	5	6
Substance specimen No.												
Gross findings												
hemorrhage foci	+	+	+	-	-	-	+	+	+	-	-	-
necrotic foci	-	-	+	-	-	-	-	-	+	-	-	-
Histological findings												
subcutaneous tissue												
cell infiltration	+	+	+	-	-	-	+	+	+	-	-	-
focal hemorrhage	-	+	+	-	-	-	-	+	+	-	-	-
cutaneous muscle												
cell accumulation	+	+	+	-	-	-	+	+	+	-	-	-
dermis												
increased histiocytes	+	+	+	-	-	-	+	+	+	-	-	-
focal hemorrhage	-	+	+	-	-	-	-	+	+	-	-	-

\*Acetic acid (0.75%). \*\*Typhoid vaccine.

**Table 5.** Individual pathological findings of New Zealand White rabbits treated intramuscularly with Typhoid vaccine (1 mg/ml)

Necropsy day	3day						6day					
	A.A.*			TV**			A.A.			TV		
	1	2	3	1	2	3	4	5	6	4	5	6
Substance specimen No.												
Gross findings												
hemorrhage foci	+	+	+	-	-	-	-	+	+	-	-	-
necrotic foci	-	-	-	-	-	-	-	-	-	-	-	-
Histological findings												
small foci of necrotic fiber	+	+	+	-	-	-	+	+	+	-	-	-
lymphocyte infiltration	+	-	+	-	-	-	-	+	-	-	-	-

\*Acetic acid. \*\*Typhoid vaccine.

inflammation, hemorrhage, infiltration of inflammation cell and myositis. From the results mentioned

above, it might be considered that typhoid vaccine have no subcutaneous and intramuscular irritation

**Table 6.** Individual pathological findings of New Zealand White rabbits treated intramuscularly with Typhoid vaccine (0.0125 mg/ml)

Necropsy day Substance specimen No.	3day						6day					
	A.A.*			TV**			A.A.			TV		
	1	2	3	1	2	3	4	5	6	4	5	6
Gross findings												
hemorrhage foci	+	+	+	-	-	-	+	+	+	-	-	-
necrotic foci	-	-	+	-	-	-	-	-	-	-	-	-
Histological findings												
small foci of necrotic fiber	+	+	+	-	-	-	+	+	+	-	-	-
lympocyte infiltration	+	+	+	-	-	-	-	-	+	-	-	-

\*Acetic acid (0.75%). \*\*Typhoid vaccine.

in rabbits.

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