Korean J. Toxicol.
Vol.2, No.1. pp. 23-30, 1986

ACUTE TOXICITY STUDY OF HEPACCINE-B(HEPATITIS B VACCINE)

Yong-Soon Lee,* Jung-Sik Cho,** Byung-Hoon Park** and Sun-Chul Kim**
*College of Veterinary Medicine, Seoul National University**
*Genetic Engineering Divison, Cheil Sugar Co., Ltd.***

ABSTRACT: *Acute toxicity study was conducted on a Hepatitis B vaccine (Hepaccine-B inj.) with mice, guinea pigs, and rabbits, in accordance with the norms suggested by the F.D.A. in U.S.A. Dose ranges were 2 doses/mouse, 5 doses/guinea pig, 10 doses/rabbit. They received the vaccine subcutaneously and intraperitoneally.*

Thereafter, all animals injected were observed of general signs daily, and of body weight for two weeks. At the end of the observation period (or at the time of death), all animals received the highest dose group were autopsied and gross observation was made on various organs and tissues. No significant toxicity was noted.

Keywords: *Acute toxicity, Hepatitis B vaccine (Hepaccine B), Acute toxicity study in mice, guinea pigs, rabbits.*

INTRODUCTION

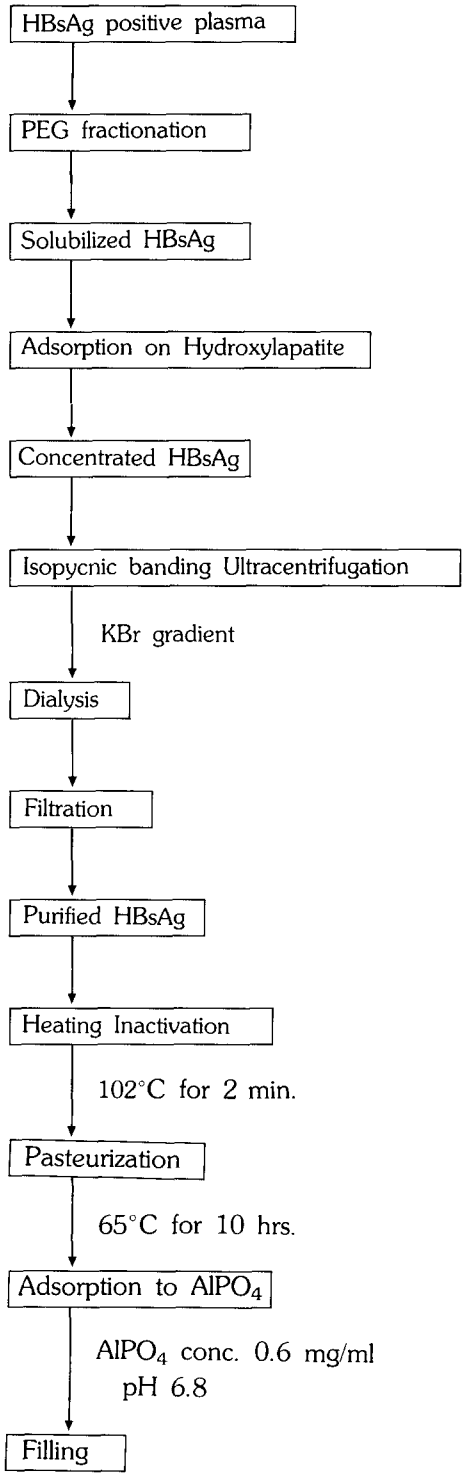
Hepatitis B virus (HBV) infection is a worldwide health problem. It often leads to cirrhosis and hepatoma.

Estimating that about 200 million (Krugman, 1982) people worldwide and 9–14% (Sun *et al.*, 1984) of Koreans are HBV-carriers and that about 50–70% of Korean adults are reported to carry anti-HBs with an increase by age, indicating that the infection appears to be very severe.

