Korean J. Toxicol.
Vol. 13, No. 3, pp. 275-279, 1997

Acute Toxicity of Combined Vaccine (KGCC-95VI) Against Japanese Encephalitis and Hantaan Virus Infection

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(Received May 29, 1997)
(Accepted September 2, 1997)

ABSTRACT *: The acute toxicity of the combined vaccine (KGCC-95VI) for the prophylaxis against Japanese encephalitis and Hantaan virus infection, recently developed by Korea Green Cross Corporation, was investigated. KGCC-95VI was administered to the Balb/c mice in two routes, orally and subcutaneously, and into the New Zealand White rabbits subcutaneously. LD_{50} was not accessible as there were no deaths in the group treated even at a dose 800 times the expected clinical dose in both animal species. Between the treated and control groups there were no statistically significant differences in body weight changes and clinical signs during the 14-day observation period, and no pathological gross findings. Accordingly KGCC-95VI is considered not to have the acute toxicity in mice and rabbits.*

Key Words *: Japanese encephalitis, Hantaan virus infection, Vaccine, Acute toxicity, LD_{50}*

I. INTRODUCTION

Japanese encephalitis virus (JEV) is widely distributed in Asia, including Korea, Japan, China, Taiwan, Philippines, far-eastern Russia, all of Southeast Asia and India (Hoke *et al.*, 1988). Mitamura *et al.* (1938) isolated the virus from the mosquito, Culex tritaeniorhynchus. It is established that pigs and birds are the principal viremic hosts and that Culex tritaeniorhynchus is responsible for transmission between these vertebrates and from them to humans(Buescher and Scherer, 1959). The inactivated vaccine using the virus propagated in the brains of the suckling mice has been available (Cho *et al.*, 1994).

Hantaan virus was originally isolated from the Korean striped field mouse, Apodemus agarius corea. The virus is one of the etiologic agents of hemorrhagic fever with renal syndrome (Hantaan virus infection, leptospirosis, rickettsial infection). The inactivated vaccine using the virus propagated in the brains of the newborn mice is being used (Shin, 1992; Lee and Ahn, 1988).

Recently Korea Green Cross Corporation developed, for the convenience in practical immunization, the combined vaccine for the prophylaxis against Japanese encephalitis and Hantaan virus infection. The efficacy of the combined vaccine was confirmed. In this study the acute toxicity of the combined vaccine was investigated using the mice and the rabbits in accordance with the guidelines for the toxicity tests of the drugs (Guidelines for Safety Tests of Drugs, 1996) provided by the Food and Drug Administration, Korea.

II. MATERIALS AND METHODS

The test material, the combined inactivated virus vaccine for Japanese encephalitis and hantaan virus-caused hemorrhagic fever with renal syndrome (referred to as KGCC-95VI hereinafter for convenience), was produced and supplied by Korea Green Cross Corporation based in Korea. Phosphate buffered saline, 1/60 M, pH 7.2, prepared

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and autoclaved at the laboratory was used as the diluent for the test material.

Both sexes of specific-pathogen-free (SPF) BALB/c mice (Laboratory of Experimental Animals, Korea) were obtained at the age of 4 weeks. All the mice were acclimatized for 1 week prior to the administration of the test material under the barrier-sustained animal room maintained at a temperature of 23±3°C, a relative humidity of 50±10% and illumination cycle of 12 hr light and 12 hr dark (light during 07:00-19:00). The mice were housed in the stainless-steel wire cages (220×410×220 mm) and fed with the rat-and-mouse pellets (Jeil Feed Co., Korea) and tap water *ad libitum*. Fifty male and fifty female mice were entered in this study. Each sex group was grouped into two by the administration routes, one for the oral administration and the other for the subcutaneous administration. In each route, twenty five mice were divided into 5 groups according to the dosage levels respectively.

