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EFFECTS OF ASPIRIN ON THE NUMBER OF PERIPHERAL WHITE BLOOD CELLS AND SPLEEN EOSINOPHILS IN GUINEA-PIGS

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Effects of lysine-acetylsalicylate, a soluble form of aspirin, on the number of peripheral white blood cells (PWBC) and on the number of eosinophil granulocytes in spleen imprints were investigated in outbred male guinea-pigs. The absolute number of PWBC and different leukocyte types were determined by standard technique in a haemocytometer. The number of eosinophils in spleen imprints was determined on 2000 nucleated cells. In a dose of 50 mg/kg or 100 mg/kg b.m., i.p., daily for five days lysine-acetylsalicylate did not change the number of PWBC, lymphocytes, neutrophils and monocytes, in comparison with the controls ($p>0.05$). However, in a dose of 50 mg/kg, b.m., i.p. daily for 5 days, lysine-acetylsalicylate produced a statistically significant increase in the number of blood eosinophils ($p<0.05$), and in a dose of 50 mg/kg, i.p., daily for ten days, lysine-acetylsalycylate produced a statistically significant increase in the number of eosinophils in spleen imprints ($p<0.01$), in comparison with the controls. The results suggest that small doses of aspirin could selectively increase blood eosinophil production or recruitment and that prostaglandins, could inhibit eosinophil production or migration in vivo.

Key words: aspirin, leukocyte, granulocyte, eosinophil, guinea-pig, spleen

INTRODUCTION

Aspirin is the well known non-steroid, anti-inflammatory and antipyretic drug. Epidemiological and experimental studies suggest long-term use of aspirin for the protection against cardiovascular disease and reduction of the risk for some types of cancer. However, precise biological mechanisms of aspirin action including its side effects are not known (Weiss, 1996).

There are contradictory data concerning the influence of acetylsalicylic acid on white blood cell proliferation and activity. Some authors have found that

acetylsalicylic acid stimulated human and rat lymphocyte proliferation *in vitro* (Brouard and Pascaud, 1993), but others reported that acetylsalicylic acid in various concentrations had no influence on lymphocyte proliferation or on the phagocytic activity of monocytes and neutrophilic granulocytes *in vitro* (Uhlenbruck *et al*., 1993) or NK cell activity (Porzsolt and Wolf, 1983). It is also known that acetylsalicylic acid, a cyclooxygenase inhibitor, inhibits prostaglandin E2 production and that prostaglandin E2 inhibits proliferation and maturation of hematopoietic stem cells (Aymard, 1983). However, *in vivo*, many other factors, such as several interleukins, or the different contribution of cyclooxygenase and lipoxygenase pathways of arachidonate metabolism in acetylsalicylic acid action, can be involved in leukocytopoiesis (Ulich *et al*., 1987). Earlier we found that lysine acetylsalicylate increased the number of basophil leukocytes in spleen imprints in immunized guinea-pigs (Pantic *et al*., 2004). In this work, we investigated the effects of a soluble form of aspirin (lysine-acetylsalicylate) on the number of peripheral white blood cells (PWBC) and eosinophil granulocytes in guinea-pigs spleen imprints.

MATERIAL AND METHODS

Experimental design

The experiments were done on outbred male guinea-pigs (about 600 grams), short haired, obtained from the Institute of Physiology animal quarters. They were maintained on a natural diet (hay, moisted oats and fresh cabbage with water *ad libitum*), at room temperature of about 22 ^{o}C, with a natural light cycle. The experimental group of animals received lysine acetylsalicylate, a soluble form of aspirin (Sanofi – Sintelabo; 100 mg lysine acetylsalicylate is equivalent 55.57 mg acetylsalicylic acid), in a dose of 50 mg or 100 mg/kg b.m., i.p., daily for 5 days. The control group received a placebo (0.9% NaCl). On the 6th day, the animals were bled by cardiac puncture with special care to perform it gently, with maximal skill, without disturbing the animals more then by venipuncture and without behavioral responses indicative of pain.

The white blood cell count

The PWBC was measured by standard technique (Seiverd, 1983) and stained blood smears (May-Grunwald-Giemsa) for differential leukocyte count were prepared. The leukocytes were counted in hamocytometer over 4 mm^2, and the differential leukocyte count was done from stained blood smears from 200 leucocytes, reading the results blindly. Then the absolute counts of different leukocyte types were calculated.

