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Flush induced by fluoroquinolones in canine skin

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Abstract. The flush induced by two fluoroquinolone antibacterial agents, balofloxacin and ofloxacin, was studied in beagle dogs. Intradermal injection of the fluoroquinolones at concentrations above 10^{-5} M produced a localized flushed area. The flush responses to fluoroquinolones were inhibited by co-administration with H_2-antagonist(s) (ranitidine or cimetidine), but not with H_1-antagonist(s) (mepyramine or chlorpheniramine). Similar inhibitory effects of these H_2-antagonists were observed for the response to histamine. The flush responses to fluoroquinolones were inhibited by a local pretreatment with compound 48/80 administered to deplete the local stores of mast cell-bound histamine. When the fluoroquinolones were orally administered at a dose of 400 mg/kg, the concentration of histamine in plasma was increased, being accompanied by systemic erythema. These results indicate that the flush induced by fluoroquinolones is mediated by histamine release from canine cutaneous mast cells and H_2-receptor stimulation.

Key words: Quinolone – Flush – Redness – Dog – Histamine

Introduction

Fluoroquinolone antibacterial agents have an extended antimicrobial spectrum and are used widely in clinical practice. However, they occasionally show adverse-effects to a low degree [1]. One of the known side effects is a skin reaction including photosensitivity [1–3].

In a preliminary study, we observed erythema of systemic skin in dogs orally treated with fluoroquinolones. Takasuna et al. [4] have shown an increase in plasma histamine in response to the oral administration of levofloxacin, an active isomer of ofloxacin. These findings led us to suggest that the erythema evoked by fluoroquinolones results from plasma histamine elevation. However, Ito et al. [5] recently reported that ofloxacin and ciprofloxacin caused vasodilation in canine vessels through an antagonistic action at α1-adrenoreceptors, suggesting another possible mechanism. Therefore, it is still unclear why fluoroquinolones induce skin reactions in dogs.

The present investigation was undertaken to study the mechanism of skin reaction caused by fluoroquinolones. We investigated the flush induced by intradermal injection of fluoroquinolones, using beagle dogs. Two fluoroquinolones – ofloxacin and balofloxacin – were employed in the present study. The former agent has been well investigated in relation to skin reactions, especially photosensitivity in mice [6, 7]. The latter drug was developed recently and has a chemical structure which is not associated with photosensitivity [8, 9].

Materials and methods

Chemicals

Balofloxacin was synthesized at the Exploratory Research Laboratories of Chugai Pharmaceutical Co., Ltd. Ofloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan) was extracted and purified from marketed tablets. Histamine dihydrochloride was purchased from Wako Pure Chemical Co. (Osaka, Japan). Compound 48/80 and ranitidine hydrochloride were from Sigma (St. Louis, MO, USA). Chlorpheniramine maleate, mepyramine maleate and dimaprit dihydrochloride were from Funakosi (Tokyo, Japan). Cimetidine (Tagamet®) was from Fujisawa Pharmaceutical Co. (Tokyo, Japan). These compounds were dissolved in Tyrode solution (NaCl 8 g, KCl 0.2 g, $MgCl_2 \cdot 6H_2O$ 0.214 g, NaH_2PO_4 0.05 g, $NaHCO_3$ 1 g, $CaCl_2$ 0.2 g/1000 ml H_2O).

Animals

Adult beagle dogs of both sexes were used. They were housed individually in cages with controlled room temperature (23 ± 2 °C), relative humidity (55 ± 10%), illumination (5 a.m. ~ 7 p.m.) and fresh air changes (14 ~ 16 times/hour). The dogs were fed with dry dog food (CD-5, Clea, Tokyo, Japan). Tap water was offered ad libitum. All animals were clinically healthy and acclimatized to the environment prior to experimentation.

Localized flush in canine skin

Anesthesia in dogs was induced intravenously with thiopental

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25 mg/kg (Ravonal®, Tanabeseiyaku Co., Osaka, Japan), and maintained with the inhalation of isofluran (Forane®, Abbot, Tokyo, Japan). In certain experiments, conscious dogs were used; we didn't find any differences in the flush responses with or without anesthesia. The agents were injected intradermally into the thoracic and abdominal skin in a volume of 50 µl with 27G×"1/2" gauge needles (0.4 mm diameter). In the case of co-administration, the agents were premixed in a syringe before injection. The area of flush was measured according to Foreman et al. [10]. Flush responses were scored as following: 0, normal; 1 very slight; 2, moderate; 3, severe. All experiments were carried out under commercial fluorescent lighting.