Both sexes of New Zealand White rabbits (Laboratory of Experimental Animals, Korea) were obtained at the age of 3 months. All rabbits were acclimatized for 1 week prior to the administration of the test material under the barrier-sustained animal room maintained at a temperature of 23±3°C, a relative humidity of 50±10% and illumination cycle of 12 hours light and 12 hours dark (light during 07:00-19:00). The rabbits were housed in the automatic washing cages (Dae-Jong, Korea) and fed with new-born calf pellets (Jeil Feed Co., Korea) and tap water *ad libitum*. Six male and six female rabbits were entered in this study. Each sex group was divided into five groups according to the dosage levels respectively.

Following the guidelines and test methods on the safety tests of the drugs provided by the Food and Drug Administration, Korea, the possibility of acute toxicity of the KGCC-95VI was investigated. The KGCC-95VI was administered into the mice orally and subcutaneously, and into the rabbits subcutaneously. The mice were observed for 2 weeks focusing on clinical appearances and body weight changes. The rabbits were observed for 1 week focusing on clinical appearances and body weight changes. After sacrificing the animals, all the organs and tissues were examined pathologically.

The mice in oral experiment, each group of male or female, were administered once orally with the dosage of 0.0, 0.000666, 0.00666, 0.0666, 0.666 and 6.66 ml/kg body weight using the stomach tube after 4-hour fasting. The mice in subcutaneous groups, male or female, were administered once subcutaneously with the same dosage to those of the oral route. The rabbit experiment, male or female, were administered once subcutaneously with the same dosage to those in mice.

Clinical observation and death checks were made daily for 14 days in mice and 7 days in rabbits. Body weights in mice were measured at the days 0, 3, 7, and 10 after the administration of the KGCC-95VI. Body weights in rabbits were measured at the days 0, 1, 4 and 7 after the administration of the KGCC-95VI. At the termination of the observation period, all the animals were sacrificed under the ether anesthesia. All tissues and organs were examined for abnormalities pathologically. Body weights were analyzed using Student's *t*-test.

III. RESULTS

In order to investigate the acute toxicity of the KGCC-95VI the acute toxicity test was performed following the guidelines and test methods for the safety tests of the drugs provided by the Food and Drug Administration, Korea. The mice were observed for the clinical signs for 14 days. In mice, both male and female, during the 14-day observation after the administration of the KGCC-95VI in two ways, there were no clinical abnormalities and death as shown in Table 1. The rabbits were observed for the clinical signs for 7 days. In rabbits, both male and female, during the 7-day observation, there were no clinical abnormalities and death as shown in Table 1.

The body weights of the mouse groups were measured at the days 0, 3, 7 and 10 after the administration of the KGCC-95VI. The body weights of the rabbit groups were measured at the days 0, 1, 4 and 7 after the administration of the KGCC-95VI. In the body weight changes in all the animal groups there were no statistically significant differences observed between the treated and the con-

Table 1. Clinical abnormalities and mortalities after administration of KGCC-95VI

Animal (route)	Dosage (ml/kg)	Clinical abnormalities		Mortality	
		Male	Female	Male	Female
Mice (oral)	0.0	0/5	0/5	0/5	0/5
	0.000666	0/5	0/5	0/5	0/5
	0.00666	0/5	0/5	0/5	0/5
	0.0666	0/5	0/5	0/5	0/5
	0.666	0/5	0/5	0/5	0/5
	6.66	0/5	0/5	0/5	0/5
Mice (subcutaneous)	0.0	0/5	0/5	0/5	0/5
	0.000666	0/5	0/5	0/5	0/5
	0.00666	0/5	0/5	0/5	0/5
	0.0666	0/5	0/5	0/5	0/5
	0.666	0/5	0/5	0/5	0/5
	6.66	0/5	0/5	0/5	0/5
Rabbits (subcutaneous)	0.0	0/5	0/5	0/5	0/5
	0.000666	0/5	0/5	0/5	0/5
	0.00666	0/5	0/5	0/5	0/5
	0.0666	0/5	0/5	0/5	0/5
	0.666	0/5	0/5	0/5	0/5
	6.66	0/5	0/5	0/5	0/5

