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ORIGINAL ARTICLE

Cutaneous anaphylactoid reaction to polyoxyethylene hydrogenated castor oil in dogs

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Abstract

Background: Polyoxyethylene hydrogenated castor oil (HCO ethoxylates) is a nonionic surfactant used as an excipient for ointments and injections in human and veterinary drugs. Several polyethylene glycol (PEG) derivatives can be obtained depending on the number of moles of ethylene oxide (EO). HCO ethoxylates have the potential to cause anaphylactoid reactions. There is little published information about these types of reactions in dogs.

Objective: To determine the potential for HCO-ethoxylate-containing drugs to cause anaphylactoid reactions in dogs, employing intradermal testing (IDT) with various concentrations of HCO ethoxylates (HCO-25, -40, -60 and -80).

Animals: Four healthy male laboratory dogs.

Materials and Methods: We performed IDT with drugs containing HCO ethoxylates and HCO ethoxylates alone to determine threshold concentrations. The IDT scores and threshold concentrations were compared. Analysis of skin biopsies from IDT sites was used to measure the percentage of degranulated mast cells. The effect of histamine at IDT sites was investigated by pre-treatment with an antihistamine.

Results: All HCO-ethoxylate-containing drugs caused a wheal-and-flare reaction. The threshold concentrations (0.001% and 0.00001%) of each HCO-ethoxylate depended on the number of moles of EO ($p<0.05$). Mast cell degranulation was enhanced by all HCO ethoxylates. The HCO-60-induced reaction was suppressed by an oral antihistamine.

Conclusions and Clinical Relevance: The threshold concentration can serve as a consideration for developing safe new drug formulations and for clinical decision-making around using drugs containing PEG derivatives. IDT is useful to predict the risk of adverse effects. Antihistamines could demonstrate a prophylactic effect.

KEYWORDS
adverse reaction, degranulation, ethoxylation, ethylene oxide, intradermal test, mast cell, PEGylation, polyethylene glycol

Yukina Sugiyama and Shinpei Kawarai should be considered joint first author.

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INTRODUCTION

Polyoxyethylene hydrogenated castor oil (HCO ethoxylates) is a nonionic surfactant used as an excipient, solubiliser and emulsifier in ointments and injectable drug formulations.[1–3] Its derivatives are obtained by reacting varying amounts of ethylene oxide (EO; C_2H_4O) with hydrogenated castor oil (HCO). This chemical process, generally called ethoxylation, or PEGylation in pharmaceuticals, attaches the strands of the polymer polyethylene glycol [$H(OC_2H_4)_nOH$] to glycerides or fatty acids – in the above case, HCO.[1–3]

Polyethylene glycol (PEG) and its derivatives are abbreviated in many ways. Polyethylene oxide (PEO) and polyoxyethylene (POE) are chemically synonymous with PEG, and these names may be used depending on the molecular weight.[1] For the sake of abbreviation, ethoxylated derivatives can be referred to as PEG, followed by a number and then combining names of glycerides or fatty acids. The number assigned represents the average number of moles of PEG that react with HCO; for example, PEG-60 HCO is prepared when 60 moles of EO are made to react with 1 mole of HCO.[1–3] The same can be applied to PEG itself, while in pharmaceutical applications, the number denotes the rounded, average molecular weight (MW; g/mol) of a given PEG compound [e.g. PEG-3350 (g/mol) is the same as PEG-75 (75 moles of EO)].[2,3] Regarding HCO ethoxylates, the numerical moles may be following the HCO without the prefix PEG, for instance, HCO-60.[1–3]

Although PEG compounds and derivatives are considered safe cosmetic ingredients and their medical utilisation is increasing, anaphylactic or anaphylactoid reactions to PEG-containing drugs have been reported in humans.[3–5] The COVID-19 prevention vaccine BNT162b2 was reported to produce an anaphylactic reaction, possibly due to a PEG compound (ALC-0159)[6] present in the vaccine formulation. To diagnose allergic reactions to PEG, skin-prick testing and intradermal testing (IDT) with the lowest, risk-avoiding concentrations of PEG, patch testing, basophil activation testing and oral provocation testing are valuable tools.[3–6]

