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# THE IMMUNOMODULATING PREPARATION UKRAIN DOES NOT INDUCE ANAPHYLACTIC SENSITIZATION IN MICE AND GUINEA PIGS

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**Summary:** *The ability of* Chelidonium majus L. *alkaloids derivative Ukrain to induce an anaphylactic sensitization was tested on mice and guinea pigs. The levels of IgE antibody in the mouse sera, and $IgG_{1a}$, $IgG_{1b}$ as well as IgE antibody levels in guinea pig sera, were evaluated by passive cutaneous anaphylaxis (PCA) tests. Ukrain alone or adsorbed on aluminium hydroxide gel (alum) introduced into BALB/c mice in several subcutaneous injections was unable to stimulate measurable anti-Ukrain IgE antibody response. Moreover, Ukrain introduced together with ovalbumin (OA) into mice in the course of immunization with OA induced lower anti-OA antibody response as compared to the response induced by OA alone. Ukrain adsorbed on alum and injected subcutaneously into guinea pigs did not induce measurable $IgG_{1a}$, $IgG_{1b}$ and IgE antibody response. The present results suggest that the immunomodulating preparation Ukrain could be therapeutically safe at least as far as its inability to induce anaphylaxis is concerned.*

## Introduction

Alkaloids from the plant *Chelidonium majus L.* conjugated to thiophosphoric acid yield a semi-synthetic compound denoted as Ukrain (1), which in preliminary clinical studies has been found to possess immunomodulatory activity (1, 2). This promising property suggests that Ukrain could be widely used in clinical practice providing that this compound does not provoke deleterious side-effects. Of primary importance is to check whether Ukrain possesses, or does not possess, the ability to induce anaphylactic sensitization. In this paper the authors present data indicating that Ukrain is unable to induce the IgE antibody response in mice and $IgG_{1a}$, $IgG_{1b}$ as well as IgE antibody responses in guinea pigs after parenteral administration.

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## Materials and methods

*Animals.* BALB/c female mice 8–10 weeks old and outbred coloured guinea pigs of both sexes weighing 300–350 g were used for immunizations. Wistar female rats weighing 180–220 g were employed for evaluation of the IgE antibody level in mouse sera. Outbred albino female guinea pigs weighing 400–500 g were employed for evaluation of the $IgG_{1a}$, $IgG_{1b}$ and IgE antibody levels in guinea pig sera.

*Immunization.* The mice were injected subcutaneously with 1 μg Ukrain (group I), or with 1 μg Ukrain adsorbed on 1 mg alum (group II). The group of mice injected with 1 μg ovalbumin (OA) (group III) and another group injected with 1 μg OA adsorbed on 1 mg of aluminium hydroxide gel (alum) (group IV) were used as reference groups. One group of mice was injected with 1 μg OA + 1 mg Ukrain. Each group consisted of 10 mice. All injections were given

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**Table I** *The lack of anti-UK IgE antibodies in the sera of UK-injected mice in comparison with the levels of anti-OA IgE antibodies in OA-immunized mice*

| | | Subcutaneous injections (doses/mouse 20g)* | | | | | |
|---|---|---|---|---|---|---|---|
| | | OA (1 μg) | UK (1 μg) | OA (1 μg) + UK (1 mg) | | OA (1 μg) + alum (1 mg) | UK (1 μg) + alum (1 mg) |
| Day of immunization | Number of mice | Antibody anti-OA | Antibody anti-UK | Antibody anti-OA | Antibody anti-UK | Antibody anti-OA | Antibody anti-UK |
| 14 | 10 | 8.82 ± 0.17 | <1.00 | 6.02 ± 0.26 | <1.00 | 9.12 ± 0.13 | <1.00 |
| 28 | 10 | 11.02 ± 0.15 | <1.00 | 8.52 ± 0.25 | <1.00 | 11.22 ± 0.10 | <1.00 |
| 70 | 10 | 11.02 ± 0.26 | <1.00 | 9.02 ± 0.21 | <1.00 | 11.32 ± 0.30 | <1.00 |

*The injections were repeated on days 7, 14, 21, 28, 57 after the first injection. The IgE levels are expressed as $\log_2$ PCA titres in the sera of mice immunized with OA and/or UK alone or mixed with aluminium hydroxide gel (alum). The mean results obtained in 10 mice ± s.e.

in 0.5 ml of physiological saline and were repeated on days 7, 14, 21, 28 and 57 after the first injection. Blood samples were obtained by puncturing the retro-orbital venous plexus at various intervals (as specified in the Results) and the sera were stored at –20°C until assayed.

Guinea pigs were injected subcutaneously into the back with 10 μg Ukrain mixed with 10 mg alum and the injection was repeated after 21 days. The animals of another group (used as reference group) were given two subcutaneous injections (at three weeks interval) of 10 μg OA mixed with 10 mg alum. On day 7 after the last injections blood samples were obtained and the sera were stored at –20°C until assayed. (3, 4)

*Passive Cutaneous Anaphylaxis (PCA)* 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 PCA reaction (blue spot of diameter ≥ 5 mm) tested on at least two rats was taken as the PCA titre. The sera of Ukrain injected mice were tested identically to the sera of OA-injected mice except that the skin reactions were evoked by intravenous challenge with 1 ml of 0.4% Evans' blue solution containing 1 mg Ukrain.