Table 2. Body weight changes after oral administration of KGCC-95VI in mice (unit : g)

Sex	Days after treatment	Dose(ml/kg)					
		0.0	0.000666	0.00666	0.0666	0.666	6.66
Male	0	22.2(4.71)	22.0(4.80)	23.0(3.95)	22.8(4.55)	22.7(4.90)	22.4(3.50)
	3	24.5(4.75)	24.7(4.50)	25.3(4.05)	24.6(3.75)	25.5(3.72)	24.9(4.20)
	7	27.4(3.95)	26.3(3.15)	27.6(2.78)	27.4(4.31)	28.3(3.87)	28.2(3.53)
	10	29.6(4.95)	29.4(4.39)	30.8(3.84)	30.0(4.05)	31.2(4.59)	30.5(2.95)
Female	0	20.1(4.74)	20.5(3.55)	20.4(4.32)	20.7(4.52)	20.4(3.97)	20.1(3.29)
	3	21.4(3.25)	22.0(4.74)	21.3(3.05)	21.6(4.37)	22.0(2.95)	21.8(3.64)
	7	22.7(2.97)	23.1(4.32)	22.8(3.68)	23.1(2.37)	23.2(4.42)	23.1(3.62)
	10	23.5(3.41)	23.9(3.12)	24.0(2.86)	23.8(2.61)	24.1(3.57)	24.3(3.65)

The numbers in the parenthesis denote the standard deviation.

Table 3. Body weight changes after subcutaneous administration of KGCC-95VI in mice (unit : g)

Sex	Days after treatment	Dose(ml/kg)					
		0.0	0.000666	0.00666	0.0666	0.666	6.66
Male	0	22.0(4.24)	22.2(3.50)	22.1(3.72)	22.5(3.54)	22.2(3.94)	22.3(3.43)
	3	24.3(3.62)	23.9(4.27)	24.2(4.35)	23.9(2.97)	24.5(3.53)	24.7(3.74)
	7	27.2(3.79)	27.0(4.86)	27.3(1.42)	27.0(0.97)	27.2(3.63)	28.0(2.96)
	10	29.5(2.42)	29.8(3.29)	30.2(2.62)	30.5(3.96)	30.2(4.04)	31.5(3.55)
Female	0	19.9(3.35)	20.4(1.96)	20.6(1.32)	19.7(1.09)	20.3(1.94)	20.5(2.43)
	3	20.8(2.76)	21.7(2.59)	22.0(2.95)	21.4(3.28)	21.4(2.31)	22.0(2.19)
	7	22.5(1.79)	22.5(3.23)	22.7(1.37)	22.5(1.42)	22.0(1.83)	23.3(2.55)
	10	23.4(3.29)	23.2(2.18)	23.9(0.65)	23.6(2.43)	23.2(1.72)	24.0(1.93)

The numbers in the parenthesis denote the standard deviation.

trol groups, male or female in both oral and subcutaneous routes, as shown in Tables 2, 3 and 4.

At the termination of the observation period the animals were sacrificed and the organs and the tissues were examined gross pathologically. In all the animals, both mice and rabbits, at the injection sites there were no abnormalities. In the mouse groups, oral and subcutaneous, there were no

Table 4. Body weight changes after subcutaneous administration of KGCC-95VI in rabbits (unit : g)

