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General Pharmacology of PEG-Hemoglobin SB1

Eun-Joo KIM*, Rae-Kyong LEE, Ji-Yeong BAK and Gyu-Kap CHOI

Toxicology Research Center, Korea Research Institute of Chemical Technology, P.O. Box 107, Yusong-Ku, Taejon 305-606, Korea

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Abstract – PEG-hemoglobin SB1 (SB1), which is a hemoglobin-based oxygen carrier, is intended to use as a safe blood substitute against brain ischemia and stroke. The general pharmacological profiles of SB1 were studied. The doses given were 0, 5, 10, 20 ml/kg and drugs were administered intravenously. The animals used for this study were mouse, rat and guinea pig. SB1 showed no effects on general behavior, motor coordination, spontaneous locomotor activity, hexobarbital sleeping time, anticonvulsant activity, analgesic activity, blood pressure and heart rate, left ventricular peak systolic pressure, left ventricular end diastolic pressure, left ventricular developing pressure, double product, heart rate, coronary flow rate, smooth muscle contraction using guinea pig ileum, gastrointestinal transport, gastric secretion, urinary volume and electrolyte excretion at all doses tested except the decrease of body temperature. These findings demonstrated that SB1 possesses no general pharmacological effects at all doses tested.

Key words □ PEG-hemoglobin SB1, Mouse, Rat, Guinea pig, General pharmacology

SB1 is a modified bovine hemoglobin (bHb) with polyethylen glycol (PEG), which yields a promising hemoglobin-based oxygen carrier, developed proprietary procedures for the large scale purification of bovine hemoglobin under conditions consistent with its use as a therapeutic agent. The use of PEG-modification is an attractive means of overcoming problems with half-life and antigenicity. PEG is widely used in the food, drug and cosmetic industries due to its non-toxic property. It has been well documented that the use of PEG modification significantly increases the circulating life and decreases the immunogenicity of proteins (Levy and Hershfield, 1988).

This study was conducted to investigate the general pharmacological proprieties of SB1 in mice, rats and guinea pigs.

MATERIALS AND METHODS

Animals

The experiments were performed on ICR mice (20~30 g), Sprague-Dawley rats (200~300 g), Hartley guinea pigs (300~350 g). The mice and rats were provided from the department of experimental animals of KRICT, guinea pigs from Gye Ryong Science System respectively. The animals are kept in a storage room under the conditions of constant temperature (20.7±0.6 °C), relative humidity (55.6±4.1%) and illumination (9 hr-light, 15 hr-dark cycle) until the day of experiment. All animals were fed a standard animal chow daily and had access to drinking water *ad libitum*. Animals were divided into groups at random.

*To whom correspondence should be addressed.

Drugs

SB1 (reddish brown solution) and Hartman's solution (vehicle control) were provided by Sun Bio Inc. Diazepam (Shionogie, Japan), hexobarbital · Na (Bayer, AG), chlorpromazine·HCl (Fluka), strychnine·HCl (Sigma) and pentylenetetrazole (Sigma) were commercially purchased. SB1, hexobarbital, chlorpromazine, strychnine and pentylenetetrazole were dissolved in distilled water and diazepam was suspended in the aqueous solution of Tween 80 (0.05%). The doses given were vehicle control, 5, 10 and 20 ml/kg and drugs were administered intravenously.

Instruments

The pH meter (Orion 720A), organ bath (Letica), transducer (Harvard), polygraph (Biopack MP 100), Na/K/Cl analyzer (Ciba corning), Langendorff apparatus (Sun Jin), pressure transducer (OHMEDA), rodent ventilator (TSE), cirulation water bath (Jeio Tech.), peristaltic pump (Jeio Tech.), motility meter (PAS, San Diego), rotarod (Ugo vasile 7600), rodent shocker (HSE), hot-plate (Letica) and thermometer (MGA-III), plethymograph (HSE) were used in this study.

Effects on general behavior

Groups of 4 male and 4 female mice were used. According to the modified method of Irwin *et al.*(1964), the general behavior of the animals was observed at 0, 0.5, 1, 2, 3, 5 hrs after drug administration.

Effects on spontaneous motility

Groups of 8 male mice were used for single dose experiment with test drugs. Mice were placed in a plastic cage (26×25×40 cm) and the locomotor activity was measured using a motility meter (PAS, San Diego) at 5 min interval for 15 min from 10 to 240 min after administration of test drugs (Svensson and Thieme, 1969).

