Melatonin treatment prevents modulation of cell-mediated immune response induced by propoxur in rats

Sanvidhan G Suke, Rahul Pathak, Rafat S Ahmed, A K Tripathi and B D Banerjee*
Environmental Biochemistry and Immunology Laboratory, Department of Biochemistry, University College of Medical Sciences and G.T.B. Hospital (University of Delhi), Dilshad Garden, Delhi 110 095, India

Received 28 September 2007; revised 26 February 2008

The effect of melatonin, a major secretory product of the pineal gland, in attenuation of propoxur (2-isopropoxy phenyl N-methyl carbamate)-induced modulation of cell mediated immune (CMI) response was studied in rats. Male Wistar albino rats were exposed to propoxur (a widely used pesticide) orally (10 mg/kg) and/or melatonin (10 mg/kg) orally for 4 weeks. CMI was measured by delayed-type hypersensitivity (DTH), leucocyte and macrophage migration inhibition (LMI and MMI) responses and estimation of cytokines TNF-α and IFN-γ levels. Rats exposed to propoxur for 4 weeks showed significant decrease in DTH, LMI and MMI responses. Propoxur also suppressed TNF-α and IFN-γ production significantly. Administration of melatonin alone caused a significant increase in DTH response. Although there were no changes in the LMI and MMI response, the cytokine levels were significantly increased, as compared to control. Co-administration of melatonin along with propoxur significantly nullified the effect of the pesticide on the CMI response, except DTH and reversed levels of cytokines to near control/normal values. Thus, melatonin treatment considerably attenuated immunomodulation caused by sub-chronic treatment of propoxur in experimental animals.

Keywords: Melatonin, Propoxur, Cell-mediated immune response, Cytokine

Experimental and epidemiological evidences suggest that many pesticides in widespread use cause immunotoxic effects (both acute and chronic), depending upon concentration and duration of exposure. The hallmark of the immune system is the maintenance of an intricate balance, inspite of its vulnerability to any chemical, including pesticides, which can cause structural and functional alterations to the system. Propoxur (2-isopropoxy-phenyl-N-methyl carbamate) is one of the widely used carbamate insecticide. The risk of human exposure to propoxur is very high, as it is applied in large quantities for public health and agricultural pest control programme. Propoxur is a non-systemic insecticide notable for its rapid knockdown. Its immunomodulatory function is probably related to the OC(O)NHCH₃ group, which is also responsible for the inhibition of acetyl cholinesterase (AChE).

Cytokines function as specific chemical mediators that ultimately regulate growth and differentiation of cells. Tumor necrosis factor-α (TNF-α), mainly a secretory product of macrophages is a pro-inflammatory cytokine with multiple roles in immunity, including rapid defense in infection. Recently, attenuation studies have focused on the protective effect of natural immunomodulators and melatonin is one such compound produced mainly as hormone by the pineal gland. It is also a normal food constituent, found in the yeast and plant material, which can influence the level of melatonin in circulation. Melatonin receptors are expressed on the lymphoid cells and are found on lymphoid tissue throughout the body. Melatonin binding may influence the response of natural killer (NK) cells, cytokine production (TNF-α and IFN-γ) and hence cell-mediated immune (CMI) response.

Since a number of pesticides are immunotoxic and melatonin, secreted by pineal gland exerts immunomodulating property, the present study has been undertaken to establish the immunosuppressive role of propoxur and its attenuation by melatonin.

Materials and Methods

Chemicals and reagents

Technical grade propoxur (purity 99.4%) was obtained through the courtesy of M/s Bayer AG, Monheim, Germany. Melatonin was procured from M/s Dabur India Ltd., Delhi, India. ELISA kits for estimation of TNF-α and interferon-γ (IFN-γ) were obtained from Diaclone Research, France. All other
reagents used were of analytical grade and obtained either from Sisco Research Laboratories, Mumbai or Qualigens Fine Chemicals, Mumbai.

Animals and treatment

Male Wistar rats weighing between 200 and 250 g were procured from the Central Animal House of the Institute and housed in standard laboratory conditions with pellet diet and water available *ad libitum*. The experimental animals were divided into four groups with eight animals in each group. Propoxur and melatonin were dissolved in groundnut oil and given orally by gavage once per day for 4 weeks. The first group received propoxur (10 mg/kg), the second melatonin (10 mg/kg) and the third daily dose of both propoxur (10 mg/kg) and melatonin (10 mg/kg). The fourth group i.e., control received groundnut oil only.