Since the 1970s to the 1980s, in anaesthetic drug developments, solubilisers of PEG-35 castor oil (Cremophor El), HCO-40 (Cremophor RH40) and PEG-20 sorbitan monooleate (Polysorbate 80) have been known to induce histamine release in dogs.[7–10] The chemical modification, such as oxethylation (or ethoxylation) and hydrogenation, of certain fatty acids has been shown to potentiate hypotension or plasma histamine release in dogs.[7–9] In dogs, acute toxicity studies showed that HCO-60 induced blood histamine release and skin mast cell degranulation.[11] This anaphylactoid reaction was specifically observed in beagle dogs, and not in monkeys, rabbits, guinea pigs or rats.[11] Drugs containing PEG derivatives carry the risk of anaphylactoid reaction in dogs and cats;[10,12–14] however, little is known about the safe amount of HCO ethoxylates in drugs containing PEG to avoid anaphylactoid reactions. The use of PEG in drugs used for people is increasing; therefore, the risk of anaphylactoid reaction may increase if dogs are administered human medications containing PEG derivatives.

The current study was inspired by an episode in a patient in the author's practice (SK, Fukushima prefecture). In this case, a dog with atopic dermatitis developed erythema and swelling after treatment with a commercial ear drop, containing triamcinolone acetonide and antimicrobials. In an attempt to confirm a hypersensitivity reaction to the product, the drop was injected intradermally. Because an anaphylactoid reaction to an excipient or other ingredient was suspected, an injectable steroid formulation also was injected intradermally for comparison. Both resulted in wheal formation at the site of injection. This phenomenon motivated the authors to design a study in which intradermal injection of PEG derivatives would be used to evaluate the potential of these compounds to induce anaphylactoid reactions.

This study was conducted to obtain basic clinical information regarding HCO-ethoxylate-related anaphylactoid reactions in dogs.

MATERIALS AND METHODS

Ethics

This research was approved by the animal ethics committee at the institute (140407–1) and was performed in accordance with the ARRIVE guidelines.

Animals

Four healthy male dogs (two beagles, dogs 1 and 2; two Shiba Inu, dogs 3 and 4) with a mean age of 7.5 years (range 6–9 years) and mean weight of 11.1 kg (range 8.2–13.5 kg) were used. These dogs were kept under suitable conditions with safe, clean and appropriate spaces and regulated feeding and temperature at the facility of laboratory animals at the institute. All tests were completed in these four dogs, with a test interval of at least 1 month.

Test compounds

The following drugs containing PEG derivatives (product/brand name, supplier) were used: ciclosporin injection (Sandimmune; Novartis Pharma), vitamin injection (Multamin; AY pharmaceuticals), tacrolimus injection (Prograf; Astellas Pharma, Inc.), oral ciclosporin capsule (Neoral; Novartis Pharma), pirenoxine eye drop (Kary Uni; Santen Pharmaceutical), ketoconazole ointment (Nizoral cream; Janssen Pharmaceutical) and betamethasone ointment (Rinderon-VG lotion; Shionogi Pharma).

The HCO ethoxylates used were HCO-25, -40 and -60 from Kao Chemicals and HCO-80 from Nikko Chemicals.

Excipients were selected based on their inclusion in common drug formulations: PEG-8 stearate (Macrogol

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400; Yoshida Pharmaceutical), benzyl alcohol (Kanto Chemical), citric acid (Fujifilm Wako Chemical), sodium dihydrogen phosphate (Nacalai Tesque), silicone resin (KM-72; Shin-Etsu Chemical), methyl parahydroxybenzoate and propyl parahydroxybenzoate (Shiseido Seiyaku). Histamine (Sigma Aldrich) and sterile saline injection (Otsuka normal saline; Otsuka Pharmaceutical) were used as positive and negative controls, respectively.

Sterile 23-gauge needles were used to aspirate compounds from capsules and bottles. Before aspiration from capsules, the compound was collected on one side by centrifuging the capsules. The paste reagents were melted at 50°C for 1 h in a water bath. Sterile saline was used to dilute all compounds. The compounds were mixed well by vortexing.

Sedation

A mixture of medetomidine (Dorbene vet; Kyoritsu Seiyaku) 0.02 mg/kg and midazolam (Dormicum; Astellas Pharma Inc.) 0.3 mg/kg was administered intramuscularly to the dogs for sedation before IDT and skin biopsy collection.[15] After the test procedure, sedation was reversed using an injection of atipamezole (Atipame, Kyoritsu seiyaku) 0.3 mg/kg i.m.

