TOXICITY OF POLYOXYETHYLENE HYDROGENATED CASTOR OIL 60 (HCO-60) IN EXPERIMENTAL ANIMALS

Akihiko HISATOMI, Mamoru KIMURA, Masashi MAEDA
Masahiro MATSUMOTO, Kaname OHARA and Hideyo NOGUCHI

*Toxicology Research Laboratories,
Fujisawa Pharmaceutical Co., Ltd.
1-6, 2-Chome, Kashima, Yodogawa-ku,
Osaka 532, Japan*

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Abstract — HCO-60, a polyoxyethylene castor oil derivative, is used as a solubilizer in the injectable formulations of lipophilic agents.

This study was performed to examine the toxicity of HCO-60 in various experimental animals including dogs, monkeys, rabbits, guinea pigs and rats. With 1.25 or 2.5 mg/kg of HCO-60 injected i.v. to dogs, blood pressure decreased, flush, swelling and itching appeared after injection, and with 10 mg/kg of HCO-60 there was additionally a decrease of spontaneous motility. In the two higher dose groups, these symptoms paralleled an increase of histamine levels. Since degranulation was observed after injection in the mast cells of the skin, but not in the liver of dogs, the histamine in the plasma was considered to be released from the mast cells of the skin. Pretreatment with diphenhydramine, a H₁-receptor antagonist, suppressed the decrease of blood pressure induced by HCO-60. These findings show that the toxicity of HCO-60 is associated with histamine release from the mast cells. No symptoms occurred in monkeys, rabbits, guinea pigs or rats with 50 or 100 mg/kg of i.v. of HCO-60, and there was no change in plasma histamine levels. This study demonstrated that the toxicity of HCO-60 is species specific to dogs among the animals tested.

Key words : polyoxyethylene hydrogenated castor oil 60, experimental animals, anaphylaxis-like symptom, blood pressure, plasma histamine, mast cell

INTRODUCTION

Polyoxyethylene hydrogenated castor oil 60 (HCO-60), a polyoxyethylene castor oil derivative, has excellent solubilizing capacity and has been used as a solubilizer in some lipophilic agent formulations (Ichibagase et al., 1990).

Nevertheless, side effects such as anaphylaxis-like symptoms have been reported in a few patients injected with drugs formulated with HCO-60. In animals, the symptom has been

observed only in dogs (Sugi et al., 1971). This study was performed to confirm that the toxicity of HCO-60 is species specific, and to examine what chemical mediator is involved in this toxicity in dogs.

MATERIALS AND METHODS

1. Animals

Male and female beagle dogs (Ichiyanagi Farm) weighing 7.8 to 13.1 kg, male and female cynomolgus monkeys (Japan Laboratory Animals

Inc.) weighing 2.9 to 4.4 kg, male New Zealand White strain rabbits (Kitayama Labes Ltd.) weighing 3.2 to 3.7 kg, male Hartley strain guinea pigs (Japan SLC Inc.) weighing 310 to 543 g and male Sprague-Dawley rats (Japan CLEA Inc.) weighing 296 to 340 g were used after acclimation for one week or longer. The animals were allowed free access to standard food for each species and tap water. Room temperature and relative humidity were 23 ± 2 °C and 55 ± 10 %, respectively. The room was lighted daily for 12 hours.

2. Test substance

As a test substance, HCO-60 (polyoxyethylene hydrogenated castor oil 60: Nikko Chemicals Co., Ltd) was dissolved in physiological saline. Antihistaminics, diphenhydramine-HCl (Katayama Chemicals Co., Ltd) and cimetidine (Fujisawa Pharmaceutical Co., Ltd.), were dissolved in physiological saline and 0.1N-HCl, respectively.