In 1965, Blumberg first discovered Australia Antigen. In 1971, Kurgman reported that heat-treated plasma containing the HB virus showed a protective efficacy against the infection by HBV when administered into the body as an active immunization. It also reduced the chronic HBV carriers. Since then, the development of vaccine for HBV infection has been proposed and two kinds of vaccines for HBV infection have already been available: formalin inactivation and heat inactivation. Clinical studies of immunogenicity and safety of the HBV-vaccines derived from plasma have been well recognized (Evelne, 1982, Reesink, 1981). Recently, we developed a vaccine with high immunogenicity by a method of two step heat-treatment; only a dose of 3 μg/ml induces better protective efficacy than 20 μ g/ml of other vaccines.

Present study aimed to investigate an acute toxicity of the vaccine (Hepaccine-B) in mice, guinea pigs, and rabbits in accordance with the norms suggested by the F.D.A. in U.S.A. (Federal Food, Drug and Cosmetics act, 1978)

Scheme 1. Procedure for production of Hepaccine-B



MATERIALS AND METHODS

Test Materials

Hepaccine-B (Lot No. 84-02) was produced by R & D center, Cheil Sugar & Co., Ltd., as the folowing (Scheme 1);

1) Purification of HBsAg
   HBsAg was purified from HBsAg positive human plasma by PEG fractionation, adsorption on hydroxylapatite and density gradient centrifugation. The purity of this purified HBsAg was 96% in SDS-PAGE.
2) Inactivation of HBsAg
   Purified HBsAg was inactivated by two step heating-inactivation process, heating at 102°C for 2 min. and pasteurization at 65°C for 10 hrs.
3) Preparation of Hepaccine-B
   Inactivated HBsAg solution was diluted with presterilized suspension of aluminum phosphate in phosphate buffered saline, pH 6.8.
   The final suspension contained 3 μg HBsAg and 0.6 mg aluminum phosphate per ml. Chemical characterization of Hepaccine-B is shown in Table 1.

Table 1. Chemical characterization of hepaccine-B.

Package	from	Ingredients	
1ml/vial	Suspension	HBsAg(inactivated)	3 μg/ml
		Aluminum phosphate	0.6mg/ml
		Thimerosal	0.1mg/ml
		Saline	q.s.

4) Quality control of Hepaccine-B
   Quality control of Hepaccine-B was conducted thoroughly in accordance with "proposed requirement for Hepatitis B Vaccine prepared from Plasma" (Requirements for Biological Substances No. 31 Revised 1983, WHO). Results of quality control of Hepaccine-B are summarized in Table 2.

Table 2. Quality control of hepaccine-B.

Agent	Method of test	Result
Hepatitis virus	Chimpanzee safety test	passed
	Serological marker	free
	DNA polymerase test	free
	Dane particle(EM picture)	free
Extraneous virus	Animal test	passed
	Egg test	passed
	Cell curture test	passed
Micro-organism	Sterility test	sterile
Pyrogen	Pyrogen test	free
Toxin	General safety test	free
Blood group substance	Blood group substance test	free

Laboratory Animals and Environmental Conditions

1) Mice

ICR–SEL mice, 4 weeks of age, of both sexes were used. These mice were purchased from Laboratory Animal Breeding Center, Seoul National University, and then bred in an experimental laboratory for a week to adapt to the experimental environment. While they were breeding in the laboratory, we selected only overtly healthy mice that increased normally in body weight. Initial mean body weights were 25.1 ± 1.6 gm for the male and 21.87 ± 1.2 gm for the female.

Experimental conditions for mice, guinea pigs and rabbits were as follows.

Temperature: 19–23°C
Relative humidity: 45–65%
Light control: 12 hrs light–dark cycle(automatic timer control).
Air change: 12–15 air changes/hr.
Ammonia gas concentration 20 ppm below.