Intradermal test

Each dog underwent a total of six IDTs: one with the excipients at one concentration each, one with the drugs at two concentrations each, one with HCO ethoxylates at nine concentrations each, one IDT with HCO ethoxylates at two concentrations each followed by skin biopsy collection, one with HCO-60 after 1 week of hydroxyzine administration, and finally, one with HCO-60 28 days after cessation of hydroxyzine.

For each IDT a 15 × 6 cm area at the dorsolateral thorax was clipped of fur and marked for injection sites at 1.5 cm intervals. The test compound (50 μL) was injected intradermally. All of the drugs were diluted to at least 1:100 from the original concentration for IDT because a false positive could be caused by an irritant, for example glycerin.[16,17] This dilution factor was determined according to the literature in human medicine.[16,18,19] The test compounds were serially diluted 1:10 in saline. The concentrations of drugs containing PEG derivatives were 1×10^{-2} v/v (1%) and 1×10^{-3} v/v (0.1%); the concentrations of HCO ethoxylates were 1×10^{-2} v/v (1%) to 1×10^{-10} v/v (0.00000001%); and positive control histamine was 1×10^{-5} w/v (0.001%) to 1×10^{-7} w/v (0.00001%). The concentrations of excipients were determined from concentrations provided by the manufacturer of the injectable steroid product used in the clinical patient mentioned in the introduction (data not shown). The excipient concentrations were diluted an additional 1:100, matching the test concentration of the drugs. The final concentration, 1×10^{-3} v/v (0.1%) to 1×10^{-6} v/v (0.001%) were used. At 15 min postinjection, wheals were evaluated based on reports published previously.[20,21] The maximum diameter (*D* in mm) was recorded and erythema (E) was assessed subjectively on a three-point scale: 1 = no erythema, 2 = weak erythema and 3 = intense erythema. The IDT score was obtained by the following formula: score = $D \times E$. The examination of *D* and *E* was performed in a blinded manner (one person preparing test compounds and injecting, and another evaluating erythema and wheal diameters after the injection).

Skin biopsy

An 8 mm biopsy punch (Kai Industry) was used to obtain biopsies from IDT sites for HCO ethoxylates and saline (negative control). Tissue samples were fixed in 10% formalin, paraffin-embedded, cut into 5 μm thick sections and stained with toluidine blue (pH 4.1; Muto Pure Chemicals).

Measurement of mast cell degranulation

Skin mast cell degranulation at the IDT site was measured by histopathological examination using toluidine blue staining (pH 4.1). The percentage of mast cell degranulation was calculated as the number of degranulated mast cells with a reduced density of cytoplasmic granules and fuzzy cell membranes. A total of 100 mast cells in the superficial dermis were counted from ≤50 fields at high power field (×400). Cells in the sections from each dog were counted by three examiners in a blinded manner.

Antihistamine administration

We compared the wheal-and-flare skin reaction for HCO-60 (1×10^{-2}, 1×10^{-3} and 1×10^{-4} v/v) and histamine (1×10^{-5}, 1×10^{-6} and 1×10^{-7} w/v) as a positive control and saline as a negative control between day (D)7 after antihistamine administration (hydroxyzine pamoate, Atarax; Pfizer Japan) and at D28 after cessation of the drug. Among HCO ethoxylates, we selected HCO-60 because it is the most frequently used in the examined PEG-containing human drugs. The dosage of 2.2 mg/kg body weight was administered orally three times daily at 08:00, 13:00 and 18:00 h for 7 days. At the end of D7 at 10:00 h, 2 h after the final administration, IDT was performed. The time of performing IDT was determined according to a past report.[20]

Statistical methods

In order to compare the IDT results and mast cell degranulation percentage, the Friedman test was applied as a nonparametric two-way ANOVA with related groups such as dogs, drugs, excipients and HCO ethoxylates.[22,23] When a significant difference was observed in each group, post hoc Bonferroni–Dunn multiple comparison analysis was performed.[24] The

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dose-dependencies, compared with the saline, were analysed using the Shirley–Williams' multiple comparison test.[22,25] To examine the suppression effect of antihistamine, a one-sided Wilcoxon rank-sum test was used for the paired samples.[22] Data are presented as average ± standard deviation (SD). All of the statistical analyses were performed using STATCEL software (Yanai H. Statcel–the Useful Addin Forms on Excel. 4th ed. Tokyo, Japan; OMS Publishing) in EXCEL (Microsoft). Statistical significance was set at $p<0.05$.