3. Experiments

1) Single i.v. injection

a) Dogs

0.625, 1.25, 2.5 or 10 mg/kg of HCO-60 was injected to dogs in groups of 3 through the cephalic vein in a volume of 5 ml/kg and at a speed of 10 ml/min. Blood pressure was measured with a continuous pressure transducer (BP-203NP, Nihon Colin Co., Ltd.) before and 10, 30 and 60 min after injection, and was expressed as mean blood pressure (Diastolic + (Systolic - Diastolic) / 3). At the same times, blood was taken from the cephalic vein and used to obtain the plasma by centrifugation for histamine assay (McBride et al., 1988) by radioimmunoassay (RIA) kit (Eiken chemical Co., Ltd). Clinical signs were observed until 60 min after injection.

b) Monkeys, rabbits, guinea pigs and rats

Groups of 3 monkeys, 5 rabbits, 5 guinea pigs and 5 rats were used. 50 mg/5 ml/kg or 100 mg/5 ml/kg of HCO-60 was injected i.v. through the cephalic vein in monkeys or ear vein in rabbits, respectively and guinea pigs were given 10 or 100 mg/5 ml/kg i.v. through the paw vein. Rats were given 10 or 100 mg/5 ml/kg of HCO-60 i.v. through the tail vein. Blood was taken for measuring plasma histamine levels before and 10, 30 and 60 min after injection.

Clinical signs were observed until 60 min after injection.

2) Histopathological observation on mast cells in the liver and skin of dog injected with HCO-60

10 mg/5 ml/kg of HCO-60 was injected i.v. to 3 dogs, and the animal was bled to death under anesthesia with thiopental sodium. The liver and skin (ear, abdomen and lip) were removed and immediately fixed in Carnoy's fluid and sections were made, stained with toluidine blue (Enerback et al., 1986) and eosin, and examined by light microscopy. The liver and skin of 3 untreated dogs were examined in the same way. Representative photos are shown in this paper.

3) Action of HCO-60 on dog blood cells in vitro

To examine whether HCO-60 induces the release of histamine from dog blood cells, blood was incubated with 0.01, 0.1 or 1 mg/ml of HCO-60 at 37°C for 15 min. Reaction was stopped by cooling with ice and the supernatant was removed by centrifugation at 3,000 r.p.m. for 10 min. Histamine levels were measured by the RIA kit.

4) Effect of antihistaminics on HCO-60-induced decrease of blood pressure in dogs

5 mg/ml/kg of diphenhydramine or cimetidine was injected i.v. through the cephalic vein of dogs, and immediately afterward, 10 mg/5 ml/kg of HCO-60 was injected in the same way at a speed of 10 ml/min. Measurement of blood pressure, blood sampling and observation of clinical signs were performed as in 3-1) -a).

RESULTS

1. Toxicity by HCO-60 in experimental animals

a) Dogs

Flush and swelling were observed immediately to 60 min after an i.v. injection of 10 mg/kg of HCO-60, and itching and decrease of spontaneous motility appeared 10 min after injection (Table 1). Blood pressure decreased immediately after injection of HCO-60. The values at 10 and 30 min were 67.7 and 65.6 % of those before the injection, respectively. Whereas, plasma histamine level elevated rapidly and showed 561 ng/ml at 10 min, and thereafter, it dropped to 42.7 ng/ml at 60 min with recovery of blood pressure (Table 2).

When 0.625 to 10 mg/kg of HCO-60 was

Table 1. Clinical signs after an i.v. injection of HCO-60.

Species	Dose (mg/kg)	Sign
Dog	0.625	None
	1.25	Flush, Swelling, Itching
	2.5	Flush, Swelling, Itching
	10	Flush, Swelling, Itching Decreased spontaneous motility
Monkey	50	None
Rabbit	100	None
Guinea pig	10	None
	100	None
Rat	10	None
	100	None

Clinical signs were observed until 60 min after injection.

Table 2. Plasma histamine and blood pressure after an i.v. injection of HCO-60.

