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Effects of chelidonic acid, a secondary plant metabolite, on mast cell degranulation and adaptive immunity in rats

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ABSTRACT

The present study evaluated the immunomodulatory effects of chelidonic acid, a secondary plant metabolite, with therapeutic potential in allergic disorders, in experimental animals. In mast cell degranulation studies, ovalbumin immunized and challenged rats, chelidonic acid (1, 3 and 10 mg/kg, i.p.) dose relatedly prevented ovalbumin challenge induced mast cell degranulation by differing degrees when compared with vehicle treated group, and these effects were comparable with prednisolone (10 mg/kg). A reduction in post-challenge mortality was also observed in all treated groups. Further, there were reductions in the blood eosinophil counts and serum IgE levels after chelidonic acid treatment. Chelidonic acid also inhibited histamine release from rat peritoneal mast cells (RPMC) in vitro, in a dose related manner. In tests for adaptive immunity, in rats immunized with sheep RBC, chelidonic acid differentially suppressed the (a) plaque forming cell (PFC) count in rat splenic cells, (b) anti-SRBC antibody titre and serum IgG levels and (c) increases in foot pad thickness in the DTH assay — all of which were comparable with prednisolone. These experimental results are discussed in light of the possible therapeutic potential of chelidonic acid in allergic disorders.

1. Introduction

Allergic disorders result from the dysregulation of the immune system and are associated with considerable morbidity. Such inappropriate immune reactions may result from exposure to foreign antigens or those misdirected against intrinsic body components leading to autoimmune disorders. Complex cellular and humoral mechanisms have been forwarded to explain such responses, and targeted drug therapy is often associated with untoward adverse effects. Further, mechanisms for explaining the etiopathogenesis of allergic responses are being revealed and hence the search for safer and effective therapeutic modalities is being constantly explored [1].

Herbal drugs are emerging as strong alternatives or adjuncts to conventional modern medical therapy. Their therapeutic efficacy combined with the comparative lack of adverse effects have prompted the generation of renewed interest in such plant based products. Further, application of modern medical technologies/strategies to authenticate the use of such agents has resulted in integration between traditional (from herbal sources) and modern medicines in a variety of disease states. The concept of 'reverse pharmacology' has added impetus to such interactive studies and a translational approach is being adopted to develop a more rational interface between traditional and modern medical concepts. Chelidonic acid is a secondary metabolite found in several plants and its presence in many alkaloid containing plants like *Chelidonium majus* (Celandine) and several others of the a Papavar species. Chelidonic acid has also been reported as the leaf closing signaling molecule for *Cassia minosodia* (an Indian medicinal plant) and has been isolated from *Sorghum vulgare* seedlings (a common food material grown in India), flowers of *Cassia spectabilis* and leaves of *Gloriosa superba*. There is the possibility of its ability to modulate the pharmacological activity of the alkaloids with which it co-exists in plants. Modern herbal pharmacologists have paid little attention to its potential as a therapeutic agent though it is a relevant active component of medicinal herbs. Interest in this molecule was triggered by the reports that it is a potent glutamic acid decarboxylase (GAD) inhibitor, and that the zinc site of the enzyme is its site of action. Structurally, this zinc site of the enzyme is analogous to many other immunological sites involved in histamine release and many other inflammatory phenomenon. There are also several reports on its zinc chelating properties. One preliminary pilot study had indicated that chelidonic acid was as effective in inhibiting histamine release as disodium cromoglycate, a mast cell stabilizer used in the prophylaxis of asthma and related allergic disorders [2–6]. In view of the above, it is possible that this plant product could have potential in the treatment of allergic disorders, and hence this study evaluated its immunomodulatory profile in experimental animals.

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2. Materials and methods

2.1. Animals

Wistar rats (180–220 g) were used for the study. The animals were maintained under standard laboratory conditions of light–dark cycle (12 h light–12 h dark) and temperature of (22 ± 2 °C) and had free access to food and water. The animal care was as per guidelines laid down by the Indian National Science Academy, New Delhi, and the study protocol was approved by the Institutional Animal Ethical Committee.

2.2. Drugs and chemicals

Chelidonic acid, Prednisolone, and Ovalbumin were procured from Sigma-Aldrich (St. Louis, USA). Aluminium hydroxide was procured from Wyeth (India). Sheep red blood cells (SRBC) were generous gifts from Dept. of Microbiology, UCMS, Delhi. Anti-IgE and anti-IgG antibody was procured from Genex Bio (New Delhi). All other routine chemicals were procured from Sisco Research Labs (Delhi). Chelidonic acid, prednisolone, and SRBC was administered intraperitoneally (i.p.), after dissolving in appropriate vehicle, in a volume of 2 ml/kg.

2.3. Mast cell degranulation studies [7]

Rats were immunized with an intraperitoneal (i.p.) injection of antigen, viz. ovalbumin (10 mg) per rat adsorbed to 10 μg of aluminium hydroxide (i.e. ovalbumin + aluminium hydroxide). The five experimental groups were as under: controls (vehicle), chelidonic acid (1, 3 and 10 mg/kg) and prednisolone (10 mg/kg), and received daily treatment (i.p.) for 14 days for all drugs. Fourteen days after the sensitization and drug treatments, the animals were challenged with an i.p. injection of ovalbumin (1 mg) in 0.5 ml of isotonic saline in all groups. Anaphylactic mortality was checked at 2 h post challenge. After 10 min, the rats were anesthetized and exsanguinated. The abdomen was opened and the mesentery was dissected away from the small intestine. Fragments of mesentery were fixed and stained for 15 min in a solution containing 50% ethanol, 10% formaldehyde, 5% acetic acid and 0.2% toluidine blue. Mesentery fragments were then mounted on a glass slide, care being taken not to fold or stretch the tissue. Mast cell degranulation was assessed by counting the % of cells with extruded granules (12 microscopic fields counted). The degranulated cells in the rats could be visualized because the granules were still stained by the dye when they were discharged from the cell and became extracellular. The number of intact and degranulated mast cells in the different treatment groups was counted microscopically using standard technique.