RESULTS

Intradermal test for excipients and excipients of PEG derivatives in drugs

The active ingredients and excipients in the drugs examined are listed in Table S1 in the Supporting information. The results compared to the IDT scores are shown in Table S2. In the IDT score, significant differences were observed within excipient groups (Friedman test, $p<0.05$), and both histamine (1×10^{-5} w/v) and HCO-60 (1×10^{-5} v/v) were different from all of the other excipients (Bonferroni–Dunn test, all $p<0.01$).

Intradermal test for drugs containing PEG derivatives

Wheal-and-flare reactions were identified at both concentrations [1×10^{-2} v/v (1%) and 1×10^{-3} v/v (0.1%)] of all drugs containing PEG derivatives using IDT (Figure 1). Significant differences in IDT score were observed within both dog and drug groups (Friedman test, $p<0.05$ and $p<0.01$, respectively); however, the differences were calculated only by comparing the drugs with each other via post hoc analysis (Table 1). All drugs (1×10^{-2} and 1×10^{-3} v/v) differed from histamine (1×10^{-5} w/v; all $p<0.01$), while betamethasone ointment (1×10^{-2} v/v; $p<0.01$), vitamin injection (1×10^{-3} v/v; $p<0.05$), ciclosporin injection (1×10^{-2} v/v; $p<0.01$) and histamine (1×10^{-5} and 1×10^{-6} w/v; both $p<0.01$) were different from the saline. Two differences were observed between drugs: pirenoxine eye drop (1×10^{-3} v/v) versus betamethasone ointment (1×10^{-2} v/v) and versus ciclosporin injection (1×10^{-2} v/v) ($p<0.01$ and <0.05, respectively; Table 1).

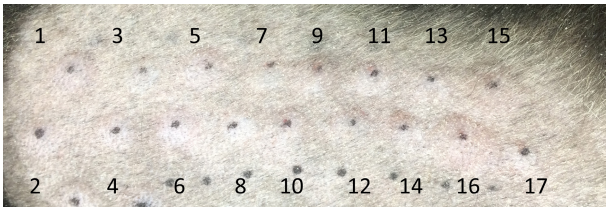


FIGURE 1 Intradermal test with drugs containing polyethylene glycol (PEG)-derivatives showing distinct wheal-and-flare reactions in dogs. 1 and 2, ciclosporin capsule; 3 and 4, ketoconazole cream; 5 and 6, tacrolimus injection; 7 and 8, pirenoxine eye drop; 9 and 10, betamethasone lotions; 11 and 12, vitamin injection; 13 and 14, ciclosporin injection; 15 and 16, histamine; and 17, saline. Concentrations: 1×10^{-3} v/v is represented by 1, 3, 5, 7, 9, 11 and 13; 1×10^{-2} v/v by 2, 4, 6, 8, 10, 12 and 14; 1×10^{-5} v/v by 15; and 1×10^{-6} v/v by 16. The results were obtained from four dogs and summarised as intradermal test (IDT) scores in Table 1.

Threshold concentrations for IDT with HCO ethoxylates

For HCO-25 and HCO-40, statistical significance as the lowest dose level, compared to saline, stood at $p<0.01$ for concentrations exceeding 1×10^{-5} v/v. In HCO-60, the statistical significance was calculated at $p<0.05$ and $p<0.01$ for concentrations of 1×10^{-6} v/v and $>1\times10^{-5}$ v/v, respectively. Among all HCO ethoxylates, HCO-80 showed the lowest threshold concentration at 1×10^{-7} v/v with $p<0.01$ (Figure 2).