Effects on motor function

Groups of 8 male mice were used for single dose experiments with SB1. The animals were sustained on the rotarod (10 rpm) for more than 3 min, were preselected and randomly distributed to 5 groups. According to the method of Dunham and Miya (1957), the animals of each group were placed on the rotarod at 0, 30, 60, 120 and 240 min after administration of test drugs. The numbers of mice falling within 1 min from a rotaring rod (10 rpm) were counted.

Effects on hexobarbital-induced sleeping time

Groups of 8 male mice were used for single dose experiments with SB1 (5~20 ml/kg, i.v.). The animals were pretreated with drugs 30 min prior to hexobarbital (70 mg/kg, i.p.) injection. Following hexobarbital injection, the duration of sleep was measured by examining the righting reflex.

Effects on body temperature

Groups of 8 male mice were used for single dose experiments with SB1 (5~20 ml/kg, i.v.). The rectal temperature was continuously monitored by an electrothermometer (MGA III) after intravenous administration of test drugs.

Acetic acid-induced writhing test

Groups of 8 male mice were used for single dose experiments with SB1. Animals were injected with 1% acetic acid (0.1 ml/10 g b.w., *i.p.*) 30 min after administration of test drugs. The number of writhings was counted for 10 min after acetic acid injection and it was compared with that produced by acetic acid alone (Koster and Anderson, 1959).

Hot-plate test

Groups of 8 male mice were used for single dose experiments with SB1. 30 min after drug administration, each mouse was placed on a 20 cm diameter hot-plate (Letica), warmed with a waterbath at 56°C. The time to the beginning of responses such as rasing and licking its feet, was measured.

Maximal electroshock-induced seizure

An electroshock (60 mA, 0.3 sec., AC) was given to the corneas of mice 30 min after the administration of test drugs. The incidence of tonic extensive convulsion and mortality was determined (Woodbury and Davenport, 1952).

Pentylentetrazol-induced seizure

Pentylentetrazol (100 mg/kg) was intraperitoneally injected 30 min after administration of test drugs. And the incidence of clonic convulsion and mortality was determined (Swinyard and Brow, 1952).

Strychnine-induced seizure

Strychnine (2 mg/kg) was intraperitoneally injected 30 min after administration of test drugs. And the incidence of tonic extensive convulsion and mortality was determined.

Effects on cardiovascular system in conscious rats

Groups of 5 male rats weighing 350~400 g were used. A chronic cannula for measuring blood pressure (BP) and heart rate (HR) had been inserted as follows: under thiopental (50 mg/kg, i.p.) anesthesia and with rats in the supine position, a small incision was made to expose the femoral artery. After a heparin-filled polyethylene tubing was reversely inserted, another end of it was passed under the skin and drawn out from the cervicodorsal region. Finally, the skin was closed. The next day, BP and HR under unrestraint were measured with a pressure transducer (Biopack, MP 100). Drugs were intravenously via femoral vein injected in a volume of 5~10 ml/kg. Data are expressed as mean percentage values ± S.E.M.

Isolated rat heart studies

For this study, isolated hearts were used according to the published methods after some modifications (Watts and Maiorano. 1987, Bolli, 1991). Male Spargue-Dawley rats weighing 300~450 g were anesthetized with sodium pentobarbital (100 mg/kg, i.p.). The tail vein was injected with heparin (20 IU/kg) and then the trachea was intubated while rats were mechanically ventilated with a rodent ventilator. Their hearts were perfused *in situ* with oxygenated modified Krebs-Henseleit bicarbonate buffer (described herein) by retrogradic aortic cannulation. The hearts were then excised and moved to the Langendorff apparatus, where they were perfused with an oxygenated modified Krebs-Henseleit bicarbonate buffer containing 116 mM NaCl, 24.9 mM $NaHCO_3$, 4.7 mM KCl, 1.1 mM $MgSO_4$, 1.17 mM KH_2PO_4, 2.52 mM $CaCl_2$, 8.32 mM glucose and 2.0 mM pyruvate at a constant perfusion pressure (75 mmHg). A water filled latex balloon attached to a metal cannula was placed in the left ventricle

