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INHIBITORY EFFECT OF THIOPHOSPHORIC ACID ALKALOID DERIVATIVES FROM *CHELIDONIUM MAJUS* L. (UKRAIN) ON OVALBUMIN ANTIGENICITY AND ANTIOVALBUMIN IgE ANTIBODY RESPONSE IN MICE

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Summary: *The ability of the* Chelidonium majus *L. alkaloid derivative Ukrain (UK) to inhibit ovalbumin-induced sensitization was tested in BALB/c and F1(BALB/c x C57BL/6J) mice. UK introduced into the mice in the mixture with antigen (ovalbumin) and adjuvant (alum) inhibited the sensitization of mice, reflected in lower anti-OA IgE antibody response and decreased antigen-induced histamine release from mast cells isolated from peritoneal cavities of sensitized mice. The effect of UK on the antigenicity of ovalbumin (OA) in anaphylaxis was tested in heterologous passive cutaneous anaphylactic (PCA) reaction on rats. The results show that the OA prepared in the mixture with UK had a decreased ability to react with anti-OA IgE antibodies raised against native OA in mice and fixed on the surface of rat mast cells in heterologous PCA reactions. The results suggest that UK pretreatment of OA may affect its antigenic property and the ability to react with anti-OA IgE antibodies raised against the native IgE molecules.*

Introduction

A semisynthetic compound obtained from alkaloids of *Chelidonium majus L.* and thiophosphoric acid triaziridide is known as immunostimulating and anticancer agent (1-3). In previous studies the authors presented data indicating that Ukrain (UK) is unable to induce the IgE antibody response in mice and IgG as well as IgE antibody responses in guinea pigs after parenteral administration (4). These results suggested that the preparation Ukrain could be therapeutically safe at least as far as its inability to induce anaphylactic sensitization is concerned. In these studies the authors have also observed that in the sera of mice injected several times (at one-week intervals) with the mixture of 1 µg ovalbumin (OA) and 1 mg UK. not only were lacking detectable anti-UK IgE antibody levels, but the levels of anti-OA IgE antibodies were significantly lower as compared to the levels of anti-OA IgE antibodies in the sera of mice injected with OA alone. These observations have suggested that UK injected in the mixture with OA may modify the antigenic property of OA or may modulate an anti-OA IgE antibody response in another way.

In the present paper we further explore the inhi-

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bitory effects of UK on anti-OA IgE antibody response in mice.

Materials and methods

Animals. Six-weeks-old female BALB/c mice and F_1 hybrids of BALB/c and C57BL/6J mice were used for immunizations. Wistar female rats weighing 180-220 g were employed for evaluation of the IgE antibody level in mouse sera.

Immunizations. The mice were injected subcutaneously with 1 μg of OA adsorbed on 1 mg of aluminium hydroxide gel (alum). Injections were given in 0.5 ml of physiological saline and were repeated on days 14 and 42 after the first injection.

In the first part of the study, five groups of mice (every group consisted of 20 mice) were tested. Control mice were injected with OA (1 μg) + alum (1 mg) given subcutaneously as described above (group 1). The mice in the next 3 groups were injected with OA + alum and were treated with UK (0.3 mg/kg) given in the 48-h intervals from the first day of immunization (day 0). UK was given in the volume of 0.3 ml (physiological saline) subcutaneously (group 2), intraperitoneally (group 3) and intravenously (group 4). The mice in the last group were injected subcutaneously with OA + alum mixed with UK (50 mg/kg) given on days 0, 14 and 42 after immunization. In the next part of the study, three groups of F1(BALB/c x C57BL/6J) mice received s.c. injections into the back of OA (1 μg) adsorbed on alum (1 mg). The first (control) group was not treated with UK and the second group was treated with UK (50 mg/kg) given in the mixture with OA and alum.The mice in the last group were injected with the same dose of UK (50 mg/kg), but UK was given separately, on the other side of the back.

Blood samples were taken at 7-day intervals by puncturing the retro-orbital venous plexus. The sera were stored at -20°C until assayed.

IgE antibody assay. The anti-OA IgE antibody levels in mouse sera were titrated by the PCA test in rats (5) using the 24-h latent period for skin sensitization. The PCA reaction was evoked by intravenous challenge with 1 mg OA in 1 ml of 0.4 % Evans' Blue solution. For each rat, control PCA with known standard IgE serum was performed for comparison with PCA titres of the tested sera. The reciprocal of the highest dilution of the serum giving the PCA reaction (blue spot of diameter ≥ 5 mm) tested on at least two rats was taken as the PCA titre. All results were expressed as $\log_2$ PCA titres ± S.E.