The anti-OA anaphylactic antibody levels in guinea pig sera were titrated by a homologous PCA test as described (3, 6). To determine IgE antibody titres, unheated samples of the sera were used with the 7 days latent period for skin sensitization. For determination of $IgG_{1a}$ and $IgG_{1b}$ antibody heated serum samples (56°C, 1 h) were used with the skin sensi-tization period of 4 h and 72 h, respectively. The skin reaction was evoked by intravenous challenge with 10 mg OA in 1 ml 0.5% Evans' blue solution. The anti-body titre was taken as the reciprocal of the highest serum dilution giving a threshold PCA reaction (blue spot of diameter ≥ 5 mm) in at least two guinea pigs. The sera of Ukrain treated guinea pigs were tested identically as the sera of OA treated guinea pigs except that the skin reactions were evoked by intravenous challenge with 1 ml 0.5% Evans' blue solution containing 10 mg Ukrain. All results were expressed as $\log_2$ PCA titres ± s.e.

## Results

The results presented in Table I show that several subcutaneous injections of mice with Ukrain alone or Ukrain adsorbed on alum did not stimulate any noticeable anti-Ukrain IgE antibody response in BALB/c mice ($\log_2$ PCA titre < 1.00) as compared to

**Table II** *The lack of anti-UK $IgG_{1a}$, $IgG_{1b}$ and IgE antibodies in the sera of UK-injected guinea pigs in comparison with the levels of anti-OA $IgG_{1a}$, $IgG_{1b}$ and IgE antibodies in OA-immunized guinea pigs*

| | Log2 PCA titre ± s.e. | |
|---|---|---|
| Antibody | anti-UK (n) | anti-OA (n) |
| $IgG_{1a}$ | 0 (n = 8) | 7.75 ± 0.25 (n = 4) |
| $IgG_{1b}$ | 0 (n = 8) | 6.25 ± 0.31 (n = 8) |
| IgE | 0 (n = 8) | 0.5 ± 0.19 (n = 8) |

n = number of guinea pig sera tested.

extremely high anti-OA IgE antibody response observed in the reference groups of mice injected subcutaneously with OA adsorbed on alum ($log_2$ PCA titres up to 11.32 ± 0.30) or with OA alone ($log_2$ PCA titres up to 11.02 ± 0.26). In the sera of mice injected with the mixture of 1 µg OA and 1 mg Ukrain the authors did not find measurable anti-Ukrain IgE antibody levels ($log_2$ PCA titres < 1.00), whereas anti-OA IgE antibody levels were well pronounced ($log_2$ PCA titres up to 9.02 ± 0.21). However, the authors observed that the levels of anti-OA IgE antibody in the sera of this group of mice were lower as compared to the levels of anti-OA IgE antibody in the sera of mice injected with OA alone (Table I).

In the next series of experiments the authors tested whether Ukrain injected subcutaneously into guinea pigs could stimulate the anti-Ukrain $IgG_{1a}$, $IgG_{1b}$ and IgE antibody production. The results presented in Table II show that Ukrain injected together with alum did not stimulate the measurable anti-Ukrain $IgG_{1a}$, $IgG_{1b}$ and IgE antibody production in guinea pigs. In the sera of animals of the reference group immunized with OA included in alum, well pronounced anti-OA $IgG_{1a}$, $IgG_{1b}$ and detectable IgE antibody levels were found.

## Discussion

Results show that the preparation Ukrain (consisting of alkaloids from the plant *Chelidonium majus L.* conjugated to thiophosphoric acid) in small doses introduced subcutaneously into mice and guinea pigs does not induce measurable production of the anaphylactic antibodies in these animals. BALB/c mice used in the present experiments are susceptible to anaphylactic sensitization and are known as high IgE antibody responders to several antigens and haptens (7). Their production of IgE antibodies in the response to ovalbumin (OA), known as a potent antigen, is high (8), and this ability of BALB/c mice was confirmed in the present study. In the same mice, the lack of IgE antibody response to Ukrain introduced together with the alum (known as a potent adjuvant for mice) was observed. Moreover, in the sera of mice injected several times (at one week intervals) with the mixture of 1 µg OA and 1 mg Ukrain, not only the lack of measurable anti-Ukrain IgE antibody levels were observed, 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. This observation suggests that Ukrain 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. This interesting property of Ukrain should be further investigated.

The results also show that Ukrain does not induce any measurable $IgG_{1a}$, $IgG_{1b}$ and IgE antibody production in guinea pigs, known to be good animal models for anaphylactic sensitization (3) and producing well pronounced levels of anaphylactic antibodies in the response to OA.

In conclusion, the present results suggest that the preparation Ukrain could be therapeutically safe at least as far as its inability to induce anaphylactic sensitization is concerned.

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