through the pulmonary vein and connected to a pressure transducer for measurement of LVP (left ventricular pressure). The hearts were allowed to equilibrate for 15 min, at which time EDP (left ventricular end-diastolic pressure) was adjusted to 10 mmHg, and this balloon volume was maintained throughout the experiment. Then, baseline contractile function, HR and CF (coronary flow) were measured. Cardiac contractile function was calculated by subtracting LVEDP (left ventricular end diastolic pressure) from LVSP (left ventricular peak systolic pressure), yielding LVDP (left ventricular developing pressure). The DP (double product), an another important parameter for assessing cardiac performance was calculated by multiplying HR by LVDP. Throughout the experiment, all these parameters were measured or calculated before and 10 min after pretreatment with drug and 30 min after the onset of reperfusion with buffer. The drugs were applied by a single dose technique.

Effects on the isolated guinea pig ileum

Male Hartley guinea pig, weighing 270~300 g were stunned by a blow on the head and bled from the carotid arteries. The longitudinal muscle preparations (about 5 cm in length) were isolated from segments of the terminal ileum. The preparations were suspended in a 10 ml water-jacked organ bath containing Kreb's solution aerated continuously with 95% O_2 and 5% CO_2 at 37°C and their responses were recorded through an isotonic transducer (Harvard). The composition of the nutrition solutions was as follows: Kreb's-Henseleit bicarbonate solution; NaCl; 6.9, KCl; 0.35, $CaCl_2$; 0.265, $MgSO_4$; 0.295, KH_2PO_4; 0.163, $NaHCO_3$; 2.1, glucose; 1.8 and EDTA; 0.009 g/L. The drugs were applied by a single dose technique. The contractile responses of drug alone and responses to acetylcholine, histamine, $BaCl_2$ were expressed as a % of the maximal response to acetylcholine (5×10^{-7} M), histamine (2×10^{-6} M) and $BaCl_2$ (2×10^{-3} M).

Effects on the gastric secretion

Groups of 5 rats weighing 150-180 g were fasted for 24 hr. Under ether anesthesia and with rats in the supine position, their abdomen was opened along the midline (Shay and Komarov, 1945). The pylorus was ligated and then drugs were intraduodenally given at a volume of 5~20 ml/kg. 5 hrs after the colsing of the abdomen, the stomach was removed. Its gastric juice was examined for the amount and total acidity by a titration method with 0.1 N NaOH using a titrator (Autoburette, Brand).

Table I. Effects of PEG-hemoglobin SB1 on general behavior in mice

SEX : ♂, ♀

Compounds	Vehicle						PEG-hemoglobin SB1 (ml/kg)																	
Dose(ml/kg, i.v.)	0						5						10							20				
Time (hr)	0	0.5	1	2	3	5	0	0.5	1	2	3	5	0	0	0.5	1	2	3	5	0	0.5	1	2	3
1. Catalepsy	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
2. Traction	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
3. Tremor	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
4. Convulsion	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
5. Exopthalmos	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
6. Piloerection	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
7. Salivation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
8. Lacrimation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
9. Diarrhea	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
10. Skin Coloration	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
11. Pinna reflex	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
12. Righting reflex	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
13. Abdominal tone	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
14. Tail elevation	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
15. Eyelid	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
16. Locomotion	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
17. Respiration rate	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
18. Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Each number represents the number of positive/tested (1-12).
The mean score; max 8 min 0 (13-17) and number of animals (18).

Effects on intestinal transport

After mice weighing 25~30 g were fasted for 20 hrs, they were used as groups of 8 animals. A charcoal meal, 5% activated carbon powder suspension in 10% gum arabic solution, was orally administered at a volume of 10 ml/kg, 30 min after the administration of test drugs at a volume of 5~20 ml/kg. 30 min later, the animals were killed by a cervical dislocation and their intestines were immediately isolated and cooled with ice. The distance between the pylorus and the top of the charcoal and the total length of the intestine were measured. A transport rate was defined as a proportion of the former to the latter.

Effects on renal function

3 groups of 8 mice weighing 25~30 g were used. Animals were acclimated to respective metabolimetry cages (Nalgene) and allowed freely to take a solid food and water for 24 hrs. After deprivation of food and water and measurment of body weight, physiological saline of 2.5%/b.w. were administered orally. And 3 hrs later 2.5% saline were administered orally and SB1 intravenously.