They were given sterile water to drink and pellet feed *ad libitum.*

2) Guinea pigs

Albino Hartley guinea pigs, 4 weeks of age, of both sexes were used. They were supplied by a commerical breeder and were kept in experimental laboratory for 2 weeks to be conditioned in the experimental environment.

Initial mean body weights, 316.56 ± 16.8 gm for the male, and 283.60 ± 14.6 gm for the female, were used. Water and feed were *ad libitum.*

In addition, vegetables such as cabbages and carrots were also supplied.

3) Rabbits

New Zealand White rabbits, 8 weeks of age, of both sexes were used. They were supplied by a commerical breeder and then conditioned in the experimental laboratory for a week. Initial mean body weights, 2.13 ± 0.27kg for the male and 2.07 ± 0.20kg for the female were used. Water and feed were *ad libitum.*

Test Methods

1) Mice

The mice were divided at random into 4 groups. Each group consisted of 10 mice. The dosages for each group are as the following:

Group Ⅰ: ½ dose of Hepatitis B Vaccine(0.5 ml)
Group Ⅱ: 1 dose of Hepatitis B Vaccine(1 ml)
Group Ⅲ: 2 dose of Hepatitis B Vaccine(2 ml)
Group Ⅳ: Placebo ($AlPO_4$ suspension, 0.6mg/ml)

This experiment was carried out with males and females, and the mice of each sex were injected subcutaneously and intraperitoneally.

Observations for any toxicological and pharmacological signs were made 4 times a day for the first week and twice a day for the second week. Body weights were measured before inoculation, then once on the 7th day and once on the 14th day after day 1. All the mice in the maximum dose group(2 ml) were killed, then autopsied on the 15th day after injection, and gross observation was conducted on organs and tissues. Also histopathological examination was carried out.

2) Guinea Pigs

The guinea pigs were divided at random into 4 groups.

Each group consisted of 5 guinea pigs.

Group Ⅰ:1 dose of Hepatitis B Vaccine (1 ml)
Group Ⅱ:3 dose of Hepatitis B Vaccine (3 ml)
Group Ⅲ:5 dose of Hepatitis B Vaccine (5 ml)

Group Ⅳ: Placebo ($AlPO_4$ suspension, 0.6 mg/ml)

This experiment was carried out with males and femals, and the guinea pigs of each sex were injected subcutaneously and intraperitoneally.

Observations for any toxicological and pharmacological signs were made twice a day for the first week and once a day for the second week.

They were weighed immediately before injection, then once on the 7th day and the 14th day to compare the difference in body weight changes between test groups and the placebo control group.

All the guinea pigs in the maximum dose group (5ml) were killed, then autopsied on the 15th day after injection, and gross observation was made on organs and tissues. In addition, histopathological examination was also made.

3) Rabbits

The rabbits were divided at random into 4 groups. Each group consisted of 5 rabbits.

Group Ⅰ: 5 dose of Hepatitis B vaccine (5 ml)

Table 3a. Acute toxicity via the subcutaneous route in the male mouse.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
½ dose (0.5ml)		25.10	30.27	34.25	+9.15
1 dose (1ml)		25.09	29.48	32.96	+7.87
2 dose (2ml)		25.05	31.08	35.14	+10.09
Placebo (2ml) control		25.11	29.58	33.47	+8.36

Table 3b. Acute toxicity via the subcutaneous route in the female mouse.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
½ dose (0.5ml)		21.59	24.97	28.35	+6.76
1 dose (1ml)		22.01	26.73	29.21	+7.20
2 dose (2ml)		21.91	25.33	28.47	+6.56
Placebo (2ml) control		21.85	25.42	28.03	+6.18

Table 3c. Acute toxicity vai the intraperitoneal route in the male mouse.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
½ dose (0.5ml)		25.09	30.14	34.21	+9.12
1 dose (1ml)		25.13	30.68	33.86	+8.73
2 dose (2ml)		25.16	30.63	34.41	+9.25
Placebo (2ml) control		25.11	30.08	34.01	+8.90

