SEX DIFFERENCES IN RAT PLATELET AGGREGATION INDUCED BY DIFFERENT STIMULI

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Introduction: Platelets are critical mediators of the physiologic response of cessation of bleeding following blood vessel injury as well as pathologic formation of blood clots. There is established evidence of gender differences in the prevalance and severity of vascular thrombosis and/or mortality, with men having a worse prognosis. Furthermore, it is interesting to note that in the literature there are conflicting data on the effect of sex on platelet aggregation. The purinergic system is involved in platelet aggregation, in fact, endothelial cell ecto-nucleoside triphosphate diphosphohydrolase 1, E-NTPDase1(CD39) plays a major role in vascular homeostasis. It rapidly metabolizes adenosine-diphosphate (ADP) released from stimulated platelets, thereby preventing further platelet activation and recruitment. To date there are no data in the literature on the effect of sex on the adenosine pathway. Another metabolic pathway is 12-lipoxygenase (12-LOX), it is mainly expressed in platelets and megakaryocytes and it has been shown that 12-LOX products play a role in the regulation of vascular and non-vascular processes. Recently an important role of 12-LOX in platelet activation has been demonstrated even though its specific role in the regulation of thrombosis is not completely clear.

Materials and methods: Wistar rats of 9weeks of age (sexually mature) of both sexes were used. The animals were anesthetized with enflurane and the blood (about 10 ml) was taken by an intracardiac injection. The blood was centrifuged to obtain a platelet-rich plasma (PRP). Platelet lysates were used to evaluate the expression of enzymes/proteins such as CD39, 12-LOX and cyclooxygenase 1(COX-1) by performing Western blot analysis. Platelets ($3x10^5/\mu l$) were stimulated with ADP or arachidonic acid (AA), at different concentrations, and the percentage of aggregation in the Born aggregometer was evaluated. In another set of experiments platelets were stimulated with ADP (1μM) in presence of the CD39inhibitor, ARL 67156.

Results: Western blot analysis performed on unstimulated platelets showed a greater expression of CD39in female rats than in males, while 12LOX was more pronounced expressed in male rats than in female. There was no sex difference in the expression of COX 1. The data obtained showed that by using ADP (1μM) platelets from male rats aggregated stronger than those from females. When platelets were pre-incubated with the CD39inhibitor ARL, they aggregated as much as the platelets from the male rats. On the other hand, the opposite results were obtained when AA (20 μM) was used as stimulus.

Discussion and conclusion: Our data show that platelets from male rats aggregated more than those of females using ADP and at the same time, when platelets were pre-incubated with ARL, there was a major response to aggregation compared to the vehicle group only in platelets from female rats. Western blot analysis confirmed that platelets from female rats expressed more CD39compared to platelets from male rats. On the contrary, when we used AA (20 μM) platelets from female rats aggregated more than those from males. Western blot analysis showed a greater expression of 12LOX in platelets from male rats while there was no difference in the expression of COX-1in platelets from male and female rats. Further experiments will be carried out to understand the molecular mechanisms underlying this sex differences