Dose (mg/kg)	Animal No.	Plasma histamine (ng/ml)				Mean blood pressure (mmHg)			
		Pre	10'	30'	60'	Pre	10'	30'	60'
10	1	0.32	550	484	110	99.7	72.7	56.7	61.7
	2	0.29	341	216	13.8	98.7	62.0	62.0	83.7
	3	0.12	792	132	4.29	90.0	60.7	70.3	75.0
	Mean	0.24	561	277	42.7	96.1	65.1	63.0	73.5
	± S. E.	0.062	130.3	106.1	33.76	3.08	3.80	3.96	6.40

injected i.v. to dogs, dose-dependent flush, swelling, itching, and changes in blood pressure and plasma histamine levels occurred in the animals dosed with 1.25 mg/kg or more (Table 1 and Fig. 1).

b) Monkeys, rabbits, guinea pigs and rats

Large doses of HCO-60 were given to monkeys, rabbits, guinea pigs and rats, but neither anaphylactic signs nor change in plasma histamine level was found in any species (Table 1 and Fig. 2).

2. Histopathological observation of mast cells

Mast cells in the liver and skin of dogs injected i.v. with 10 mg/kg of HCO-60 were examined histopathologically. Mast cells in the livers were normal and were similar to those in the untreated dogs (Photo 1), whereas those in

the skin showed degranulation (Photo 2). This suggests that HCO-60 acts on the mast cells of the skin but not of the liver, and in this way induces histamine release from only these cells.

3. Action of HCO-60 on dog blood cells in vitro

Blood taken from dogs treated without HCO-60 was incubated with 0.01, 0.1 or 1 mg/ml of HCO-60. There was no histamine release from the blood cells including the platelets (Table 3).

4. Effect of antihistaminics on HCO-60-induced decrease of blood pressure in dogs

Pretreatment of dogs with 5 mg/kg diphenhydramine improved itching and decreases in spontaneous motility and blood pressure following an injection of HCO-60 (Fig. 3), but not flush and swelling.

Cimetidine 5 mg/kg had no effect on these changes (Fig. 4), but a combination of diphenhydramine and cimetidine further ameliorated the symptoms, as did diphenhydramine only (Fig. 5).

Pretreatment with diphenhydramine, including a combination of diphenhydramine and cimetidine, also tended to suppress the increase in plasma histamine level induced by HCO-60, but cimetidine did not have this effect (Fig. 3, 4, 5).

Fig. 1 Changes of blood pressure and plasma histamine levels after an i.v. injection of HCO-60 in dogs. Blood pressure and plasma histamine levels were measured at 10 min after injection of HCO-60. : Values are expressed as the mean $\pm$ S. E. (n=3)

Fig. 2 Plasma histamine levels in standard laboratory animals treated with HCO-60. : Values are expressed as the mean of 3 monkeys, 5 rabbits, 5 guinea pigs, 5 rats and 3 dogs.

A) Control

B) HCO-60 treatment

Photo 1. Histopathological findings of liver of dog dosed i.v. with 10 mg/kg of HCO-60. : Arrows show mast cells. Toluidine blue and eosin stain, $\times 750$

A) Control

B) HCO-60 treatment

Photo 2. Histopathological findings of skin of dog dosed i.v. with 10 mg/kg of HCO-60. : Arrows show mast cells. Toluidine blue and eosin stain, $\times 750$

Table 3. HCO-60-induced histamine release from blood cells of dogs.

HCO-60 conc. (mg/ml)	Histamine conc. in supernatant (ng/ml ; Mean $\pm$ S.E., n=5)
0	0.59 $\pm$ 0.077
0.01	0.63 $\pm$ 0.081
0.1	0.61 $\pm$ 0.098
1	0.58 $\pm$ 0.082

Histamine levels in blood were 1.80 to 12.9 ng/ml.