Depletion of mast cell histamine

Depletion of local histamine stores bound to cutaneous mast cells was performed according to the method of Hägermark et al. [11] and modified. In this study, compound 48/80 (10 µg/ml) was repeatedly injected into the same thoracic sites and abdominal skin until the flush response was no longer observed (four times on two consecutive days).

Cutaneous blood flow

The change in canine cutaneous blood flow was measured by means of a laser flowmeter (ALF21, Advance, Tokyo, Japan). A probe (S-Type, Advance) was attached to the skin at the point where the agents were injected, and cutaneous blood flow was monitored.

Determination of plasma histamine level

Blood from dogs treated orally with fluoroquinolones was collected from their cephalic vein into heparinized tubes. Plasma was separated by centrifugation at approximately 2000×g for 10 min at 4 °C. Concentration of histamine in the plasma was analyzed by the method of fluorometry coupled with a high-performance liquid chromatography system which consisted of a pump (Model 510, Waters, Milford, USA) with a column (Inertsil ODS-2, GL Sciences, Tokyo, Japan). The mobile phase, containing 500 ml of 5% H_3PO_4, 520 ml of CH_3CN and 5 g SDS, was pumped at a flow rate of 0.8 ml/min. A fluorescence detector (RF-535, Shimazu Co., Kyoto, Japan) was operated at an excitation wavelength of 355 nm and an emission wavelength of 430 nm.

Statistical analysis

Analysis of flush scores was done using the Wilcoxon test.

Results

Flush induced by fluoroquinolones

As shown in Table 1, intradermal injection of balofloxacin or ofloxacin produced a localized flushed area at doses of 10^{-5} M and higher. The flush appeared within 5 or 10 min after injection, and then gradually subsided for the subsequent 30 min of observation. Tyrode solution alone did not produce any responses.

The data on flush area (Table 1) parallelled those on flush score (data not shown). The following experiments were therefore carried out on the basis of flush score.

Inhibitory effects of histamine antagonists

Table 2 demonstrates the inhibitory effects of histamine antagonists on the flush responses. The flush responses to balofloxacin was inhibited by co-administration with ranitidine and cimetidine (H_2-receptor antagonists), but not with mepyramine and chlorpheniramine (H_1-receptor antagonists). The flush-evoked by ofloxacin was also blocked by ranitidine. Similar inhibitory effects of these histamine antagonists were observed for the response to histamine.

As expected, dimaprit which is a known H_2-receptor agonist was as potent as histamine in causing flush. Compound 48/80 (mast-cell histamine liberator) elicited flush, and the flush response was almost completely abolished by ranitidine.

The flush response in the skin pretreated with compound 48/80

After local histamine depletion with the histamine liberator compound 48/80, flush response could not be evoked by the injection of fluoroquinolones, whereas the histamine-induced flush was similar at the histamine-depleted and the control sites (Fig. 1).

Cutaneous blood flow measured by the laser flowmeter

The changes in cutaneous blood flow measured by the laser flowmeter is shown in Figure 2. The intradermal injections of both fluoroquinolones (10^{-4} M) increased cutaneous blood flow at the injected sites in a similar manner to histamine (10^{-5} M). Tyrode solution alone caused no change in cutaneous blood flow.

Oral administration of fluoroquinolones to beagle dogs

Figure 3 shows the increase in plasma histamine and concomitantly observed erythema (redness) of the skin when the fluoroquinolones were administered orally at a dose of 400 mg/kg. Two of the four dogs treated with balofloxacin showed erythema in systemic skin, especially on the facial and abdominal skin. Such erythema was also seen in all dogs treated with ofloxacin. The fluoroquinolone-induced erythema was accompanied by plasma histamine elevation.

