IMMUNE RESPONSES INDUCED IN HENS AFTER ORAL ADMINISTRATION OF BOVINE SERUM ALBUMIN IN COMBINATION WITH AN EXTRACT OF UNCARIA TOMENTOSA

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Abstract

A dry extract from the bark of Uncaria tomentosa was evaluated as a potential immunomodulator in various formulations. Hens were immunised three times orally with a bovine serum albumin (BSA) supplemented with 7, 70, and 280 mg of the dry extract of the bark. Specific IgA antibodies in saliva and IgY antibodies in yolk were examined by ELISA. It was found that the birds inoculated with BSA in addition to 280 mg of Uncaria tomentosa bark extract had a higher amount of BSA-specific IgA antibodies in the saliva and BSA-specific IgY antibodies in yolk, than hens that received lower doses of the extract. Furthermore, the hens immunised with BSA alone had significantly lower immune responses to BSA than hens immunised with BSA supplemented with the bark extract. These results suggest that dry extract from bark of Uncaria tomentosa is useful as an oral adjuvant or immunomodulator for hens.

Key words: hens, Uncaria tomentosa, bovine serum albumin, immunisation, immune response.

A majority of laboratory animals are used as models of man in biomedical research. A significant proportion however, is used simply to produce biological reagents. The use of animals for antibody production may be associated with ethical problems and the immunisation schemes and can be more or less aggressive depending on the site of administration, frequency of boosting, and type of immunopotentiating adjuvant.

Mucosal immunity, in which secretory IgA antibodies play the main important role, is established via the mucosa-associated lymphoid tissues such as those in the respiratory and gastrointestinal tracts (9). The gut-associated lymphoid tissues (GALT), particularly the Peyer’s patches, as inductive tissues, contain a large number of IgA precursor B cells, and the stimulation of them with orally administered antigens may lead to the dissemination of B and T cells to mucosal effector tissues such as the lamina propria region of intestinal, respiratory, and genitourinary tracts and various secretory glands for subsequent antigen-specific IgA antibody responses (14).

Plants are invaluable sources of new drugs. There is an ever-growing interest in the investigation of different species of plants to identify their potential therapeutic applications, such as immunomodulatory activity. This increasing interest is due to a tremendous historical legacy in folk medicine to use plants as medicines due to their easy availability, cost effectiveness, and presumed safety (12).

The woody vine Uncaria tomentosa (Willd.) DC. (Rubiacea), a Peruvian plant commonly known as “cat’s claw”, “vilcacora”, or “uña de gato”, is widely used for the treatment against cancer and various infections (7, 8, 10). Numerous reports have shown that extracts of the bark from U. tomentosa possess not only anti-inflammatory activity, but also anti-viral, anti-mutagenic, and anti-oxidant activities (1, 2, 5, 11). In addition, such extracts have been reported to enhance phagocytosis (15) and to stimulate the production of IL-1 and IL-6 in rat alveolar macrophages (9).

The aim of the present study was to assess the efficacy of oral immunisation of chickens with bovine serum albumin (BSA) in combination with extract of U. tomentosa as a mucosal adjuvant. The efficiency of the systemic and mucosal immunospecific response was evaluated by measurement of BSA antibodies (IgY in yolk and IgA in saliva).

Material and Methods

Animals and their management. Fifteen 25-week-old outbred ISA hens were obtained from the
breeding unit of the laboratory of animal resources of the Institute of Immunology (Vilnius, Lithuania). The hens were kept singly in 1 m x 1 m floor pens equipped with nest boxes, in a standard animal room with a 17/7 h light/dark cycle. As bedding, woodchips of deciduous trees after sterilisation at 120°C and pressure 1.5 kg/cm² during 20 min were used. The bedding was changed twice weekly. The temperature in the room was 20°C ± 2°C, with a relative humidity within the range of 55%-60% and the noise level was maintained below 50 dB. The chicken feed was based on granulated forage (“Biosynthesis” AB Vilnius, Lithuania). It consisted of dry matter (88%), crude protein (20%), fat (3%), and carbohydrate (4%). The feed was balanced for vitamins and micronutrients, and the moisture content did not exceed 12%. Water was provided ad libitum.

**Antigen.** A commercial preparation of BSA (Sigma, USA) was used as the antigen.

**Adjuvant.** The stem bark of *U. tomentosa* was used as the adjuvant. A commercial preparation of BSA containing emulsion (0.5 ml) was mixed with 7.0 mg of *U. tomentosa* extract. The total volume of 1 ml was administered by oral gavage. The treatment groups were:

- **Group A.** BSA alone. The mixture containing 200 mg of BSA in 0.5 ml of phosphate-buffer saline (PBS), pH 7.2, was emulsified with an equal volume of olive oil (Extra Virgin, Carapelli Firenze, Italy). The total volume of 1 ml was administered by oral gavage.
- **Group B.** BSA + 7.0 mg *U. tomentosa*. The BSA containing emulsion (0.5 ml) was mixed with 7.0 mg of the dry extract of *U. tomentosa*.
- **Group C.** BSA + 70.0 mg *U. tomentosa*. The BSA containing emulsion (0.5 ml) was mixed with 70 mg of the dry extract of *U. tomentosa*.
- **Group D.** BSA + 280 mg *U. tomentosa*. The BSA containing emulsion (0.5 ml) was mixed with 280.0 mg of the dry extract of *U. tomentosa*.
- **Group E.** The suspension containing 200 mg of BSA in 0.5 ml PBS was emulsified with an equal volume of Freund’s incomplete adjuvant (Bio-Rad, USA) and the total volume of 1 ml was administered intramuscularly into four site of each hen. Inoculation was performed three times (at days 1, 14, and 28).