Table 3d. Acute toxicity via the intraperitoneal route in the female mouse

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
½ dose (0.5ml)		21.90	26.87	28.57	+6.67
1 dose (1ml)		21.69	26.73	28.51	+6.82
2 dose (2ml)		21.87	25.60	28.75	+6.88
Placebo (2ml) control		21.87	25.53	28.57	+6.70

*Mean body weight

Table 4a. Acute toxicity via the subcutaneous route in the male guinea pig.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
1 dose (1ml)		316.17	367.67	374.86	+58.63
3 dose (3ml)		315.17	366.87	377.92	+62.75
5 dose (5ml)		313.22	365.44	338.22	+75.00
Placebo (5ml) control		314.54	367.52	375.36	+60.82

Table 4b. Acute taxicity via the subcutaneous route in the female guinea pig.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
1 dose (1ml)		281.47	318.46	346.71	+65.24
3 dose (3ml)		283.61	319.58	347.98	+64.37
5 dose (5ml)		284.04	314.46	340.26	+56.22
Placebo (5ml) control		279.66	317.32	344.06	+64.40

Table 4c. Acute toxicity via the intraperitoneal route in the male guinea pig.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
1 dose (1ml)		317.86	365.41	380.72	+62.86
3 dose (3ml)		315.65	355.23	381.44	+65.79
5 dose (5ml)		319.44	357.48	383.88	+64.44
Placebo (5ml) control		319.06	361.40	383.54	+64.48

Table 4d. Acute toxicity via the intraperitoneal route in the female guinea pig.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
1 dose (1ml)		284.56	326.43	351.83	+67.27
3 dose (3ml)		282.64	324.96	350.57	+67.93
5 dose (5ml)		286.63	327.7	349.93	+63.30
Placebo (5ml) control		284.10	321.28	350.78	+66.68

*Mean body weight

Group Ⅱ: 7 dose of Hepatitis B vaccine (7 ml)
Group Ⅲ: 10 dose of Hepatitis B vaccine (10 ml)
Group Ⅳ: Placebo ($AlPO_4$ suspension, 0.6mg/ml).
This experiment was carried out with males and females, and the rabbits of each sex were injected subcutaneously and intraperitoneally.
Observations for any toxicological and pharmacological signs were made twice a day for the first week and once a day for the second week.
Body weights were measured immediately before injection, then once on the 7th day and the 14th day to compare the body weight changes between test groups and the placebo control group.
All the rabbits in the maximum dose group (10 ml) were killed, then autopsied on the 15th day after injection, and gross observation was made on organs and tissues. In addition, histopathological examination was carried out.

Table 5a. Acute toxicity via the subcutaneous route in the male rabbit.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
5 dose (5ml)		2.119	2.235	2.355	+0.236
7 dose (7ml)		2.137	2.326	2.387	+0.25
10 dose (10ml)		2.060	2.2175	2.350	+0.29
Placebo (10ml) control		2.125	2.2525	2.3475	+0.2225

Table 5b. Acute taxicity via the subcutaneous route in the female rabbit.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
5 dose (5ml)		2.073	2.2927	2.3257	+0.2527
7 dose (7ml)		2.069	2.3024	2.3816	+0.3126
10 dose (10ml)		2.095	2.3075	2.5625	+0.4675
Placebo (10ml) control		2.070	2.2125	2.2675	+0.1975

Table 5c. Acute toxicity via the intraperitoneal route in the male rabbit.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
5 dose (5ml)		2.1265	2.2730	2.4245	+0.2980
7 dose (7ml)		2.1360	2.3026	2.4986	+0.3626
10 dose (10ml)		2.1825	2.2900	2.5200	+0.3375
Placebo (10ml) control		2.1425	2.4275	2.4600	+0.3175

Table 5d. Acute toxicity via the intraperitoneal route in the female rabbit.