2.4. Estimation of IgE and IgG levels

The blood samples collected were processed as per standard methodology for the assay of the immunoglobulins (IgE and IgG) using commercially available assay kit (Shibayagi Co., Ltd.). This was performed by enzyme linked immunosorbent assay (ELISA) using the sandwich method. Briefly the samples and biotin conjugated anti IgE antibody are incubated in monoclonal antibody coated wells. After 2 h of incubation HRP conjugated avidin is added and incubated for 1 h. The HRP conjugated avidin is reacted with a chromogenic substrate reagent and reaction is stopped and the absorbance of yellow product formed is measured using the software based microplate reader at 450 nm. Results are expressed in ng/ml. Similar procedure was also followed for IgG estimation.

2.5. Histamine assay from rat peritoneal mast cells (RPMC) in vitro [8]

Rats were anesthetized by anesthetic ether and injected with 50 ml of Tyrode buffer (137 mM NaCl, 5.6 mM glucose, 12 mM $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4, and 0.1% gelatin) into the peritoneal cavity, and the abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells were aspirated by a Pasteur pipette. The peritoneal cells were sedimented at 150 g for 10 min at 4 °C and resuspended in Tyrode buffer. Mast cells were separated from the major components of rat peritoneal cells. Mast cell preparations were assessed by toluidine blue staining. Histamine content was measured by the *o*-phthaldialdehyde spectrofluorometric procedure. RPMC suspensions (54×10^5) were reacted with chelidonic acid 1, 3, and 10 mg/ml and with prednisolone 10 mg/ml for 10 min at 37 °C then treated with 48/80 1 μg/ml. The reaction was stopped by cooling the tubes on ice. The cells were separated from the released histamine by centrifugation at 400*g* for 5 min at 4 °C. Residual histamine in the cells was released by disrupting the cells with 1% Triton-X 100 and centrifuged at 400*g* for 5 min at 4 °C. In brief, the culture supernatant (250 μl each) were mixed with 225 μl of 0.1 N HCl and 25 μl of 60% perchloric acid and centrifuged 22,000 *g* at 4 °C for 20 min. The collected supernatant (400 μl), water (1.5 ml), and *n*-butanol (5 ml) were put into a tube containing NaCl (0.6 g) and 5 N NaOH (250 μl), and mixed for 20 min and centrifuged at 385*g* for 5 min at 4 °C. The collected supernatant (4 ml) and heptane (5 ml) were put into a tube containing 1.5 ml of 0.1 N HCl, and mixed for 20 min and centrifuged as above. The liquid collected from bottom level (150 μl), 1 N NaOH (30 μl), and 7.5 μl of 1% ophthaldialdehyde (OPT) were put into optiplate 96-well plates (Costar) and incubated for 3 min at 30 °C. To stop the reaction, 3 N HCl (15 μl) was added and the light emission was measured in a spectrofluorometer. The fluorescence intensity was measured at 440 nm (excitation at 360 nm) by a spectrofluorometer.

2.6. Tests for adaptive immunity

2.6.1. Antibody response [9]

Animals were immunized with sheep red blood cells (SRBC, 0.5×10^9 cells/ml/100 g) on day 0 then they then received vehicle or chelidonic acid (1, 3 and 10 mg/kg) from the day 1 to 5. On the day 6, all rats were anesthetized with ether and blood was collected from the retro-orbital plexus using the microcapillary technique. The serum was assayed for hemagglutination titre was follows: two fold dilutions (0.025 ml) of sera were made in microtitre plate with saline. To each well 0.025 ml of 1% (v/v) SRBC was added. The plate were incubated at 37 °C for 1 h and then observed for hemagglutation by using the double dilution technique. The highest dilution giving haemagglutination was taken as the antibody titre. The antibody titres were also expressed in graded manner ($-\log_2$), the minimum dilution (1/2) being ranked as 1, and the mean ranks of different treatment groups were compared for statistical significance.

2.6.2. Plaque forming cell assay [10,11]

The plaque forming cell (PFC) assay was carried to assess the ability of the spleen to form antibody forming cells. The animals were immunized with SRBC on day 0 and sacrificed on the day 4 under anesthesia. The spleen was removed and Spleen cell suspensions are prepared by gently tamping the spleen through a 60-mesh stainless steel screen, and collecting the cells in balanced salt solution (BSS). The spleen cells were washed and made up to 15 ml with BSS. SRBC was washed twice and made up to a 10% concentration. Complement (Gibco) was diluted 1/20 with BSS. All stock solutions were kept on ice water until used. All the cells were transferred to a conical test tube and the volume adjusted to 15 ml. The tube filled with the cell suspension was centrifuged. The supernatant was aspirated and discarded. The remaining pellet, constituted of intact spleen cells, was resuspended in fresh balanced salt solution. The tube was once more centrifuged; the supernatant was also discarded and the pellet is resuspended in more balanced salt solution. The cells were washed this way three times with balanced salt solution. The test consisted of mixing 0.05 ml of spleen cells, 0.070 ml of SRBC and 0.5 ml of the complement solution in a test tube at 37 °C. The whole