Skin mast cell degranulation at the site of IDT with HCO ethoxylates

The results compared to the percentage of mast cell degranulation are shown in Figure 3 and Table S3. Significant differences within dog ($p<0.05$) and HCO-ethoxylate ($p<0.05$) groups were detected by Friedman test. A post hoc analysis confirmed significant differences only within the HCO-ethoxylate group, particularly between the saline and HCO ethoxylates of HCO-25 at the concentration of 1×10^{-2} v/v ($p<0.05$), HCO-40 at 1×10^{-2} v/v ($p<0.01$) and 1×10^{-3} v/v ($p<0.01$), HCO-60 at 1×10^{-2} v/v ($p<0.01$), and HCO-80 at 1×10^{-2} v/v ($p<0.01$) and 1×10^{-3} v/v ($p<0.01$; Figure 3 and Table S3).

Suppressive effect of oral antihistamines on skin reactions to HCO-60

Results shown in Figures 4 and 5, and Table S4, demonstrate a statistically significant difference ($p<0.05$) between the post-antihistamine and post-cessation IDT scores for histamine at the concentrations of 1×10^{-5} w/v, 1×10^{-6} w/v and 1×10^{-7} w/v and HCO-60 at 1×10^{-4} v/v.

DISCUSSION

We examined the potential for drugs containing PEG derivatives to cause anaphylactoid skin reactions in dogs. Using IDT, we found that all drugs examined produced a wheal-and-flare reaction (Figure 1). The excipients in the drugs differed from each other, and the drugs were diluted 1:100 by saline to eliminate possible reaction to the excipients. HCO ethoxylates induced anaphylactoid reactions, whose potential was confirmed by the threshold concentration. Because polyoxyethylene hydrogenated castor oils are utilised as nonionic solubilisers in the formulations of lipophilic drugs, including steroid hormones, antibiotics, immunosuppressants and vitamins,[4,7,9,14] adverse

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TABLE 1 Results of intradermal testing (IDT) for drugs containing polyethylene glycol (PEG)-derivatives.

Numbers[a]	Active ingredient[b]	Concentration[c]	PEG derivatives (concentration in the drug)[c]	IDT score[d] (mean ± SD)	$p<0.05$*	$p<0.01$*
1	Ciclosporin	1×10^{-2} v/v	HCO-40	25.0±8.3		15
2		1×10^{-3} v/v		22.3±6.4		15
3	Ketoconazole	1×10^{-2} v/v	HCO-60, PEG-20 Cetyl Ether	20.8±7.4		15
4		1×10^{-3} v/v		21.3±6.8		15
5	Tacrolimus	1×10^{-2} v/v	HCO-60 (80 mg/0.4 mL)	22.0±8.4		15
6		1×10^{-3} v/v		24.5±7.0		15
7	Pirenoxine	1×10^{-2} v/v	HCO-60	17.3±4.8		15
8		1×10^{-3} v/v		9.3±3.1	13	9,15
9	Betamethasone, Gentamicin	1×10^{-2} v/v	HCO-60, PEG-8 stearate/ Macrogol ester	35.3±10.0		8,15.17
10		1×10^{-3} v/v		24.3±6.8		15
11	Vitamins	1×10^{-2} v/v	HCO-60 (50 mg/5 mL), Polyoxyethylene (160) polyoxypropylene (30) glycol (10 mg/5 mL)	24.3±7.1		15
12		1×10^{-3} v/v		29.0±9.2	17	15
13	Ciclosporin	1×10^{-2} v/v	PEG-35 castor oil (3.25 g/5 mL)	32.0±10.7	8	15,17
14		1×10^{-3} v/v		19.0±2.2		15
15	Histamine	1×10^{-5} w/v		67.0±8.2		All of 1 to 14,16,17
16		1×10^{-6} w/v		38.0±4.7		8,15,17
17	Saline	1 v/v		5.8±1.5	12	9,13,15,16

[a]Same numbers are applied in Figure 1. The numbers indicates following drugs (application, brand name, supplier): 1 and 2, oral use (capsule), Neoral, Novartis Pharma Japan; 3 and 4, ointment (cream), Nizoral cream, Janssen Pharmaceutical; 5 and 6, injection, Prograf, Astellas Pharma, Inc; 7 and 8, ointment (eye drop), Kary Uni, Santen Pharmaceutical; 9 and 10, ointment (lotion), Rinderon-VG lotion, Shionogi Pharma; 11 and 12, injection, Multamin, AY pharmaceuticals; 13 and 14, injection, Sandimmune, Novartis Pharma Japan; 14 and 15, positive control, Histamine, Sigma Aldrich; and 16 and 17, negative control, Otsuka normal saline.

