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Primary Skin and Eye Irritation Study of Combined Vaccine (KGCC-95VI) Against Japanese Encephalitis and Hantaan Virus Infection

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ABSTRACT *: The primary skin and eye irritancy 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. The KGCC-95VI was applied to the back skins of the New Zealand White rabbits. The rabbits were observed for 72 hours and did not exhibit erythema, eschar and edema. The eyes of the rabbits were exposed to the KGCC-95VI. The rabbits were observed for 7 days and did not exhibit any ocular findings on cornea, iris and conjuntivae. The KGCC-95VI is considered not to have the primary skin and eye toxicity in rabbits.*

Key Words *: Japanese encephalitis, Hantaan virus infection, Vaccine, Skin irritancy, Eye irritancy*

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 prepared from 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, rickettial 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 primary skin and eye irritation tests of the combined vaccine was performed using the rabbits in accordance with the guidelines on the safety 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 and autoclaved at the laboratory was used as the

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diluent for the test material.

Male 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 and fed with new-born calf pellets (Jeil Feed Co., Korea) and tap water ad libitum. Six male rabbits entered in the primary skin irritation test and nine male rabbits in the eye irritation test.

Following the guidelines on the safety tests of the drugs provided by the Food and Drug Administration, Korea, the possibility of skin and eye irritancy of the KGCC-95VI was investigated. For the primary skin irritation test, the backs of six rabbits were clipped free of hairs, two sites at each side of the back, by 2.5 cm×2.5 cm. The two sites were abraded by the hypodermic needle drawn across the skin repeatedly in a way that the stratum corneum is opened but no bleeding was produced. And the other two sites remained intact. One human dose (0.5 m*l*) of the KGCC-VI was soaked into the surgical gauze secured in place with bands (Microfoam, 3M) and applied onto the abrased and the intact sites respectively. Separately 0.5 m*l* of phosphate buffered saline was applied for the control in the same way as the test material. The entire trunk of the animal was wrapped with the rubberized cloth. After 24 hours after application, the wrappings and the adhesive bands were removed. The applied sites were washed gently with the saline. The applied sites were evaluated for erythema and edema at hours 24, 48 and 72 after the application. The skin irritation was evaluated according to the Scoring System (Draize Scoring System) specified in the guidelines on the safety tests of the drugs.

For the eye irritation test, nine male rabbits without any background ocular findings were used. The left eyes of the rabbits were exposed to 0.1 m*l* of the KGCC-95VI on the cul-de-sac of the conjunctiva. Both eyes of three rabbits were irrigated for one minute with 20 m*l* of the lukewarm sterile phosphate buffered saline 25 seconds after the exposure, Both eyes of the remaining six rabbits were not irrigated. The right eyes of the rabbits were used as the control. The abnormalities of the eyes were observed at days 1, 2, 3, 4 and 7. The observation was evaluated according to the Grading the Ocular Lesions specified in the guidelines on the safety of the drugs.

III. RESULTS

In order to investigate the possibility of the KGCC-95VI to irritate the skin and eye the primary skin and eye irritation tests were performed following the guidelines on the safety tests of the drugs provided by the Food and Drug Administration, Korea. In the primary skin irritation test, no clinical findings and death caused by the KGCC-95VI were observed during the 72-hour observation period. In the eye irritation test, no ocular findings and death caused by the KGCC-95 VI were observed during the 7-day observation period . The skin reaction was evaluated according to the Draize scoring system in the rabbits. The scoring is given in Table 1. In the eye irritation test, the ocular findings were evaluated according to the grading system. The scoring is given in Tables 2 and 3.

Table 1. Evaluation of Primary Skin Irritation

Description	Mean Score After Application (Hours)		
	24	48	72
Erythema and Eschar Formation	0	0	0
Edema Formation	0	0	0

Table 2. Evaluation of Eye Irritation (Irrigated)

Description	Mean Score After Exposure (Days)				
	1	2	3	4	7
1. Cornea					
Opacity	0	0	0	0	0
Area Involved	0	0	0	0	0
2. Iris	0	0	0	0	0
3. Conjunctivae					
Redness	0	0	0	0	0
Chemosis	0	0	0	0	0
Discharge	0	0	0	0	0

Table 3. Evaluation of Eye Irritation (non-Irrigated)

Description	Mean Score After Exposure (Days)				
	1	2	3	4	7
1. Cornea					
Opacity	0	0	0	0	0
Area Involved	0	0	0	0	0
2. Iris	0	0	0	0	0
3. Conjunctivae					
Redness	0	0	0	0	0
Chemosis	0	0	0	0	0
Discharge	0	0	0	0	0

IV. DISCUSSION

The practical use of Japanese encephalitis vaccine purified from infected mouse brains started in 1966 in Japan, and 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 thus 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 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 of a vaccine is at least 10 times higher than 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.

In the primary skin irritation test, during the 72-hour observation period there occurred no death. One human dose (0.5 m*l*) of the KGCC-95VI was applied to the skin of the rabbits, corresponding to 30 times the expected clinical dose. The skin reaction was evaluated according to the Draize scoring system in albino rabbits which was adopted and specified in the guidelines on the safety tests of the drugs provided by Food and Drug Administration, Korea. As shown in Table 1, during the 72-hour observation period, there observed no erythema, eschar or edema. This strongly suggests that the KGCC-95VI has no primary skin irritancy in the rabbits. Lee *et al.*(1993) reported the primary skin irritation test of sodium salt of linear alkylbenzenesulfonic acid (LAS-Na) and sulfur-containing fatty acid methyl ester sodium salt. They reported these two chemicals had, even statistically non-significant, the slight skin irritancy. This might ascribe to the nature of the test material, which are chemical themselves. In this study the test material was the viral proteins, which were not chemicals but biosynthesized proteins. It is generally granted that the proteins are not irritant. Hence, the KGCC-95VI did not exhibit any primary skin irritancy.

In the eye irritation test, during the 7-day observation period there occurred no death. The eyes of the rabbits were exposed to 0.1 m*l* of the KGCC-95VI. The ocular findings were evaluated according the grading system which was adopted and specified in the guidelines on the safety tests of the drugs provided by Food and Drug Administration, Korea. As shown in Tables 2 and 3, during the 7-day observation period, there observed no ocular findings on the cornea, iris and

conjunctivae. This strongly suggests that the KGCC-95VI has no ocular irritancy in the rabbits. Lee *et al.* (Lee *et al.*, 1993) reported the eye irritation test of sodium salt of linear alkylbenzenesulfonic acid (LAS-Na) and sulfur-containing fatty acid methyl ester sodium salt. They reported these two chemicals had, even statistically non-significant, the very slight ocular irritancy. This might ascribe to the nature of the test material, which are chemical themselves. In this study the test material was the viral proteins, which were not chemicals but biosynthesized proteins. It is generally granted that the proteins are not irritant. Hence, the KGCC-95VI did not exhibit any ocular irritancy.

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

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