Quantitative evaluation of PCA reactions. In order to test whether UK affects the cutaneous anaphylactic reaction, the group of 9 rats was injected intracutaneously with sera containing mouse IgE antibody diluted in physiological saline as specified in the Results. After a 24-h latent period for skin sensitization, the cutaneous anaphylactic reaction was evoked by intravenous injection of 1 mg OA in 1 ml of 0.4% Evans' blue solution. One group of rats was injected intravenously with 3 mg of UK (dissolved in 1 ml of physiological saline) 30 min before antigen challenge. In a second group of rats, UK (3 mg) was injected together with OA and Evans'blue solution (the mixture was prepared 30 min before intravenous injection). A third group of rats was not treated with UK and was injected intravenously with OA and Evans' blue solution only. The rats were killed 30 min after the challenge and the skin of each reaction locus was removed for the determination of extravasated dye. The blueing reaction intensity was determined by dye extraction with KOH as described (6). Briefly, pieces of the skin containing extravasated dye were soaked overnight in stoppered glass tubes containing 1 ml of N KOH at 37 °C. Then, 9 ml of a mixed solution of 0.6 N H_3PO_4 and acetone (5:13) was added to the tubes. The tubes were shaken vigorously for a few seconds and centrifuged at 3000 rpm for 15 min. Absorbance of supernatant was measured spectrophotometrically at 620 nm.

In-vitro *antigen-induced histamine release from mast cells of sensitized mice.* Mast cells were

obtained by simple lavage of peritoneal cavities of mice with buffered salt solution, and tested for OA-induced histamine release as previously described (7). Briefly, several 180-μl samples of cell suspension containing approximately 5 x 10^5 mast cells/ml were incubated in a shaker bath at 37°C for 10 min, challenged with 20 μl of OA in various concentrations (as specified in the Results) and further incubated for 6 min. The reaction was stopped by adding 1.8 ml ice-cold medium, and samples were separated by centrifugation into cell and supernatant fractions. Mast cells were disrupted by addition of distilled water. Both supernatants and cell fractions were assayed for histamine by the fluorescence method of May *et al.* (8).

Histamine release was expressed as percentage of the supernatant histamine to the total histamine after deduction of spontaneous release found in controls.

Results

In the first part of the study, the effect of low doses of UK (0.3 mg/kg) given into BALB/c mice subcutaneously, intraperitoneally, or intravenously in 48-h intervals throughout the entire period of immunization on the anti-OA IgE antibody response was compared with the effect of two high doses (50 mg/kg) of the compound in the mixture with OA and alum given at the times of immunization only (days 0 and 14). The results presented in Fig. 1 show that on-going IgE antibody formation was readily inhibited by UK injected subcutaneously in two high doses (50 mg/kg) in the mixture with OA and alum and given at the time of immunization. Low doses of UK (0.3 mg/kg) given subcutaneously or intraperitoneally at 48-h intervals throughout the period of immunization were less effective. Only UK in low doses introduced intravenously induced detectable inhibition of anti-OA IgE antibody response (Fig. 1).

Significant inhibition of IgE antibody response after treatment of mice with UK mixed with the antigen (OA) raised the question whether UK affects antigenic properties of OA. Therefore in the next part of the study we compared the effect of UK injected into F_1 (BALB/c x C57BL/6J) mice subcutaneously in the dose of 50 mg/kg in the mixture with OA and alum with the effect of the same dose of UK injected separately at the time of immunization (on days 0 and 14). The results presented in Fig. 2 clearly show that significant inhibition of on-going IgE antibody response was induced only in the mice injected with UK in the mixture with OA and alum. Mast cells of these mice tested *ex vivo* for the level of sensitization released significantly less histamine in the response to antigen challenge, showing weaker cell sensitization as compared to mast cells of mice not treated with UK or the mast cells of mice treated with UK injected separately (Fig. 3).