Sex	Days after treatment	Dose(ml/kg)					
		0.0	0.000666	0.00666	0.0666	0.666	6.66
Male	0	1875(59.7)	1850(67.3)	1920(97.4)	1875(59.3)	1840(29.5)	1835(75.2)
	1	1880(45.2)	1850(51.6)	1930(58.4)	1875(42.7)	1850(48.2)	1840(25.3)
	4	1920(73.5)	1870(31.6)	1945(15.3)	1910(22.50)	1860(33.1)	1875(19.3)
	7	1955(65.2)	1885(45.7)	2005(32.8)	1940(32.9)	1875(22.7)	1885(24.1
Female	0	1660(23.7)	1645(46.2)	1680(31.6)	1710(29.4)	1670(43.7)	1685(36.4)
	1	1660(16.2)	1655(32.9)	1690(11.7)	1720(11.6)	1685(32.7)	1690(25.3)
	4	1705(33.5)	1695(28.6)	1720(21.5)	1750(32.5)	1700(27.4)	1720(34.1)
	7	1750(32.5)	1710(32.9)	1770(33.8)	1805(22.5)	1765(36.4)	1790(25.8)

The numbers in the parenthesis denote the standard deviation.

pathologal findings. In the subcutaneously administrated rabbit groups, there were also no pathologal findings.

IV. DISCUSSION

The practical use of Japanese encephalitis vaccine purified from infected mouse brains started in 1966 in Japan, has led to a rapid increase in the number of the vaccinated people and a rapid reduction in the incidence of this disease (Oya, 1987). At the beginning of the use of the vaccine, the virus was purified by alcohol-protamine precipitation and centrifugation. The vaccine contained a high level of impurities and its potency was very low. Recently the manufacturing procedures employs many sophisticate methods such as ultracentrifugation and ultrafiltration to reduce the impurities and improve the potency (Umenai *et al.*, 1985).

In 1988, Lee and Ahn and Yamanishi *et al.* reported the development of inactivated vaccines against HFRS with Hantaan virus infection. Lee and Ahn (1988) inoculated Hantaan virus isolated from an HFRS patient into the suckling rat brains and purified and inactivated with the methods to prepare Japanese encephalitis virus mouse brain vaccine with a slight modification. Yamanishi *et al.*, (1988) inoculated Seoul virus isolated from a rat tumor into the suckling mouse brains. The available evidences appeared that these vaccines induced protective immunity in mice.

Currently some combined vaccines are available. The toxoid vaccines against tetanus and diphtheria and inactivated pertussis whole cell vaccine were combined. The live attenuated virus vaccine against measles, mumps and rubella were combined. The combined vaccines have many advantages over the corresponding monovalent vaccines in the practical use such as manufacturing costs, transportation, storage and administration. The combined vaccines may result in a substantially reduced number of contacts with health care workers to immunize against those diseases. The cost of administration a vaccine is at least 10 times the cost of the vaccine (Douglas, 1993). Although it is unlikely that this ratio will hold for many other vaccines, reducing the number of visits of health care workers for vaccine administration could clearly result in great savings.

During the observation period there occurred no death in both mice and rabbits. The expected clinical dose is 0.5 ml (8.3 μl/kg body weight). In this study the maximum dosage of the KGCC-95VI administered into the animals was 6.66 ml/kg body weight, corresponding to 800 times the expected clinical dose. The dosages administered to the experiment animals were 0.0, 0.000666, 0.00666, 0.0666, 0.666 and 6.66 ml/kg body weight. As there was no death in this study, the lethal dose 50% (LD_{50}) of the KGCC-95VI was not accessible. It is estimated that the LD50 is more than 800 times than the expected clinical dose. In 1995 Lim *et al.* reported the acute toxicity of Hantaan virus vaccine (HRccine) in rats and rabbits. In their report, they concluded that the Hantaan virus vaccine was quite safe because there were no signs of acute toxicity even with the dosage of 600 times that of expected clinical dosage. During the observation period the body weight changes were monitored. There was no abnormality in the body

weight gains in the treated groups comparing to those in the control group. All the tissues and organs showed no abnormalities in the gross pathological examination.

In conclusion, the combined vaccine (KGCC-95VI) against Japanese encephalitis and Hantaan virus infection showed no signs of the acute toxicity and is considered not to have the acute toxicity in mice and rabbits.

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