[b]Lists of active ingredient and excipients are shown in Table S1. The concentration, provided from the drug information are shown.

[c]The dilution factors are determined according to the literature in human medicine [18–20].

[d]The mean ± SD IDT score, obtained to multiply a diameter of wheals by erythematous score are calculated from the results of IDT in four dogs.

*The statistically significant results ($p<0.05$ and 0.01), obtained by post hoc analysis of Bonferroni–Dunn test after the significance ($p<0.05$) of Friedman test are shown. The figures in the column are same as the figures shown in the column of numbers; the drugs shown by the numbers are significantly different against the drugs in the rows.

effects from HCO ethoxylates should be considered when anaphylactoid reactions are encountered with these drug formulations.

We examined not only injections, but also capsules, eye drops and ointments. The PEG-derivative-containing drugs for injection, especially those containing high concentrations, such as Prograf and Sandimmune (Table 1), should be prescribed for careful use because adverse reactions have been reported with drugs containing the same PEG derivatives or the drug itself.[10,12-14] Although it is used as an infusion fluid (50 mg HCO-60 dissolved at 250–500 mL) in small animal practices in Japan, Multamin elicited skin reactions in the present study (Table 1). It has been described that male beagle dogs (n = 5) did not show any clinical signs of abnormalities after receiving total parenteral nutrition with Multamin-dissolved infusion solution, with concentration 50 mg HCO-60 dissolved at 5 mL, 0.1 mL/kg for 40 days (including a vitamin washout period).[26] Therefore, the anaphylactoid reaction may be relatively tolerable at low and long-lasting doses. Intradermal testing is conducive to recognising the risk for anaphylactoid reactions in cases that require the use of drugs containing PEG derivatives, as well as with preventive IDT for concerns of L-asparaginase in humans (LEUNASE Injection; Kyowa Hakko Kirin).[27]

For the oral route, a study found that HCO-40 is safe for dogs at concentrations of 1.0%, 2.5% and 5.0% because no abnormalities were shown during 6 months of administration in physiological, haematological and biochemical parameters and histopathological analysis.[1,2] For the cutaneous route, we performed patch testing directly using HCO-60 in two dogs; however, no erythematous changes were observed 24 and 48 h after the challenge (data not shown). The oral formulation, eye drops and ointment are considered relatively safe because these human formulations are often used in dogs.[28] If anaphylactoid reactions are experienced after use on erosive and ulcerative lesions with impaired skin barrier function, HCO-ethoxylate reactions might be considered.

The results in the present study indicate that the potency of anaphylactoid skin reaction appears molar amount-dependent. It has been reported that the oxethylation (or ethoxylation) of PEG-35 castor oil (Cremophor El)-derivatives enhanced the plasma histamine release response in dogs.[7,8] Our results corresponded to past reports,[7,8] indicating that the number of moles of EO reacting with HCO could be important for anaphylactoid reactions in dogs. In the present study, PEG-8 stearate (1×10^{-3} v/v) did not elicit anaphylactoid reactions (Table S2). Chemically, PEG stearates (synonyms: macrogol stearates) are PEG mono- and diester polymers

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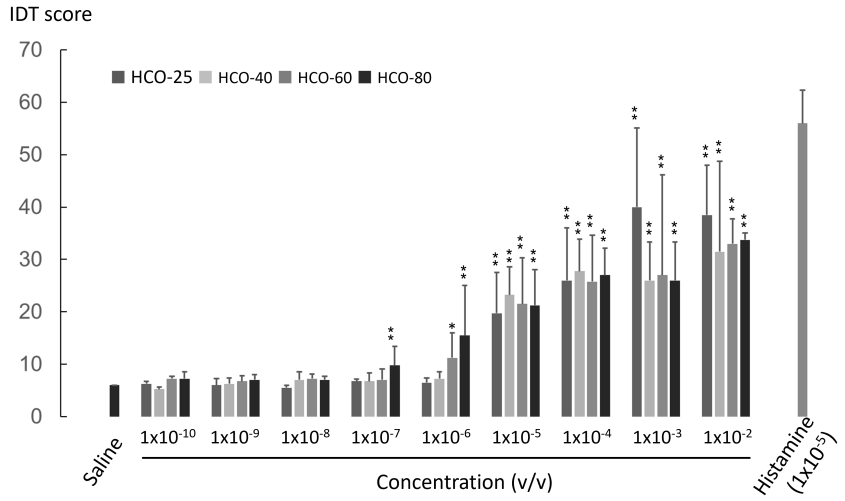