Discussion

It is assumed that the flush induced by histamine is predominantly an H_1-receptor effect in human skin [10, 12]. In the present study using beagle dogs, the flush response to histamine was inhibited by H_2-receptor antagonists, but not by H_1-receptor antagonists. These data suggest that the flush response to histamine in canine skin is mediated by H_2-receptors. Actually, dimaprit (H_2-receptor agonist) also evoked a localized flushed area equiactive to histamine. The involvement of H_2-receptors

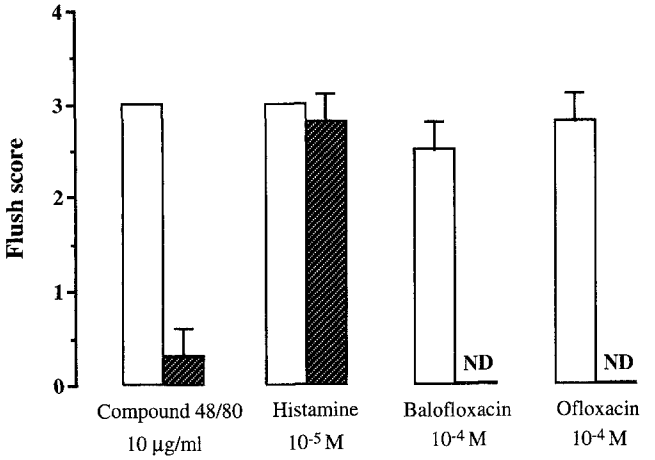

Fig. 1. Flush responses of compound 48/80, histamine, balofloxacin and ofloxacin in normal skin (open columns) and compound 48/80 pretreated skin (hatched columns). ND = not detected. Each value represents the mean with S.E. of 4 beagle dogs.

in this flush response is also corroborated by previous investigators [13–16], who demonstrated that H_2-receptors contribute to the vasodilating action of histamine in the canine vessel.

As expected, intradermally administered compound 48/80 (mast-cell histamine liberator) evoked flush at the injection site and this response was almost completely inhibited by ranitidine. Therefore, histamine is conceivably the major chemical mediator released from canine cutaneous mast cells.

Like histamine and compound 48/80, the two fluoroquinolones elicited flush at the injection sites. This flush was accompanied by increases in cutaneous blood flow. This indicates that the fluoroquinolone-induced flush is related to vasodilation, and confirms our optical observations.

H_2-receptor antagonists inhibited flush responses to fluoroquinolones, suggesting that this receptor played an important role in the reaction. Pretreatment with compound 48/80 to deplete the local stores of mast cells-bound histamine also inhibited the flush to fluoroquinolones. In addition, the oral administration of fluoroquinolones (400 mg/kg, p.o), though a high dose,

Table 2. Effect of histamine antagonists on the flush induced by intradermal injections of fluoroquinolones.

Agents	Flush score
Balofloxacin 10^{-4} M	2.3 ± 0.3
Balofloxacin 10^{-4} M + Mepyramine 10^{-5} M	2.0 ± 0.0
Histamine 10^{-5} M	3.0 ± 0.0
Histamine 10^{-5} M + Mepyramine 10^{-5} M	3.0 ± 0.0
Mepyramine 10^{-5} M	0.0 ± 0.0
Balofloxacin 10^{-4} M	3.0 ± 0.0
Balofloxacin 10^{-4} M + Chlorpheniramine 10^{-5} M	2.8 ± 0.3
Histamine 10^{-5} M	3.0 ± 0.0
Histamine 10^{-5} M + Chlorpheniramine 10^{-5} M	2.8 ± 0.3
Chlorpheniramine 10^{-5} M	0.0 ± 0.0
Balofloxacin 10^{-4} M	2.8 ± 0.3
Balofloxacin 10^{-4} M + Ranitidine 10^{-5} M	0.0 ± 0.0*
Histamine 10^{-5} M	2.8 ± 0.3
Histamine 10^{-5} M + Ranitidine 10^{-5} M	0.0 ± 0.0#
Ranitidine 10^{-5} M	0.0 ± 0.0
Balofloxacin 10^{-4} M	3.0 ± 0.0
Balofloxacin 10^{-4} M + Cimetidine 10^{-5} M	0.0 ± 0.0**
Histamine 10^{-5} M	3.0 ± 0.0
Histamine 10^{-5} M + Cimetidine 10^{-5} M	0.3 ± 0.3#
Cimetidine 10^{-5} M	0.0 ± 0.0
Ofloxacin 10^{-5} M	3.0 ± 0.0
Ofloxacin 10^{-5} M + Ranitidine 10^{-5} M	0.0 ± 0.0**
Dimaprit 10^{-5} M	2.8 ± 0.3
Compound 48/80 10 μg/ml	2.8 ± 0.3
Compound 48/80 10 μg/ml + Ranitidine 10^{-5} M	0.3 ± 0.3#