In the next experiments we tested whether the inhibitory effect of UK introduced in the mixture with antigen and alum on mast-cell sensitization level persists after the booster injection of antigen and adjuvant without UK. The results presented in Table I show that peritoneal mast cells of F_1 (BALB/c x C57BL/6J) mice treated with UK introduced in the mixture with antigen and adjuvant released significantly less histamine in the *in-vitro* antigen-induced anaphylactic reaction as compared with mast cells of mice immunized with the same dose of OA and alum but not treated with UK. However, the booster injection of both groups of mice with the mixture of antigen and adjuvant only resulted in significant reduction of the difference in the level of antigen-induced histamine release between mast cells of these groups of mice. Similar results were obtained when BALB/c mice were tested in identical experimental conditions (results not shown).

In order to see whether UK is able to affect the anaphylactic reaction *in vivo*, we sensitized the skin of rats with several dilutions of mouse IgE antibody-containing sera as described in Materials and methods. After a latent period the PCA reaction was challenged by intravenous injection of Evans' blue solution containing antigen alone (1st group) or antigen in the mixture with UK (2nd group). The 3rd group of rats was injected intravenously with UK 30 min before antigen challenge performed as in the 1st group. The results presented in Table II show that in the

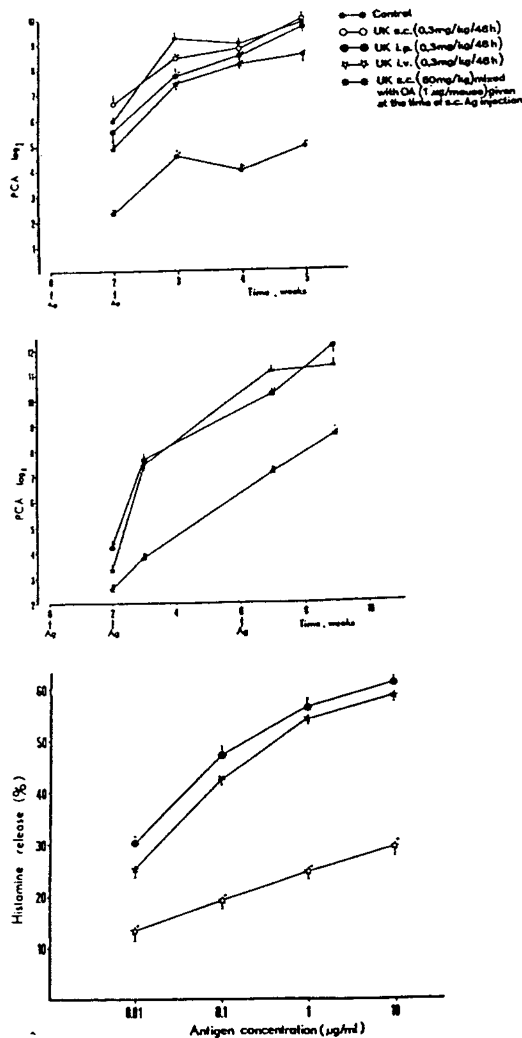

Fig. 1 Kinetics of anti-OA IgE antibody responses in BALB/c mice treated with UK in the doses of 0.3 mg/kg given subcutaneously (s.c.), intraperitoneally (i.p.), or intravenously (i.v.) during the entire period of immunization, or treated with two high doses of UK (50 mg/kg) injected s.c. in the mixture with OA and alum at the time of immunization (indicated as Ag and arrows). Results are the means + S.E. for n=20 animals. Small black points indicate a significant difference from the control response in mice nontreated with UK ($p < 0.05$).

Fig. 2 Kinetics of anti-OA IgE antibody responses in F(BALB/c x C57BL/6J) mice injected s.c. with OA + alum (black stars), injected s.c. with OA + alum and treated with UK (50 mg/kg) given s.c. on the other side of the back (black points), or injected s.c. with the mixture of OA, alum and UK (50 mg/kg) (white stars). The time of the injections is indicated as Ag and arrow. Results are the means + S.E. for n=10 animals. Small black points indicate a significant difference from the control response in mice nontreated with UK ($p < 0.05$).

Fig. 3 Antigen-induced histamine release from mast cells isolated from peritoneal cavities of Fl(BALB/c x C57BL/6J) mice immunized with OA + alum (black stars) and treated with UK as indicated in the legend to Fig. 2 (black points - release from mast cells of mice treated with UK given separately, white stars - release from mast cells of mice treated with UK given in the mixture with OA and alum). Mast cells were harvested after 7 weeks of immunization. The results represent the means from 4 duplicate experiments + S.E. Small black points indicate a significant difference from the results obtained with mast cells of control mice, non-treated with UK ($p < 0.05$).