An additional experiment

In a separate experiment, the experimental group of animals received lysine acetylsalicylate in a dose of 50 mg/kg, i.p., daily for ten days. The control group of animals received a placebo. On the 5th day of the experiment all animals were immunized with sheep red blood cells. On 11th day from the beginning of the experiment, the animals were sacrificed, the spleens were taken and spleen

section imprints were taken and stained with May-Grunwald-Giemsa stains. The number of eosinophil granulocytes from stained spleen imprints was obtained on 2000 nucleated cells reading the results blindly.

Statistical analyses

Statistical analysis was done by Student's t-test and "U" criterion (Wilcoxon-Mann-Whitney).

RESULTS

Table1 and the Figure 1 show that lysine acetylsalicylate, given to guinea pigs in doses of 50 mg or 100 mg/kg b.m., i.p., daily for 5 days did not change significantly the absolute number of PWBC, lymphocytes, neutrophils, basophils and monocytes, in comparison with the controls ($p>0.05$). However, in a dose of 50 mg/kg b.m., i.p., daily for 5 days, lysine acetylsalicylate produced a statistically significant increase in the number of blood eosinophils in guinea-pigs in comparison with the controls ($p<0.05$). In a dose of 100 mg/kg b.m., i.p., daily for 5 days lysine acetylsalicylate produced an apparent increase in the number of blood eosinophils in comparison with the controls (+180%), but the difference was not statistically significant ($p<0.05$), probably due to high natural variability in the number of eosinophils.

Lysine acetylsalicylate, given to guinea-pigs in a dose of 50 mg/kg b.m., i.p., daily for ten days produced (Table 1) a statistically significant increase in the number of eosinophil granulocytes in spleen imprints in comparison with the controls ($p=0.01$).

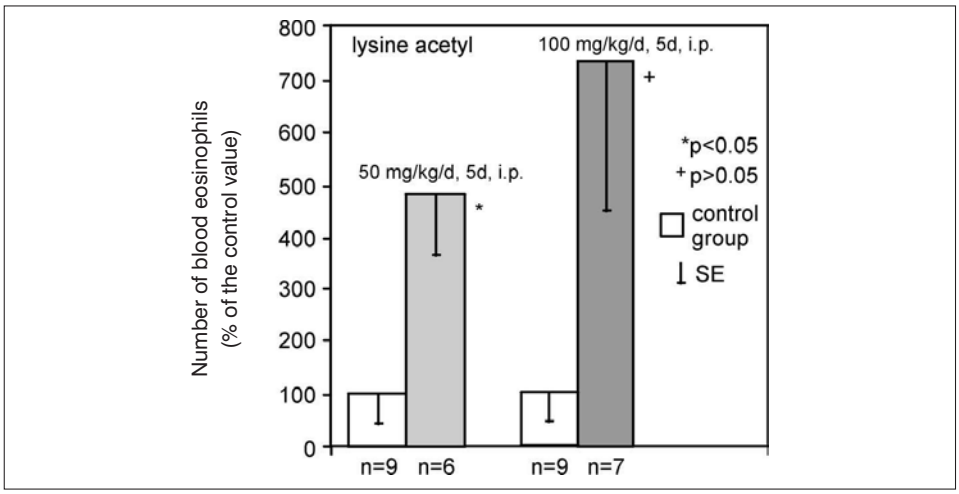

Figure 1. Effect of lysine-acetylsalicylate on the number of blood eosinophils in guinea-pigs

Table 1. Effects of aspirin (as lysine acetylsalicylate) on the number of peripheral white blood cells (WBC) and the number of eosinophil granulocytes in spleen imprints in guinea-pigs[o]