Flush was scored 5 min after injection. The agents were premixed in the syringe before injection and injected simultaneously in a volume of 50 μl. Each value represents the mean ± S.E. of 4 beagle dogs. *$p < 0.05$, **$p < 0.01$, significantly different from balofloxacin or ofloxacin #$p < 0.05$, from histamine or compound 48/80 (Wilcoxon test).

Table 1. Localized flush area (mm^2) in the canine skin intradermally injected with balofloxacin and ofloxacin.

Agents	Concentration	Time after injection (min) 5	10	15	20	25	30
Balofloxacin	3×10^{-4} M	31.2 ± 15.1	35.0 ± 15.3	36.5 ± 16.4	24.3 ± 12.0	18.3 ± 11.0	5.3 ± 5.3
	10^{-4} M	22.3 ± 11.1	31.7 ± 13.5	28.3 ± 11.8	13.1 ± 6.9	11.8 ± 7.5	6.0 ± 6.0
	3×10^{-5} M	0	0	0	0	0	0
	10^{-5} M	0	3.4 ± 3.4	3.7 ± 3.7	5.0 ± 5.0	3.5 ± 3.5	4.4 ± 4.4
	3×10^{-6} M	0	0	0	0	0	0
	10^{-6} M	0	0	0	0	0	0
Ofloxacin	3×10^{-4} M	51.5 ± 14.5	57.6 ± 12.4	54.5 ± 20.4	36.8 ± 17.5	30.5 ± 17.7	29.9 ± 17.4
	10^{-4} M	45.2 ± 12.2	47.5 ± 11.8	38.4 ± 12.8	26.1 ± 12.0	19.0 ± 12.4	18.6 ± 11.9
	3×10^{-5} M	24.7 ± 8.4	25.6 ± 10.4	10.4 ± 7.2	6.4 ± 6.4	6.7 ± 6.7	9.4 ± 9.4
	10^{-5} M	0	2.3 ± 2.3	4.1 ± 4.1	2.9 ± 2.9	3.8 ± 3.8	3.9 ± 3.9
	3×10^{-6} M	0	0	0	0	0	0
	10^{-6} M	0	0	0	0	0	0
Tyrode	———	0	0	0	0	0	0

Each value represents the mean ± S.E. of 4 beagle dogs, in which the flush was observed.

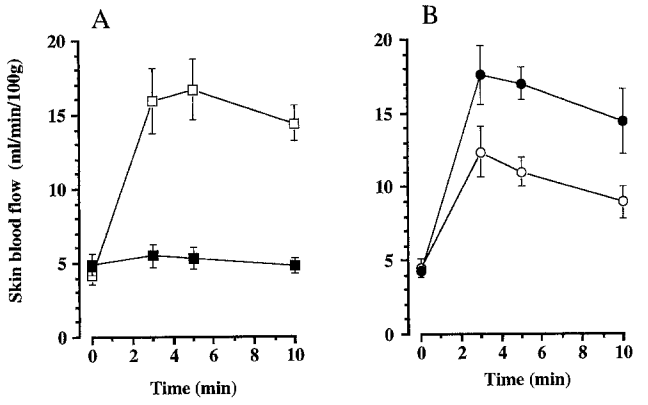

Fig. 2. Time-course of the skin blood-flow measured by laser flowmeter. (A) shows the blood flow changes after intradermal injections of histamine 10^{-5} M (open squares) and Tyrode (closed squares), and (B) shows the blood flow changes after intradermal injections of balofloxacin 10^{-4} M (open circles) and ofloxacin 10^{-4} M (closed circles). Each value represents the mean ± S.E. of 4 beagle dogs.