Table I *The effect of UK treatment of F1(BALB/c x C57BL/6J) mice (s.c. injection of OA + Alum + UK) on ex-vivo antigen-induced histamine release from their peritoneal mast cells tested before and after booster immunization of the mice with OA + alum, but without UK.*

Antigen concentration µg/ml	Percent of antigen-induced histamine release			
	Peritoneal mast cells from:			
	Control mice		UK - treated mice*	
	Before booster immunization	After booster immunization	Before booster immunization	After booster immunization (without UK)
0.01	25.3 ± 1.2	18.8 ± 2.0	13.2 ± 1.5	14.6 ± 0.9
0.1	42.6 ± 0.9	19.4 ± 1.0	19.4 ± 1.0	26.6 ± 2.1
1.0	53.4 ± 0.3	52.2 ± 1.6	24.9 ± 0.9	47.8 ± 1.3
10.0	58.4 ± 0.7	61.5 ± 3.0	29.7 ± 1.2	60.7 ± 0.9

* UK dose - 50 mg/kg.
The results represent the means from 4 duplicate experiments ± S.E.

Table II *UK-induced inhibition of PCA reaction intensity in rats (for details see Materials and methods). Each result represents the mean of 9 PCA reactions ± S.E.*

Dilutions of IgE-containing serum	PCA reactions challenged by intravenous injection:				
	OA+EB* (amount of dye extracted, μg/ml)	OA+UK+EB (amount of dye extracted, μg/ml)	Percent inhibition	UK injection 30 min before OA+EB (amount of dye extracted, μg/ml)	Percent inhibition
1 : 80	139,9 ± 8,1	70,9 ± 5,1	49,4	105,7 ± 4,4	24,6
1 : 160	83,4 ± 7,7	50,0 ± 3,4	40,1	65,9 ± 4,0	21,0
1 : 320	65,7 ± 6,2	37,2 ± 2,3	43,5	51,3 ± 3,1	21,9
1 : 640	44,2 ± 3,4	27,5 ± 1,9	37,8	35,7 ± 2,5	19,3
1 : 1280	18,8 ± 2,6	11,8 ± 1,6	37,1	13,4 ± 1,7	28,5

*EB - Evans' blue dye.

group of rats challenged with antigen mixed with UK the intensities of PCA reactions was diminished at all dilutions of IgE-containing sera used. Injection of UK 30 min before antigen challenge resulted in PCA diminution as well, althought the percent of this diminution was lower.

Discussion

Our results show that UK can effectively inhibit the IgE antibody response when administered to mice in the mixture with antigen (OA) and adjuvant (alum). Separate introduction of UK resulted in slight inhibition of anti-OA IgE antibody response only when UK was injected intravenously in several low doses. The results suggest that UK may modify the antigenic property of OA, which induces lower anti-OA IgE antibody titres in the sera of treated mice. Decreased anti-OA IgE antibody production in UK-treated mice might contribute to the lower level of their mast-cell sensitization reflected in decreased histamine release after *in-vitro* antigen challenge. However, the inhibitory effect of UK on IgE-induced mast cell sensitization was diminished after subsequent injection of mice with the mixture of OA and alum without UK.

It has been previously shown that common allergens such as OA can be made less allergenic by chemical modification of allergen molecules (9-11). Generally, chemically modified antigens are thought to lose the major antigenic determinant in the native antigen (12). Whether UK is able to modify antigenic determinants in OA molecules must be further investigated.

Our results show that OA prepared in the mixture with UK had a decreased ability to react with mouse anti-OA IgE antibodies, raised against native OA and fixed on the surface of rat mast cells in heterologous PCA reactions. Earlier reports showed that treatment of OA with urea (9) or with glutaraldehyde (10) resulted in the decrease of its reactivity toward anti-OA IgG and IgE antibodies directed against the native OA molecules. It has been also reported that formaldehyde pretreatment of allergen resulted in decrease of its antigenicity (ability to bind antibodies raised against native allergen) (13). It was suggested that loss of reactivity was attributed to a decrease in the avidity of binding of the modified allergen (14). Further studies are necessary to clarify the inhibitory effect of UK pretreatment of OA on its

antigenic property and the ability to react with anti-OA IgE antibodies raised against the native OA molecules.

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