	Aspirin-treated group	Control group	Changes in % of controls
	Mean ± SEM	Mean ± SEM	
Lysine acetylsalicylate 50 mg / kg, i.p., daily for 5 days			
WBC (x 10^9 / L)	8.80 ± 0.500 (9)	8.71 ± 0.495 (8)	+1.03%
Lymphocytes (x 10^9/L)	3.52 ± 0.413 (9)	3.74 ± 0.409 (8)	– 5.88%
Neutrophils (x 10^9/L)	4.06 ± 0.517 (9)	4.19 ± 0.427 (8)	– 3.10%
Eosinophils (x 10^9/L)	0.69 ± 0.218 (9)	0.14 ± 0.081 (6)*	+392.86%
Basophils (x 10^9/L)	0.06 ± 0.028 (9)	0.01 ± 0.009 (8)$^+$	+500.00%
Monocytes (x 10^9/L)	0.47 ± 0.074 (9)	0.43 ± 0.059 (8)	+9.30%
Lysine acetylsalicylate 100 mg / kg, i.p., daily for 5 days			
WBC (x 10^9 / L)	8.31 ± 0.655 (9)	8.42 ± 0.639 (9)	– 1.31%
Lymphocytes (x 10^9/L)	5.26 ± 0.626 (9)	4.95 ± 0.357 (9)	+6.26%
Neutrophils (x 10^9/L)	2.33 ± 0.412 (9)	2.93 ± 0.801 (9)$^+$	– 20.48%
Eosinophils (x 10^9/L)	0.32 ± 0.124 (9)	0.044 ± 0.0227 (7)$^+$	+627.27%
Basophils (x 10^9/L)	0.061 ± 0.0277 (9)	0.062 ± 0.0213(9)	– 1.61%
Monocytes (x 10^9/L)	0.33 ± 0.106 (9)	0.58 ± 0.129 (9)$^+$	– 43.10%
Lysine acetylsalicylate 50 mg / kg, i.p., daily for 10 days			
Eosinophils in spleen imprints (on 2000 nucleated cells)	3.62 ± 0.73 (8)	1.25 ± 0.412 (8)**	+189.60%

[o] Number of animals in parenthesis; *$p < 0.05$; **$p = 0.01$; $^+p > 0.05$

DISCUSSION

Since daily doses of 50 mg/kg b.m. and 100 mg/kg b.m. lysine acetylsalicylate i.p., used in this experiment, contain 27.78 mg and 55.56 mg acetylsalicylic acid per kg b.m., respectively, it appears that the doses of aspirin used in these experiments were low for guinea-pigs. Literature data show that the analgesic and antipyretic dose of acetylsalicylic acid in guinea-pigs is 269 mg /kg i.p., that LD50 is 500 mg/kg i.p. in rats and 495 mg/kg i.p. in mice (Barnes and Eltherington, 1966). Therefore, if the doses of aspirin used in these experiments were in the range of small doses for guinea-pigs, then this could be one of the possible explanations for the absence of significant changes in the absolute numbers of PBWC, neutrophils, and monocytes. In this case, we could assume that the blood eosinophils (and possibly monocytes) could be more sensitive to the actions of acetylsalicylic acid, than the blood neutrophils and monocytes. The highly significant change in the number of spleen eosinophils in the same

direction could support these explanations. On the other hand, responsiveness of eosinophils to chemoatractants could be more complex and specific. It was found that eosinophil chemotaxis toward eotaxin was amplified up to six fold in the presence of PGD2, the effect only seen in eosinophils, not in neutrophils and basophils, and that PGD2 might be an initial chemoatractant which augments the responsiveness of eosinophils to other chemoatractants (Schratl *et al*., 2006). PGD2 is a major mast cell product that acts via two receptors, DP and CRTH2. The latter mediates chemotaxis of eosinophils, basophils and Th2 lymphocytes (Hirai *et al.*, 2001; Fujishima *et al*., 2005; Schratl *et al*., 2007). In addition, it was found that DP receptor plays an important role in eosinophil trafficking, that PGD2 induced the rapid release of eosinophils from human bone marrow and that human eosinophils expressed DP receptor, but at a lower level than CRTH2 (Schratl *et al*., 2007).

The registered increase in the number of blood eosinophils in aspirin treated guinea pigs could be the result of increased eosinophil proliferation or recruitments, or both, due to inhibition of prostaglandin synthesis through cyclooxygenase inhibition by aspirin. It is known that prostaglandin E2 increases intracellular cyclic AMP levels in target cells, and that the agents which increase intracellular cAMP level decrease cell proliferation. Finally, prostaglandins of E-series were postulated to inhibit hematopoietic cells proliferation and maturation (de Silva *et al*., 2003), and the process appears to be signalled through EP2 receptors. It was been found that dibutyryl cyclic AMP and prostaglandin E2 block neutrophil recruitment and that indometacin or aspirin enhanced by two fold neutrophil recruitment (Concalves de Moraes *et al*., 1996).