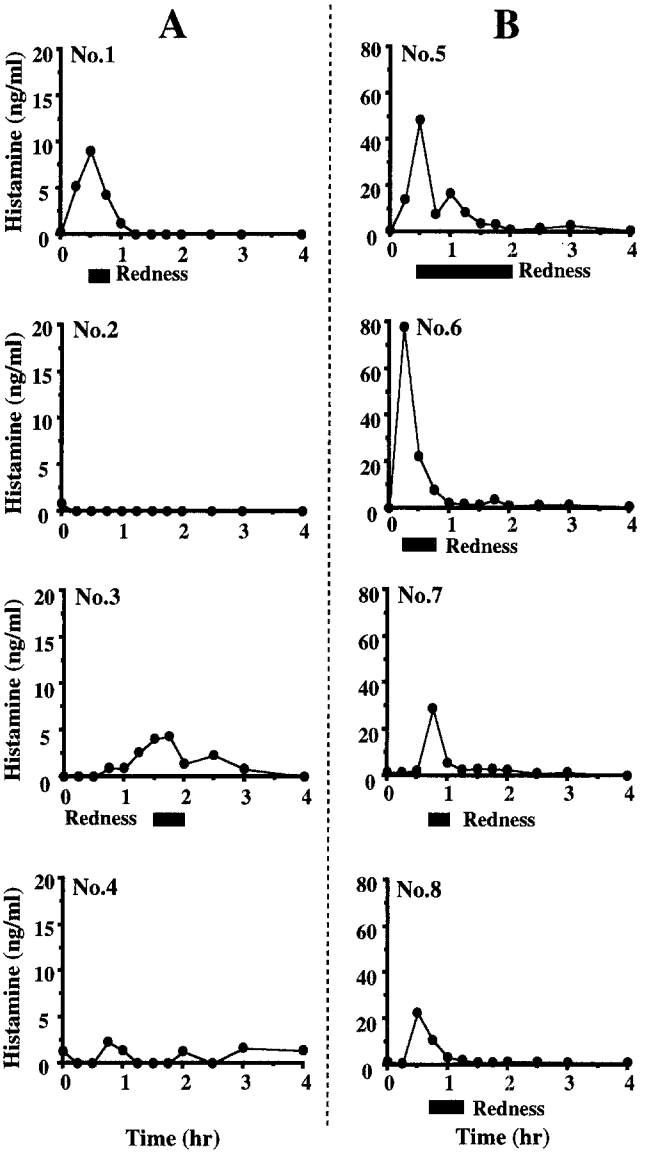

Fig. 3. Changes in plasma histamine levels and appearance of erythema (redness) after oral administration of fluoroquinolones to eight beagle dogs. Eight beagle dogs were randomly divided into two groups, and balofloxacin and ofloxacin at doses of 400 mg/kg were administered to the groups (A) and (B), respectively. Erythema was observed in systemic skin, especially on facial and abdominal skin.

well above that used clinically (approximately 5-10 mg/kg, p.o), caused increases in plasma histamine concentration and systemic redness, which was considered to be erythema. These findings suggest that the fluoroquinolones can release histamine from canine cutaneous mast cells and produce flush through actions at the H_2-receptor.

These data thus implicate a contribution of histamine to the side-effects of fluoroquinolones and probably rule out a direct action of fluoroquinolones on blood vessels. A recent report by Ito et al. [5], however, has shown that ofloxacin and ciprofloxacin possess antagonistic actions on α1-adrenoceptor in the smooth muscle from canine mesenteric artery. They reported that their potency to cause antagonism was approximately 10^{-5} M, which compares well with our results on canine skin. Therefore, there are other mechanisms by which fluoroquinolone induced vasodilation can occur. One possible explanation for the discrepancy may be the difference in the vessels studied; Ito et al. used the isolated mesenteric artery, while we carried out experiments on skin. Thus, further studies should be carried out to elucidate tissue specific differences.

Wagai and his coworkers [6] have reported that ofloxacin causes erythema associated with photosensitivity under UV irradiation in a murine model. Balofloxacin has a chemical structure which rules out photosensitisation; this agent has a methoxyl substituent at position 8 of the quinolone nucleus, which renders it very stable to UV irradiation [8]. The complete absence of photosensitivity is supported by an in vivo experiment using mice [9]. In the present study, balofloxacin, like ofloxacin, caused a skin reaction, but this was unrelated to photosensitivity.

We used beagle dogs in our study. Dogs are probably an ideal model for in vivo studies not only by virtue of their size but also because of their similarity to man with respect to allergic reactions and histamine release [17, 18]. However, it is necessary to pay attention to their relevance to human responses, since a species-difference was suggested in that the flush of histamine is mainly an H_1-receptor effect in humans, whereas it is an H_2-effect in dogs.

The present data indicate that intradermal administration of fluoroquinolones causes flush through an action at the H_2-receptor. This phenomenon raises a further question as to how fluoroquinolones cause histamine release from mast cells. In general, drug-induced histamine release is caused by the following mechanisms; 1. allergic reaction involving IgE, 2. non-immunologic reaction involving neural components and substance P, 3. direct action of drug on mast cells. It is likely that the induction of histamine release by fluoroquinolones will be clarified soon.

References

[1] Janknegt R. Fluoroquinolones. Adverse reactions during clinical trials and postmarketing surveillance. Pharm Weekbl 1989;11:124–7.

[2] Feist H. Sequential therapy with i.v. and oral ofloxacin in lower respiratory tract infection: A comparative study. Infection 1991;19:S380–3.

[3] Dávila I, Diez ML, Quirce S, Fraj J, De La Hoz B, Lazaro M. Cross-reactivity between quinolones. Report of three cases. Allergy 1993;48:388–90.

[4] Takasuna K, Kasai Y, Usui C, Takahashi M, Hirohashi M, Tamura K, et al. General pharmacology of the new quinolone antibacterial agent levofloxacin. Arzneim Forsch Drug Res 1992;42:408–18.

[5] Ito K, Murakami K, Tamura K. α1-Adrenoceptor-blocking activity of ofloxacin and ciprofloxacin in isolated vascular smooth muscles. Arch Int Pharmacodyn 1993;325:86–95.

[6] Wagai N, Yamaguchi F, Sekiguchi M, Tawara K. Phototoxic potential of quinolone antibacterial agents in Balb/c mice. Toxicol Lett 1990;54:299–308.

[7] Wagai N, Tawara K. Quinolone antibacterial-agent-induced cutaneous phototoxicity; ear swelling reactions in Balb/c mice. Toxicol Lett 1991;58:215–33.

[8] Matsumoto M, Kojima K, Nagano H, Matsubara S, Yokota T. Photostability and biological activity of fluoroquinolones substituted at the 8 position after UV irradiation. Antimicrob Agents Chemother 1992;36:1715–9.

[9] Marutani K, Matsumoto M, Otabe Y, Nagamuta M, Tanaka K, Miyoshi A, et al. Reduced phototoxicity of a fluoroquinolone antibacterial agent with a methoxy group at the 8 position in mice irradiated with long-wavelengh UV light. Antimicrob Agents Chemother 1993;37:2217–23.

[10] Foreman JC, Jordan CC, Oehme P, Renner H. Structure-activity relationships for some substance P-related peptides that cause wheal and flare reactions in human skin. J Physiol 1983;335:449–65.

[11] Hägermark Ö, Hökfelh T, Pernow B. Flare and itch induced by substance P in human skin. J Invest Dermatol 1978;71:233–5.

[12] Owen DAA. Histamine receptors in the cardiovascular system. Gen Pharmacol 1977;8:141–56.

[13] Black JW, Owen DAA, Parsons ME. An analysis of the depressor responses to histamine in the cat and dog: Involvement of both H_1- and H_2-receptors. Br J Pharmacol 1975;54:319–24.

[14] Powell JR, Brody MJ. Identification and specific blockade of two receptors for histamine in the cardiovascular system. J Pharmacol Exp Ther 1976; 196:1–14.

[15] Pawlik W, Tague LL, Tepperman BL, Miller TA, Jacobson ED. Histamine H_1- and H_2-receptor vasodilation of canine intestinal circulation. Am J Physiol 1977;233:E219–24.

[16] Dobbins DE, Swindall BT, Haddy FJ, Dabney JM. Blockade of histamine-mediated increases in microvascular permeability by H_1- and H_2-receptor antagonists. Microvasc Res 1981;21:343–50.

[17] Ennis M, Amon EU and Lorenz W. Histamine release from canine lung and liver mast cells induced by radiographic contrast media. Agents Actions 1989;27:101–3.

[18] DeMora F, García G, Ferrer L and Arboix M. Canine cutaneous mast cells dispersion and histamine secretory characterization. Vet Immunol Immunopathol 1993;39:421–9.