Estimation of immunological parameters

Levels of TNF-α and INF-γ were measured by ELISA using standard kits. Delayed-type hypersensitivity (DTH) was determined according to the method of Institoris. Each rat was immunized subcutaneously with keyhole limpet hemocyanin (KLH) at the base of tail by 0.3 ml of antigen preparation on 1st day of the treatment. On 14th day, the reaction was challenged by injecting 20 µg KLH in 50 µl normal saline in left hind foot pad as a test and with normal saline in right hind foot pad as control. Foot pad thickness was measured just before and 24 h, after challenge by antigen using dial calipers. Results were expressed as percentage increase in footpad thickness over vehicle treated control values.

Leucocyte migration inhibition (LMI) and macrophage migration inhibition (MMI) were measured as described previously. In brief, the rats were sensitized with 0.5 ml of ovalbumin (50 µg/ml) mixed with Freund’s complete adjuvant. On day 21st, 4-5 ml of heparinized blood sample was taken and mixed with 2 ml 3% dextran, followed by incubation for 45 min at 37°C. The leucocyte rich plasma was taken out into a siliconized tube and centrifuged at 900 rpm for 5 min. Washing of pellets was done with lactalbumin hydrolysate medium with Hank’s balance salt solution, adjusting the final cell concentration to $15 \times 10^6$ cells/ml. The microlitre capillaries plugged at one end with plasticine were filled with cell suspension and centrifuged at 500 rpm for 5 min. The capillaries were cut at cell-fluid interface and affixed with silicon wax in pyrogen-free perspective chamber plates and filled with lactalbumin hydrolysate enriched medium with 5% fetal calf serum containing antigen (ovalbumin, 1 µg/ml), closed with coverslip ensuring no air bubble and incubated for 20 h at 37°C in humid atmosphere.

The area of migration in control as well as antigen chamber was recorded on a centimeter graph paper with aid of a camera lucida and calculated as percentage migration inhibition expressed as $100 - \left( \frac{\text{area of migration in antigen chamber}}{\text{area of migration in control chamber}} \right) \times 100$.

Statistical analysis

One-way ANOVA with Tukey’s multiple comparison procedure as post-hoc test was used for comparison of data. $P$ value of less than 0.05 was considered as the level of significance for all statistical tests.

Results and Discussion

Pesticides have been associated with immunotoxicity; however, most of the information is available on organochlorine and organophosphate compounds. In a recent study, we reported that melatonin protects against propoxur-induced oxidative stress and suppression of humoral immune response as measured by serum antibody titre and splenic plaque forming cell assay. The present study was designed to investigate the effect of sub-acute exposure of propoxur on CMI response (cytokine levels, LMI and MMI) in experimental animals and to determine whether melatonin could attenuate these effects.

Rats exposed to propoxur at a dose of 10 mg/kg per day for 4 weeks exhibited no symptoms of overt toxicity, neurotoxicity or mortality. No significant difference was noted in body weight between control and treated rats, suggesting that propoxur at these levels did not produce any physical stress, which might be responsible for the observed effect on immune parameters.

The effect of propoxur and melatonin on CMI response was evaluated using DTH reaction, LMI and MMI responses. DTH response was used to measure a secondary cellular response that appeared 48 to 72 h after antigen exposure. Rats exposed to propoxur and subsequently immunized by KLH showed a marked decrease in DTH response, while co-administration of propoxur with melatonin showed a slight increase, as compared to propoxur-treated
group (Table 1). Melatonin alone caused a significant increase in foot pad thickness (DTH response), but failed to offer much protection in the presence of propoxur. Though there was an increase in DTH response as compared to propoxur-treated group, it did not reach control levels. During CMI response, in contact with an appropriate antigen, sensitized T-lymphocytes release a number of molecular mediators collectively known as ‘lymphokine’. LMI factor, one such lymphokine inhibits migration of neutrophils and MMI factor inhibits migration of macrophages. In the present investigation, there was a significant effect of propoxur on percent LMI and MMI. Melatonin per se did not show significant modulation as compared to control, but in combination with propoxur prevented propoxur-induced decrease in LMI and MMI, indicating its protective role (Table 1).

TNF-α, mainly a secretory product of macrophages acts on all cells (except erythrocytes), where it enhances leucocyte cytotoxic enhancement of NK cell function etc. Exposure of rats to sub-chronic dose of propoxur showed a significant decrease in TNF-α and INF-γ levels, as compared to controls. Melatonin alone increased the TNF-α and INF-γ levels and also showed modulation, when co-administered with propoxur. Some workers have reported that melatonin at physiological concentration did not induce production of these cytokines by peripheral blood mononuclear cells (PBMC) in culture, but caused a dose-related inhibition in production of both cytokines if the PBMC were stimulated with phytohaemagglutinin. In the present study, the dose of melatonin used and the experimental conditions were entirely different, hence the observed difference.
References