Recently, it was elucidated that prostaglandin E2 suppresses, while indomethacin and aspirin enhance, eosinohil production in murine liquid bone marrow cultures; indomethacin and aspirin act through blockage of PGE2 and through cysteinyl leukotrienes production (Elsas *et al*., 2008). It is also known that that inhibition of COX is associated by concurrent increased production of leukotrienes (Leone *et al*., 2007). When COX1 is inhibited by aspirin, PGE2 synthesis stops and synthesis of leukotrienes occurs (Stevanson and Zuraw, 2003).

Trying to explain the absence of the changes in the number of blood neutrophils in aspirin-treated guinea-pigs, in addition to low doses of aspirin we have also considered the possibility that two opposite tendencies might be taking place: inhibition of prostaglandin E2 series synthesis with consequent stimulation of neutrophil production, and aspirin triggered lipoxin that can prevent neutrophil recruitment *in vivo* (Clish *et al*., 1999; Serhan *et al*., 2001; Qui *et al*., 2001).

The spleen is a normal active hematopoietic tissue in guinea-pigs. The significant increase in the number of eosinophil granulocytes in aspirin-treated guinea-pigs (reading the results blindly) could be the result of increased eosinophil proliferation in the spleen due to the aspirin induced blockage of local prostaglandin synthesis.

In a dose of 50 mg/kg/d, i.p., for 5 days, lysine acetylsalicylate produced a high, but statistically nonsignificant increase in the number of blood basophils (+500%) in comparison with the controls, possibly due to high natural variations

in the number of these cells. Though we did not find changes in the number of blood basophils in animals treated with lysine acetylsalicylate in a dose of 100 mg/kg it is of interest to mention our earlier finding that lysine acetylsalicylate in a dose of 100 mg/kg, b.m., i.p., for five days, produced a significant increase in the number of *splenic* basophils in *immunized* guinea-pigs. Since basophils possess CRTH2 receptors that mediate PGD2-dependant cell migration (Hirai *et al.*, 2001; Schratl *et al*., 2007), our increase in the number of basophils in aspirin-treated animals might be biologically significant. Our present findings on the selective increase in the number of blood eosinophils (and possibly blood basophils) induced by aspirin is in agreement with the findings of Hirai *et al*. (2001) that prostaglandin D2 selectively induced chemotaxis in helper type 2 cells, eosinophils, and basophils via CRTH2 receptor.

In conclusion, lysine acetylsalicylate given to guinea pigs in small doses, produces a selective increase in the number of blood and spleen eosinophils, probably through stimulation of cell production or recruitment. The number of neutrophils, monocytes and lymphocytes did not change, probably because of the too small doses of aspirin and differences in responsiveness of different blood cells to products of arachidonate metabolism.

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DEJSTVO ASPIRINA NA BROJ LEUKOCITA PERIFERNE KRVI I BROJ EOZINOFILNIH GRANULOCITA SLEZINE

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SADRŽAJ

Ispitivano je dejstvo lizin-acetilsalicilata (rastvorljivog oblika aspirina) na broj leukocita periferne krvi (PWBC) i na broj eozinofilnih granulocita u otiscima slezine neinbrednih zamorčića muškog pola. Apsolutni broj leukocita i različitih tipova leukocita određivan je standardnom tehnikom u hemocitometru. Broj eoz-

inofila u imprintima slezine određivan je na 2000 jedarnih ćelija čitajući rezultate slepo. U dozi od 50 mg/kg ili 100 mg/kg, t.m., i.p., dnevno u toku pet dana, lizin-acetilsalicilat nije doveo do promene broja leukocita, limfocita, neutrofila i monocita, u poređenju sa kontrolama ($p>0,05$). Međutim u dozi od 50 mg/kg, t.m., i.p., tokom pet dana, lizin-acetilsalicilat je doveo do statistički signifikantnog povećanja broja eozinofila krvi ($p<0,05$), a u dozi od 50 mg/kg, t.m., i.p., tokom deset dana, lizin-acetilsalicilat je doveo do statistički visoko signifikantnog povećanja broja eozinofila u otiscima slezine ($p<0,01$) u poređenju sa kontrolama. Rezultati sugeriraju da bi male doze aspirina mogle selektivno povećati produkciju i/ili mobilizaciju eozinofila krvi i da bi prostaglandini, produkti metabolizma arahidonske kiseline, mogli inhibirati eozinofilnu produkciju i/ili migraciju *in vivo*.