Inhibitory effects of BAY 60-2770 in human eosinophil chemotaxis and murine allergic inflammation

Lineu Baldissera-Jr, Dalize Maria Squebola, Marina Ciarallo Calixto, André Lisboa Rennó, Gilberto De Nucci, Edson Antunes

State University of Campinas - Faculty of Medical Sciences - Department of Pharmacology, Campinas, Sao Paulo, Brazil

Eosinophil recruitment to inflamed lung is a hallmark of allergic asthma, where the chemokine eotaxin acts as selective eosinophil chemoattractant released upon allergen challenge (Holgate, 2008). Soluble guanylyl cyclase (sGC) plays a key antiinflammatory role by inhibiting leukocyte recruitment (Ahluwalia et al., 2004), and increased cGMP intracellular levels inhibit eosinophil chemotaxis (Thomazzi et al., 2004, 2005). Therefore, this study aimed to investigate the inhibitory effects of BAY 60-2770 (NO- and haem-independent sGC activator) in eotaxin-induced human eosinophil chemotaxis and pulmonary allergic inflammation in mice.

Human blood eosinophils (4x10^6 cells/mL) were isolated from healthy volunteers by negative immunomagnetic anti-CD16 microbeads separation, after approval from National Committee for Ethics in Human Research - CONEP (nº 44347) and written informed consent from subjects. Cells were incubated with BAY 60-2770 (1-10 µM; 30 min, 37°C) and allowed to migrate toward eotaxin (300 ng/ml; 1 h, 37°C) in chemotaxis chamber. Our data showed that human eosinophil migration to eotaxin was significantly inhibited (p<0.001; n=5) by BAY 60-2770 (10 µM: 17.1±4.8 eosinophils/HPF) compared with vehicle (0.1% DMSO)-treated cells (81.4±6.2 eosinophils/HPF).

In separate experiments, male C57BL6 mice were subjected to immunization (100 µg of OVA, s.c.; day 0 and 7) and challenge with OVA (10 µg; twice a day, 6 h between challenges), after approval of by The Ethical Principles in Animal Research (nº 2622-1). After 48 h post-OVA challenge, total and differential cell counts in bronchoalveolar lavage (BAL) fluids, peripheral blood and bone marrow cells were obtained. Chronic oral treatment with BAY 60-2770 (1 mg/kg/gavage; 14 days) significantly reduced (p<0.001; n=7-8) the eosinophil counts in BAL fluid (1.7±0.4 x 10^6/mL) compared with untreated mice (6.0±0.7 x 10^6/mL) that was accompanied by a decreased IL-5 levels (13.6±2.0 vs 32.8±3.6 pg/mL for treated and untreated group respectively; p<0.05, n=6). Histological analysis showed reduced eosinophil peri-bronchiolar infiltration in treated group (4.1±1.2 eosinophil/HPF; p<0.05; n=3) compared with untreated group (22.4±1.2 eosinophil/HPF). Blood eosinophilia triggered by OVA challenge (0.42±0.11 x 10^5 eosinophil/mL) was also reduced by BAY 60-2770 treatment (0.11±0.02 x 10^5 eosinophil/mL; p<0.05; n=7). The bone marrow eosinophilopoiesis was also reduced by BAY 60-2770 treatment (1.4±0.15 vs 0.7±0.14 x10^6/mL for untreated and treated groups, respectively; p<0.001; n=7-8).

Our findings that BAY 60-2770 inhibits human eosinophils chemotaxis and allergic pulmonary eosinophilic inflammation suggest that this sGC activator may be of therapeutic value in the treatment of asthma.

References:


