

DIISOPROPYLFLUOROPHOSPHATE-EVOKED INHIBITION OF ANAPHYLACTIC HISTAMINE RELEASE FROM HUMAN SKIN: DECREASE OF THE INHIBITION BY STORING THE SKIN SPECIMENS

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Abstract. Antigen-induced histamine release from human skin slices passively sensitized with reaginic serum *in vitro* was inhibited by DFP, suggesting involvement of serine esterase activation in the reaction. The magnitude of DFP-evoked inhibition of the histamine release was not the same in each skin sample and no correlationship was observed between the magnitude of the DFP-evoked inhibition of the histamine release and that of the histamine release in the absence of DFP. The magnitude of the histamine release in the presence of DFP was smaller in the fresh skin than in the stored skin, whereas that in the absence of DFP was greater in the former than in the latter. The inhibitory effect of cytochalasin B on the anaphylactic histamine release from fresh and stored human skin was the reverse of the effect of DFP. The present results indicate that the magnitude of anaphylactic histamine release from human skin does not match that of the activation of serine esterase in the reaction, suggesting the possibility that there may be some factor modulating the activation of serine esterase in the anaphylactic reaction in human skin.

Key words: Anaphylactic histamine release; Diisopropylfluorophosphate; Cytochalasin B

Diisopropylfluorophosphate (DFP) inhibits antigen-induced histamine release from chopped guinea pig lung (2), rat mast cells (7), chopped human lung (8), human leukocytes (10) and sliced human skin (11). It has been assumed that activation of serine esterase is essential for IgE-mediated histamine release, since DFP, a specific inhibitor of serine esterase (6), inhibits the histamine release when sensitized target cells or tissues are challenged with antigens in the presence of DFP (3). In a previous study, DFP inhibited the antigen-induced histamine release from human skin slices passively sensitized with reaginic serum *in vitro* (11). The magnitude of the inhibition was not identical in each skin sample, even when the experiments were performed under

the same conditions. The inhibition ranged from 0 up to 95% in the presence of 10^{-3} M of DFP and the magnitude of the DFP-evoked inhibition was not dependent upon that of the histamine release in the controls in which the sensitized human skin slices were incubated with antigens in the absence of DFP. These findings suggest that the magnitude of the anaphylactic histamine release from human skin does not always match that of the activation of serine esterase.

As previously reported, human skin specimens stored at 4°C for 24 hr can be sensitized passively with reaginic serum *in vitro* and release histamine upon challenge with antigens as also does fresh skin. However, the magnitude of the histamine release from fresh skin was greater than that from stored skin (12). In the present study, in order to investigate the relationship between activation of serine esterase and histamine release under anaphylactic reaction in human skin, the magnitude of DFP-evoked inhibition of the histamine release from fresh skin was compared with that from stored skin obtained from the same donor.

MATERIALS AND METHODS

Human skin. Apparently healthy human breast skin removed at mastectomy was used. After excision, the subcutaneous fat was removed using surgical scissors. Unless otherwise stated, the skin was stored at 4°C until use.

Reaginic serum. Reaginic serum was obtained from a grass pollen antigen-sensitive patient. The concentration of IgE in the reaginic serum was 950 ng total IgE/ml. The serum was stored at -20°C until use.

Sensitization, histamine release and DFP-evoked inhibition. The antigen-induced histamine release from human skin passively sensitized with reaginic serum was effected by the method described in detail elsewhere (5). The skin was sliced into 500 μ m thick slices using a hand micro-

Table I. *Effect of DFP on the anaphylactic histamine release from fresh and stored human skin in vitro*

Exp. no.	Fresh skin			Stored skin		
	Antigen-induced histamine release (%)	DFP-evoked inhibition (%)		Antigen-induced histamine release (%)	DFP-evoked inhibition (%)	
		10 ⁻⁴ *	10 ⁻³ *		10 ⁻⁴ *	10 ⁻³ *
1	16.9	22.5	58.0	8.4	1.2	6.0
2	31.3	2.2	19.8	22.5	-2.2	-1.1
3	28.8	38.5	62.0	20.4	13.2	26.5
4	26.7	-1.9	9.7	28.4	2.3	-3.0

* Concentrations of DFP (M).

tome. Each skin slice was incubated in 2 ml of reaginic serum diluted with Tyrode solution (1:30), using a shaking water bath. The duration of incubation was 120 min and the temperature was 37°C. After incubation, the sensitized skin slices were washed in Tyrode solution and incubated in triplicate in 4 ml of Tyrode solution containing group B₂ grass pollen antigen (Bencard Ltd., Brentford, Middlesex, England) in the presence or absence of DFP (Sigma Chemical Co., Saint Louis, USA) for 15 min at 37°C, using a shaking water bath. The final concentration of antigen was 100 µg/ml. The reaction was terminated by decantation of the supernatant. The histamine content of the supernatant was determined, in duplicate, by bioassay, using an isolated atropinized guinea pig ileum preparation. Each supernatant was treated with 1 N NaOH and neutralized by the procedure of Perera & Mongar (9) to destroy DFP prior to the bioassay, since DFP in concentrations used in the present experiments interfered with the bioassay. The determination of the residual histamine content of the skin slices was carried out by boiling the postincubation slices in 5 ml Tyrode solution for 5 min in a boiling water bath, followed by bioassay after cooling. The effect of DFP on the histamine release was expressed as percentage inhibition. The concentration of DFP used in the present experiments did not affect the spontaneous histamine release from the skin slices.

RESULTS

The effect of DFP on anaphylactic histamine release from fresh and stored skin was studied by the

following method. A fresh human skin sample obtained at mastectomy was divided into two parts. One fragment was immediately sliced and sensitized with reaginic serum, followed by antigen challenge in the absence of or presence of DFP. The other fragment was stored at 4°C for 24 hr in a small airtight container to prevent drying of the specimen. Sensitization and antigen-induced histamine release in the absence or presence of DFP in the stored skin was carried out under the same experimental conditions as applied to the fresh skin.

The results are summarized in Table I. In three out of four experiments using different skin specimens, the magnitude of antigen-induced histamine release from fresh skin was greater than that from stored skin. The magnitude of inhibition of histamine release effected by 10⁻⁴ or 10⁻³ M DFP was reduced markedly in all experiments by storing the skin specimens at 4°C for 24 hr.

In order to confirm the specificity of the results mentioned above, the following experiment was carried out. As previously reported, cytochalasin B, which is a pharmacologically active fungal metabolite derived from the mould *Helminthosporium dematioides* (1), inhibits anaphylactic histamine release from human skin (13). The inhibitory effect

Table II. *Effect of cytochalasin B on the anaphylactic histamine release from fresh and stored skin in vitro*

Exp. no.	Fresh skin		Stored skin	
	Antigen-induced histamine release (%)	Cytochalasin B-evoked inhibition (%)	Antigen-induced histamine release (%)	Cytochalasin B-evoked inhibition (%)
1	25.3	10.3*	17.9	31.8*
2	27.0	32.2**	17.6	40.3**
3	15.4	0**	18.4	13.6**

Concentration of cytochalasin B: * 2 × 10⁻⁶ M, ** 2 × 10⁻⁵ M.

of cytochalasin B on the histamine release was tested in the fresh and stored skin by the method described in detailed elsewhere (13). The sensitized skin slices were preincubated at 37°C for 90 min in 2 ml of Tyrode solution containing 2×10^{-6} or 2×10^{-5} M of cytochalasin B (Imperial Chemical Industries Ltd., Cheshire, England). The slices were then challenged in the presence of cytochalasin B by adding 2 ml of antigen solution (200 µg/ml in Tyrode solution) and incubation was continued for a further 15 min at 37°C. As shown in Table II, the inhibition of histamine release effected by cytochalasin B in the stored skin was greater than that in the fresh skin in all of three experiments, contrary to the results of DFP experiments.

DISCUSSION

It is now widely accepted that antigen-antibody (IgE) reaction on the target cells activates a cellular enzyme system, probably serine esterase, which is concerned with the release of pharmacologically active substances. The esterase exists in or on the target cells in a DFP-resistant form until activated by antigen-antibody interaction (3).

As previously reported, the antibody involved in the histamine release from passively sensitized human skin *in vitro* is IgE (5), and the histamine release is inhibited by DFP (11). The magnitude of the DFP-evoked inhibition of the IgE-mediated histamine release from human skin was not the same in each skin sample obtained from a different donor under the same experimental conditions and there was no correlation between the magnitude of the inhibition and that of the histamine release in the absence of DFP (11).

In the present studies, the magnitude of DFP-evoked inhibition of histamine release was markedly decreased by storing the skin at 4°C for 24 hr. In all of four experiments, the inhibition was smaller in the stored skin than in the fresh skin under the same experimental conditions, although the histamine release from the former was smaller than that from the latter in the absence of DFP in 3 out of 4 experiments (Table I). It has been reported that as DFP inhibits the anaphylactic histamine release only when present at the time of antigen exposure, it acts on the serine esterase activated by the union of antigen with cell-bound IgE-antibody (3). Therefore, the present findings that the DFP-evoked inhibition of the anaphylactic histamine re-

lease in the stored skin was smaller than that in the fresh skin raises the possibility that the magnitude of antigen-induced serine esterase activation in the target cells may be greater in the stored skin than in the fresh skin. If so, the results indicate that magnitude of IgE-mediated histamine release from human skin does not parallel that of the activation of serine esterase.

Although it is unclear why storage of the skin at 4°C for 24 hr causes decrease of DFP-evoked inhibition of the IgE-mediated histamine release in human skin, there may be some factor modulating the activation of serine esterase in or on the target cells in the skin tissues and such factor may decay when the skin is stored at 4°C for 24 hr. In addition, it cannot be excluded that uptake of DFP by target cells (presumably mast cells) in the stored skin may be smaller than that in the fresh skin, as also that the release of histamine from the mast cells in the former is smaller than that in the latter, since the results reported by Darżynkiewicz & Barnard suggest that DFP must be taken up by mast cells in order to exert its inhibitory effects (4). The mast cells are embedded in the skin tissue and some change of the skin tissue by storage may hinder access of the inhibitor to the mast cells. One can, however, exclude this theory, since cytochalasin B-evoked inhibition of the histamine release in the stored skin was greater than that in the fresh skin (Table II), contrary to the DFP-evoked inhibition. The function of microfilaments, which is inhibited by cytochalasin B, in the target cells to release histamine in the anaphylactic reaction may be impaired by storage of the skin. It would be of great interest to know what factor modulates activation of serine esterase in IgE-mediated anaphylactic reaction in skin. This problem is under investigation in our laboratory.

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