Animals were returned at once and their urine was collected for 4 hrs. Urinary electrolytes, Na, K and Cl were measured with Na/K/Cl analyzer (Ciba corning)

RESULTS AND DISCUSSION

Effects on general behavior

SB1 at 5, 10 and 20 ml/kg did not show any effects on general behavior (Table I).

Effects on spontaneous motility

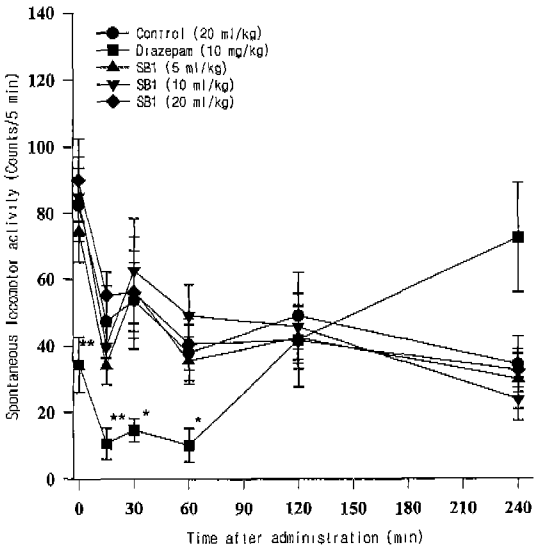

Fig. 1. Time-course of changes in spontaneous locomotor activity following an intravenous administration of PEG-hemoglobin SB1 to mice. Each point is the mean and S.E.M. (n=8). Significant difference from control group(*p<0.05, **p <0.01).

SB1 produced no changes in locomotor activity of mice at 5, 10 and 20 ml/kg (Fig. 1).

Effects on motor function

SB1 did not affect the motor coordination in mice at 5, 10 and 20 ml/kg (Table II).

Effects on hexobarbital-induced sleeping time

SB1 at 5, 10 and 20 ml/kg did not prolong the sleeping time in mice (Table III).

Table II. Effects of PEG-hemoglobin SB1 on motor coordination

Drugs	Dose (ml/kg)	Route	Animal No.	Incidence of ataxia (number of mice)					
				0 min	15 min	30 min	60 min	120 min	240 min
Vehicle	20	i.v.	8	0	0	0	0	0	0
PEG-hemo-	5	i.v.	8	0	0	0	0	0	0
globin SB1	10	i.v.	8	0	0	0	0	0	0
	20	i.v.	8	0	0	0	0	0	0
Diazepam	6 mg/kg	p.o.	8	0	7	7	5	2	0

Table III. Effects of PEG-hemoglobin SB1 on hexobarbital-induced hypnosis

Drugs	Dose (ml/kg)	Route	Animal No.	Sleeping time (min)	Control ratio (%)
Vehicle	20	i.v.	8	49.8±5.0	100
PEG-hemoglobin SB1	5	i.v.	8	57.6±8.8	116
	10	i.v.	8	56.1±10.5	113
	20	i.v.	8	60.3±12.9	121
Chlorpromazine	6 mg/kg	p.o.	8	189.4±43.1**	380

Each value represents the mean±S.D.

Significant difference from control group (**; p<0.01).

Table IV. Effects of PEG-hemoglobin SB1 on body temperature

Drug	Dose (ml/kg)	Route	temperature(°C) after administration					
			0 min	15 min	30 min	60 min	120 min	240 min
Vehicle	20	i.v.	36.6±0.5	37.5±0.3	37.3±0.2	37.3±0.3	37.0±0.3	36.6±0.4
PEG-hemo-	5	i.v.	36.8±0.4	37.1±0.4	37.1±0.4	37.2±0.5	37.4±0.3	36.9±0.3
globin SB1	10	i.v.	37.0±0.5	36.9±0.5	37.1±0.4	37.3±0.3	37.6±0.5**	37.0±0.4
	20	i.v.	36.8±0.3	37.3±0.4	37.3±0.4	37.3±0.4	37.7±0.2**	37.1±0.4*

Each value represents the mean±S.D.
Significant difference from control group (*; $p<0.05$, **; $p<0.01$).

Table V. Effects of PEG-hemoglobin SB1 on analgesia using acetic acid

Drugs	Dose (ml/kg)	Route	Animal No.	Wriths (No)
Vehicle	20	i.v.	8	13.5±3.4
PEG-hemo-	5	i.v.	8	14.3±3.5
globin SB1	10	i.v.	8	15.0±3.4
	20	i.v.	8	14.9±3.8
Ketoprofen	10 mg/kg	p.o.	8	6.1±3.6**

Each value represents the mean±S.D.
Significant difference from control group (**; $p<0.01$).

Table VI. Effects of PEG-hemoglobin SB1 on analgesia using hot-plate

Drugs	Dose (ml/kg)	Route	Animal No.	Licking time(sec)
Vehicle	20	i.v.	8	4.2±0.6
PEG-hemo-	5	i.v.	8	4.2±1.0
globin SB1	10	i.v.	8	4.5±1.4
	20	i.v.	8	4.4±0.8
Codeine	100 mg/kg	p.o.	8	13.2±6.1**

Each value represents the mean±S.D.
Significant difference from control group (**; $p<0.01$).

Table VII. Effects of PEG-hemoglobin SB1 on convulsion induced by pentylentetrazole

Drugs	Dose (ml/kg)	Route	Convulsion (No)	TE-time (sec)	Protection(%)
Vehicle	20	i.v.	8/8[a]	57.6±8.8[b]	0
PEG-hemo-	5	i.v.	8/8	59.9±21.1	0
globin SB1	10	i.v.	8/8	61.5±20.8	0
	20	i.v.	8/8	67.6±42.1	0
Diazepam	10 mg/kg	p.o.	0/8	-	100

[a] the number of positive/tested.
[b] the the mean±S.D.

Table VIII. Effects of PEG-hemoglobin SB1 on convulsion induced by strychnine

Drugs	Dose (ml/kg)	Route	Convulsion (No)	TE-time (sec)	Protection(%)
Vehicle	20	i.v.	8/8[a]	164.5±39.5[b]	0
PEG-hemo-	5	i.v.	8/8	148.0±30.6	0
globin SB1	10	i.v.	8/8	169.9±27.1	0
	20	i.v.	8/8	170.4±27.1	0
Diazepam	10 mg/kg	p.o.	4/8	286.8±32.8**	50.0

[a] the number of positive/tested.
[b] the mean±S.D.
Significantly difference from control group (**; $p<0.01$).

Table IX. Effects of PEG-hemoglobin SB1 on electroshock-induced convulsion

Drugs	Dose (ml/kg)	Route	Convulsion (No)	Protection (%)
Vehicle	20	i.v.	8/8[a]	0
PEG-hemo-	5	i.v.	8/8	0
globin SB1	10	i.v.	8/8	0
	20	i.v.	8/8	0
Diazepam	20 mg/kg	p.o.	8/8	0

[a] the number of positive/tested.

Effects on body temperature

SB1 produced the decrease of body temperature 30 min after drug injection at the doses of 10 and 20 ml/kg, but the symptoms recovered 1 hr later to a normal. At doses of 5 ml/kg, PEG-hemoglobin SB1 did not exert the decrease of body temperature (Table IV).

Acetic acid-induced writhing test

SB1 at 5, 10 and 20 ml/kg did not show any analgesic effects (Table V).

Hot-plate test

SB1 at 5, 10 and 20 ml/kg did not show any analgesic effects (Table VI).

Pentylentetrazole-induced seizure

SB1, administered intravenously at 5, 10 and 20 ml/kg, exhibited no effects on seizure and mortality induced pentylentetrazole (Table VII).

Strychnine-induced seizure

SB1, administered intravenously at 5, 10 and 20 ml/kg, exhibited no effects on seizure and mortality induced strychnine (Table VIII).

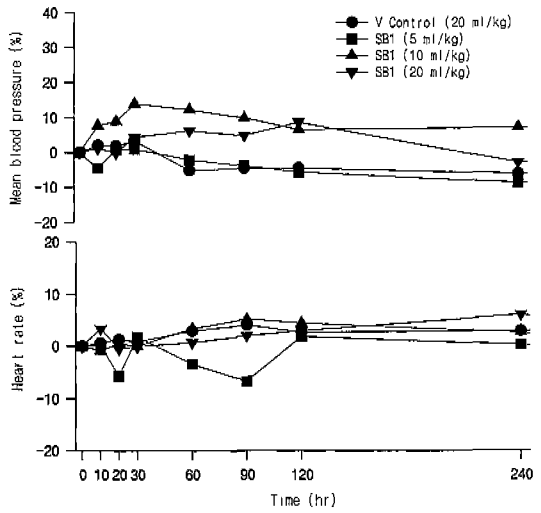

Fig. 2. Effects of PEG-hemoglobin SB1 on mean blood pressure and heart rate in conscious rats.

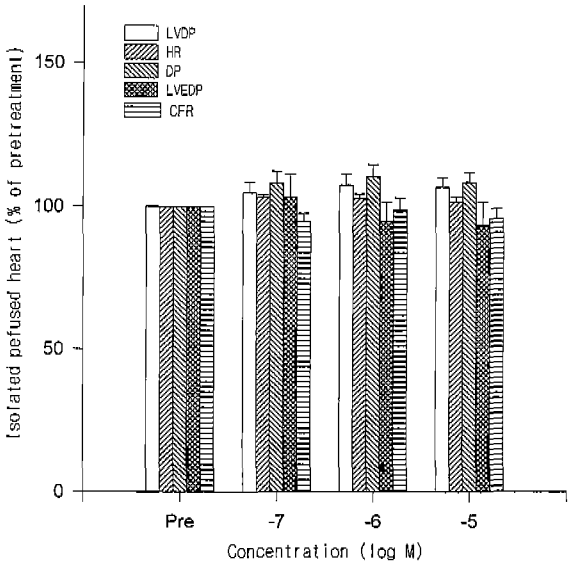

Fig 3. Effects of PEG-hemoglobin SB1 on isolated perfused heart in rats. Each column indicates the mean and S.E.M.(n=8).

Maximal electroshock-induced seizure

SB1, administered intravenously at 5, 10 and 20 ml/kg, exhibited no effects on seizure and mortality induced by electroshock (Table IX).

Effects on cardiovascular and respiratory system

SB1 administered intravenously at 5, 10 and 20 ml/kg, showed no effects on blood pressure and heart rate in rats. With respect to the respiration, PEG-hemoglobin SB1 had no influence in guinea pigs (Fig. 2, Table X).

Effects on isolated hearts

As shown in Fig. 3, SB1 at the concentration ranging from 10^{-8}~10^{-5} mol/L showed no influence on LVP, LVDP, HR, DP, LVEDP and CFR in rat hearts.

Effects on isolated smooth muscles

SB1 at concentration ranging from $1\times10^{-7}\sim1\times10^{-5}$ mol/L had no effect on the contractile response of isolated guinea pig ileums to acetylcholine (5×10^{-7} M), histamine (2×10^{-6} M) and $BaCl_2$ (2×10^{-3} M) (Table XI).

Effects on gastrointestinal transport

SB1 had no influence on the charcoal transport in mice at the doses 5, 10 and 20 ml/kg (Fig. 4)

Effects on the gastric secretion

SB1 did not show the antisecretory action at doses ranging

Table X. Effects of PEG-hemoglobin SB1 on respiration rate in guinea pigs

Drugs	Dose (ml/kg)	Respiration (rate/min)					Tidal volume (ml)				
		0	15	30	60	120	0	15	30	60	120
Vehicle	20	105.2±5.9	117.0±16.9	102.3±16.6	103.9±8.9	98.8±9.4	2.9±0.2	2.5±0.2	2.9±0.4	2.8±0.4	2.9±0.2
PEG-	5	102.7±4.2	99.6±5.5	98.9±3.3	101.8±4.5	96.3±1.6	2.4±0.7	2.1±0.3	2.6±0.6	2.2±0.4	2.2±0.3
hemo-	10	106.7±7.3	114.4±11.1	107.0±4.8	111.8±8.0	102.5±4.6	2.5±0.5	2.1±0.5	2.0±0.2	2.5±0.3	2.2±0.2
globinSB1	20	109.3±9.1	112.2±11.4	99.2±6.2	99.1±4.9	96.8±6.6	2.8±0.7	2.1±0.5	2.4±0.2	2.3±0.6	2.4±0.4

Each value represents the mean±S.D.

Table XI. Effects of PEG-hemoglobin SB1 on isolated guinea pig ileum

Drugs	Log [M]	Contractile responses (% of pre-drug response)			
		Alone	ACh.	His.	$BaCl_2$
		Mean	Mean	Mean	Mean
Vehicle	0	0.00±0.0	146.5±17.3	148.3±22.1	124.8±16.9
PEG-hemo- globin SB1	-7	0.00±0.0	152.3±10.8	138.6±29.2	133.9±16.5
	-6	0.00±0.0	156.4±11.9	129.1±21.8	134.2±8.2
	-5	0.00±0.0	161.5±14.4	132.9±21.9	131.5±20.5
No. of animals		8	8	8	8

Each value represents the mean±S.D.

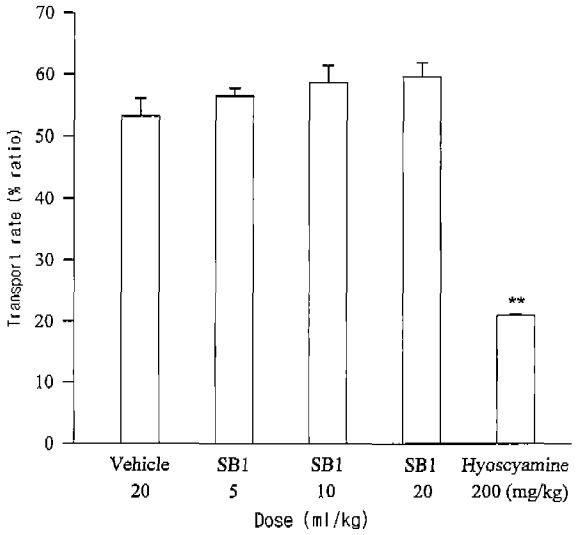

Fig. 4. Effects of PEG-hemoglobin SB1 on intestinal transport in mice. Each column indicates the mean and S.E.M.(n=8). Significant difference from control group(**; p<0.01).

to 5~20 ml/kg (Table XIII).

Effects on the renal function

Intravenous administeration of SB1, showed no effects on urinary volume, pH and excretion of electrolytes, Na, K and Cl (Table XIII).

The purpose of the present study was to examine the pharmacologic properties of high dosage of SB1 in an attempt to gain some insight into the potential side effects on the cardiovascular, central nervous and the other organ systems, resulting from the secondary pharmacologic activity of high doses of the agent.

At the doses approximately 2 times the anticipated clinical dose of SB1, central nervous system, cardiovascular system, gasrtointestinal system and renal function were generally normal. Based upon these studies, SB1 appears to possess no general pharmacologic activity in animals.

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Table XII. Effects of PEG-hemoglobin SB1 on gastric secretion in rats

Drugs	Dose (ml/kg)	Route	No. of animal	Volume(ml)	pH	Total acidity (μEq)
Vehicle	20	i.v.	5	11.9±1.9	1.44±0.24	1087.6±111.7
PEG-hemo-	5	i.v.	5	10.7±0.7	1.10±0.07	893.1±87.8
globin SB1	10	i.v.	5	10.5±1.1	1.24±0.17	929.2±145.0
	20	i.v.	5	12.5±1.1	1.12±0.04	1209.2±136.6
Atropine	1 mg/kg	p.o.	5	5.8±1.2**	1.20±0.00	587.9±149.2**

Each value represents the mean±S.D.
Significant difference from control group (**; p<0.01).

Table XIII. Effects of PEG-hemoglobin SB1 on renal function

Drugs	Dose (ml/kg)	Route	pH	Urine volume(ml)	Na^+(mmol/l)	Cl^- (mmol/l)	K^+(mmol/l)
Vehicle	20	i.v.	7.0±0.0	2.8±0.3	80.7±3.2	105.3±8.1	44.3±9.6
PEG-hemo-	5	i.v.	7.0±0.0	2.3±0.5	54.7±10.1	71.0±3.6	37.0±13.1
globin SB1	10	i.v.	7.0±0.0	2.7±0.3	64.7±28.1	84.0±32.2	28.0±2.6
	20	i.v.	7.0±0.0	3.9±1.2	55.3±31.1	88.3±12.7	30.3±10.2
Furosemide	1 mg/kg	p.o.	7.5±0.0	6.0±1.8**	133.0±4.0*	61.7±22.2*	30.3±6.5

Each value represents the mean±S.D.
Significant difference from control group (*; p<0.05, **; p<0.01).

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