FIGURE 2 Intradermal test results for serially diluted polyoxyethylene hydrogenated castor oil (HCO ethoxylates). The intradermal test (IDT) score was obtained by multiplying the diameter of the wheal by the erythema score (1 to 4) is shown. Each bar represents the mean±SD of four dogs. IDT scores were measured in a blinded manner. Statistically significant results compared to saline: *, $p<0.05$; **, $p<0.01$).

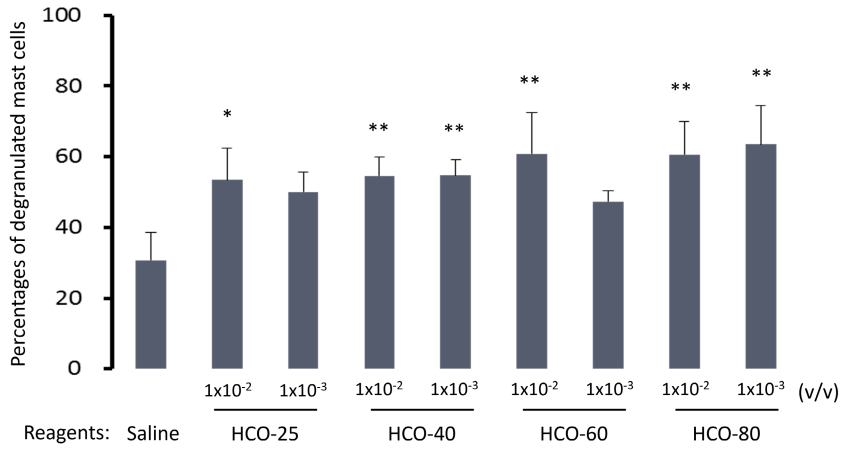


FIGURE 3 Mast cell degranulation induced by polyoxyethylene hydrogenated castor oil (HCO ethoxylates). Percentages of degranulated mast cells were calculated for each dog, blindly counted by three examiners. Each bar represents the mean±SD of four dogs. Statistically significant results compared to saline: *, $p<0.05$; **, $p<0.01$).

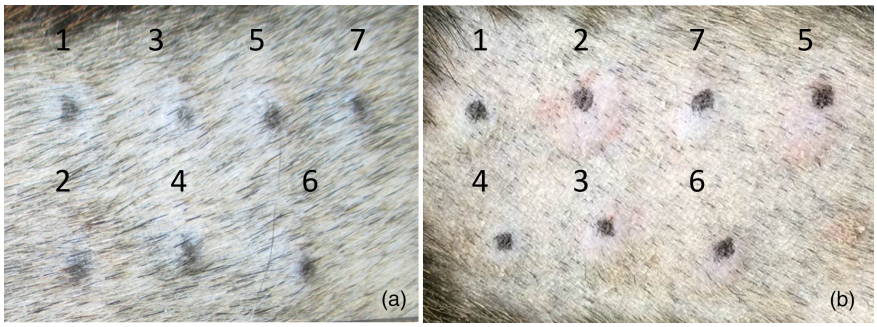


FIGURE 4 Results of intradermal testing (IDT) for hydrogenated castor oil ethoxylate (HCO)-60 with or without oral antihistamine administration. Hydroxyzine was administered at 2.2 mg/kg body weight orally three times daily, and IDT was performed (a) 2 h after the final administration on Day (D)7 and (b) on D28 (21 days after cessation). 1, saline; 2, histamine 1×10^{-5} w/v; 3, histamine 1×10^{-6} w/v; 4, histamine 1×10^{-7} w/v; 5, HCO-60 1×10^{-2} v/v, 6, HCO-60 1×10^{-3} v/v, 7, HCO-60 1×10^{-4} v/v.

of stearic acids, while polyoxyethylene hydrogenated castor oil is ethoxylated triglycerides.[1] It has been reported that nonionic PE6800 (copolymer of PEG and polypropylene glycol with approximately 40% EO) of a non-fatty acid base did not release plasma histamine in dogs.[8] Therefore, specific chemical features, not only ethoxylation but also glyceride structure, are responsible for the anaphylactoid reactions caused by PEG derivatives in dogs.[8]