Group	Days	Body weight check*			Δg
		0 day	7th day	14th day	
5 dose (5ml)		2.0270	2.2580	2,3507	+0.3237
7 dose (7ml)		2.0760	2.2620	2.3747	+0.2987
10 dose (10ml)		2.0675	2.2500	2.3450	+0.2775
Placebo (10ml) control		2.0750	2.3400	2.3800	+0.305

*Mean body weight

RESULTS

Toxicological Signs

No mortality and no behavioural, anorectic, respiratory, motor or autonomic disturbance, and no salivation, diarrhea were observed for 2 weeks in mice, guinea pigs and rabbits.

Body Weight Changes

In mice, guinea pigs and rabbits, significant difference in body weight change between test groups and placebo control group was not seen. Results of body weight changes are shown in Table 3,4,5. No significant body weight changes different between test and control animals were noted.

Gross Findings

No significant gross changes in test groups in comparison with those of control group were observed at the maximum dose levels.

DISCUSSION

Generally, an acute toxicity study is conducted to measure the dose of any drug to cause 50% deaths of subjects; i.e. the median lethal dose(LD_{50}), and to help determine the dose level for subacute or chronic toxicity study.

But in cases of a drug with a low toxicity, determine LD_{50}, it is a principle to administer a maximum dose level which can be mechanically administered to test animals to determine LD_{50}. This is called Bulk toxicity (Guide Lines for Toxicity Studies of Drugs, 1984).

In most toxicity studies, the dose level is set up on the basis of body weight(kg) and surface area(cm^2) and then correlation of dose–response between men and test animals should be considered (John *et al.*, 1980). The difference of dose–response, i.e. sensitivity between men and test animals is not usually well informed in most new drugs. For toxicity studies of new drugs of which the sensitivity difference is unknown, the first dosage to be administered is determined on the basis of the body weight of the test animal compared to 60 kg of human body weight. The sensitivity of humans to toxicity is about 10 times as high as that of rodent. In the acute toxicity study of Hepaccine–B, we administered maximum dosage 4800 times more than one human dose by weight for humans into mice, 1000 times into guinea pigs, and 300 times into rabbits, but no toxicological signs revealed.

REFERENCES

Blumberg, B.S., Alter H.J. and Visnich, S.(1965): A "New" antigen in leukemia sera, *J.A.M.A.*, 191:541.

Eveline, E. Reevink–Brongers(1982): Preparation and Evaluation against Hepatitis B; *in* Viral Hepatitis, 1981, Int'l Symposium, (Eds.) Wolf szmuness, Harvey J. Alter and James E. Maynard, The Franklin Institute Pres Philadelphia, PA.

Federal Food, Drug and Cosmetics act: Federal Register, Vol. 43, No. 247:60013–60020, Friday, December 22, 1978.

Guide lines for toxicity studies of drugs; Notification No. 118 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, Japan, February 15, 1984.

John D., Curtis D.K. and Mary, O.A. (1980): *Toxicology* (2nd Ed.), Macmillan Publishing Co., Inc., New York.

Krugman, S.(1982): The Newly licensed hepatitis B vaccine: Characteristic and indications for use, *J.A.M.A.*, 247:2012.

Krugman, S., Giles, J.P. and Hammond, J.(1971): Viral hepatitis type B. (MS–2 strain): Studies on active immunization, *J.A.M.A.*, 217:41.

Reesink, H.W., Reevink–Brongers, E.E. and Brummelnuis, H.G.J., (1981): Heat inactivated HBsAg as a vaccine against hepatitis B. *Antiviral Research*, 1:13–15.

Sun, H.S., Chung, H.K., Shin, K.S. and Kim, K.H.(1984): Immune response of hepatitis B vaccine, Asian Pacific Assoc. for the study of the liver, Scientific Meeting (1984.1.12–1.14: Bangkok, Thailand)