Fig. 3 Effect of anti-histaminics on changes of blood pressure and plasma histamine levels in dogs treated with HCO-60. Ten mg/kg of HCO-60 was injected i.v. to dogs treated with 5 mg/kg of diphenhydramine (DH). : Values are expressed as the mean $\pm$ S. E. (n=3)

Fig. 4 Effect of anti-histaminics on changes of blood pressure and plasma histamine levels in dogs treated with HCO-60. Ten mg/kg of HCO-60 was injected i.v. to dogs treated with 5 mg/kg of cimetidine (CM). : Values are expressed as the mean $\pm$ S. E. (n=3)

Fig. 5 Effect of anti-histaminics on changes of blood pressure and plasma histamine levels in dogs treated with HCO-60. Ten mg/kg of HCO-60 was injected i.v. to dogs treated with 5 mg/kg of diphenhydramine (DH) and 5 mg/kg of cimetidine (CM). : Values are expressed as the mean $\pm$ S. E. (n=3)

DISCUSSION

Although polyoxyethylene castor oil derivatives such as HCO-60 and Cremophor EL are used as solubilizers in some injectable formulations, they have induced such adverse effects as anaphylactic symptoms in a few patients (Ichiki et al., 1992; Howrie et al., 1985). Some studies about these effects have been performed in experimental animals and have demonstrated an association with elevated histamine for Cremophor EL (Lorenz et al., 1977; Ennis et al., 1986), but the mechanism has not been clarified.

In the present study, we examined the toxicity of HCO-60, which is one of several polyoxyethylene castor oil derivatives, in standard laboratory animal species. Only the dogs showed toxic signs, and these were primarily associated with an elevation of plasma histamine. This suggests a species specific toxicity which is mediated by histamine. The mast cells are primarily the cells which store histamine throughout the body. As their population is variable, this determines the histamine levels in various tissues (Metcalf et al., 1981; Irani et al., 1989). In the present study, HCO-60 induced degranulation of mast cells in the skin but not in the liver, and did

not induce histamine release from blood cells including platelets. Accordingly, HCO-60 would act on the mast cells in the skin and there induce histamine release. The morphology of the mast cells in the skin and liver is generally similar, but those in the skin are more sensitive to degranulation. Diphenhydramine, a H_1 -receptor antagonist, alleviated the decrease of blood pressure and some other toxic effects of HCO-60, but cimetidine, which is a H_2 -receptor antagonist, had no effect. This suggests that H_1 -receptor is the site of mediation of HCO-60 toxicity.

Diphenhydramine is a classic H_1 -antagonist, and has been shown to inhibit antigen-induced histamine release in human leukocytes (Lichtenstein et al., 1975) and compound 48/80 stimulated histamine release in rat mast cells (Mota et al., 1960). In the present study, diphenhydramine partially inhibited HCO-60-induced histamine release, but cimetidine did not. Accordingly, diphenhydramine may act not only on H_1 -receptor in various organs, but also on the histamine release mechanisms in the mast cells.

In clinical use of drugs containing HCO-60, anaphylaxis-like symptoms such as flush and decrease of blood pressure occur with an incidence of about 0.1 %. Although these adverse reac-

tions were more common in studies with dogs, HCO-60 was reported to elicit skin reaction in healthy subjects given Pransnitz-Küstner test using sera from a patient who had anaphylactic shock by drug containing HCO-60 and also positive prick test (Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, 1986). This seems to suggest that HCO-60 has antigenicity, but in other patients with anaphylaxis the prick test was negative against HCO-60.

Considerably earlier (1973), Werner reported that Cremophor EL which is similar in structure to HCO-60 induced this allergic skin reaction in human subjects on first exposure to this solubilizer (Werner et al., 1973). Therefore, the anaphylaxis-like response in the above cited case was not necessarily immunologic. In dogs, anaphylaxis-like signs occurred on first exposure to HCO-60 and accordingly would be derived from "Pharmacological" histamine release, not an immunological source.

In summary, dogs were far more sensitive than monkeys, rabbits, guinea pigs and rats to the toxicity of HCO-60. In dogs, HCO-60 mainly elevated plasma histamine which in turn manifested as anaphylaxis-like symptoms mediated by H_1 -receptor.

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