We observed the suppressive effect of antihistamines on HCO-60-induced anaphylactoid skin reactions. An increase in the blood histamine concentration

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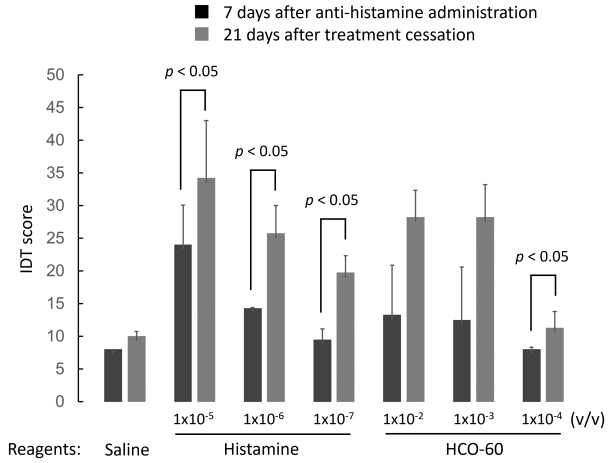


FIGURE 5 Suppression effects of wheal-and-flare reactions to hydrogenated castor oil ethoxylate (HCO)-60 by oral antihistamines. Changes in the intradermal test (IDT) score, obtained by multiplying the diameter of the wheal by the erythema score (1 to 4) are shown. Each bar represents the mean±SD of four dogs. Hydroxyzine was administered at 2.2 mg/kg body weight orally three times daily, and IDT was performed 2 h after the final administration. One-sided Wilcoxon rank-sum test was used for the analysis of paired samples. Statistical significance: *, $p<0.05$.

and hypotension has been reported after intravenous injection of HCO ethoxylates to dogs.[7–9,11] Moreover, hypotension was prevented by intravenous diphenhydramine administration before the HCO-60 intravenous administration.[11] These findings suggest that prior oral antihistamine treatment effectively prevents anaphylactoid reactions caused by HCO injection in dogs.

The direct effect on mast cells has been reported in vitro and complement activation has been suspected,[3,9] yet the mechanisms through which HCO ethoxylates induce histamine release and the receptor involved by PEG derivatives during mast cell degranulation remain unclear. Compound 48/80 is a potent chemical inducer of mast cell degranulation, and it has been shown to relay the signalling leading to mast cell degranulation via binding to mas-related G protein-coupled-receptor X2 (MRGPRX2) in humans or MRGPRB2 in mice.[29,30] It has been reported that in a unique nonresponsive phenotype of compound 48/80 in beagle dogs, compound 48/80 did not induce anaphylactoid skin reaction as per IDT, while HCO-60 did.[31] The significant difference in IDT score and percentage of mast cell degranulation within the dog groups in the present study indicate there is the possibility that individual differences in the response of HCO-ethoxylate could be present. Individual differences might be one reason why we experienced the uncommon anaphylactoid reaction to an ear drop.

CONCLUSION

We showed that HCO ethoxylates and the drugs containing PEG derivatives-induced wheal-and-flare skin reactions in dogs. The determined threshold concentration can serve for understanding anaphylactoid reactions induced by drugs containing PEG derivatives. Intradermal tests are useful to predict the risk of effects. Antihistamines could demonstrate a prophylactic effect. Further research is required to reveal the exact mechanism by which HCO ethoxylates activate mast cells, identify chemical features with potential for anaphylactoid reactions and provide new strategies for the prevention of canine anaphylactoid reactions caused by PEG derivatives.

AUTHOR CONTRIBUTIONS

Yukina Sugiyama: Writing – original draft; data curation; visualization. **Shinpei Kawarai:** Conceptualization; methodology; data curation; writing – review and editing; writing – original draft; funding acquisition; project administration; formal analysis; investigation; validation; visualization; resources; software. **Shinichi Ansai:** Supervision; validation. **Pradeep Bist:** Supervision; writing – review and editing; validation. **Soman N. Abraham:** Validation; supervision. **Takuya Maruo:** Supervision; validation; writing – review and editing; project administration; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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SUPPORTING INFORMATION

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