Groups A, B, C, and D were immunised with the antigen mixture in a dose of 1 ml/hen administered by oral gavage. The birds were not anaesthetised during immunisations.

**Collection of samples.** Eggs and saliva were collected for antibody measurement weekly, beginning 7 d after the first injection, and stored at 4°C and -20°C, respectively. The purification of IgY from egg yolk was performed as described earlier (13). Saliva secretion was collected by absorbent filter papers (Whatman No.1, Sigma). Pre-weighed two wicks were placed under the tongue of the hen for approximately 20 s. The wicks were weighed to measure the amount of saliva. The saliva was extracted by adding 400 µl of PBS containing 0.1% Tween 20, pH 7.2, to the Eppendorf tube with the paper wicks and incubating the mixture with slow shaking at 20°C for 2 h. After this, the extract was used for analysis.

**Determination of antibodies.** BSA-specific IgA antibodies in saliva and BSA-specific IgY antibodies in yolk were analysed by ELISA as described earlier (4). The antibody titres were expressed, as the reciprocal of the highest dilution of saliva or yolk and the optical density (492 nm), which was 2-fold higher than that of the negative samples. The titres were converted to a base-2 logarithmic scale.

**Ethical committee approval.** We have performed the experiment after having received the permission No. 0086 of the Ethics Committee on the Use of Laboratory Animals, at the State Food and Veterinary Service (Vilnius, Lithuania).

**Statistical analyses.** The mean of the IgA and IgY antibody titres were compared using two-tailed Student’s *t*-test. All values were expressed as geometric mean titres ± standard deviation and were considered to be statistically significant at *P*<0.05

**Results**

The IgA antibody response after immunisation with BSA in combination with *U. tomentosa*. Fig. 1 shows that the IgA titres in group D were significantly higher than those in groups A, B, C, and E at 2, 3, 4, 5, and 6 weeks after the last immunisation and also that there were significant differences in IgA titres among groups A, B, C, and E, except for the first two weeks of the observation. Notably, IgA titres were significantly increased within one to six weeks after the last immunisation only in groups B, C, and D. Furthermore, the titres of the IgA were below log₂ 2.5 before immunisation (at day 0, Fig. 1).

The IgY antibody response after immunisation with BSA in combination with *U. tomentosa*. It was recorded that the IgY titres in group E were significantly higher than those in other groups at all time-points of the observation after the last immunisation (Fig.2). In addition, the difference was found in the IgY titres among all groups at 4, 5, and 6 weeks after the last immunisation. In groups B, C, D, and E, IgG titres were significantly increased within one to six weeks after the last immunisation. In fact, the titres of the IgY were below log₂ 5.0 before immunisation (at day 0, Fig. 2).
**Fig. 1.** Saliva IgA antibody response in hens after oral and intramuscular immunisations with BSA alone or mixed with *U. tomentosa*. Asterisks indicate groups the values of which differ significantly from those of other groups on the same day.

**Fig. 2.** Yolk IgG antibody response in hens after oral and i.m. immunisations with BSA alone or mixed with *U. tomentosa*. Asterisks indicate groups the values of which differ significantly from those of other groups on the same day.
Discussion

In this study, we have demonstrated that oral immunisation with BSA in combination with a dry extract of *U. tomentosa* could prime the immune system for both secretory and systemic booster of antibody responses to later repeated oral immunisations. Furthermore, the addition of the extract into the immunisation mixture emulsified with an olive oil had a prolonged enhancing effect on the immune response in birds. To obtain a stable, water-in-oil emulsion, various kinds of emulsions with different oil samples were evaluated. With this type of emulsion, the antigen in the internal aqueous phase is released slowly into the biological fluids. Basically, the emulsification system used for the immunisation includes an oil phase, an aqueous phase, and an emulsifying agent, which should favour the formation of water-in-oil emulsion (6). In our studies, probably the water-in-oil emulsion contained high local concentration of olive oil trapped in a droplet together with antigen that is normally deposited in a mucosal tissue. These findings suggest that repeated immunisation with the BSA in combination with dry extract of the *U. tomentosa* as mucosal adjuvants may induce the mechanism of the immune memory and develop the immune response in animals for a long time (3).

Our results show that water-in-oil emulsions based on extracts containing biologically active components from plants may be effective when used in various formulations as immunostimulating complexes.

References