Differential Regulation Of Chemokine Expression And Recruitment Of Leukocytes In Females Underlies Sex-differences In Ischaemia/Reperfusion Injury

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Background & Aim: Ischaemia/reperfusion (I/R) injury is implicated in several clinical conditions including stroke and myocardial infarction, conditions where incidence and severity is substantially lower in females. I/R injury is characterised by the mobilisation and recruitment of leukocytes, a pro-inflammatory response that is mediated by several pathways including the release of chemokines. Leukocytes, in particular neutrophils (PMNs) and monocytes, are recruited into the injured tissue during reperfusion through a sequential cascade of rolling, adhesion and emigration. The aim of this study is to determine whether differences in regulation of chemokine generation and leukocyte recruitment are the causal link between female sex and protection from I/R injury.

Methods: A loop of mesentery from male and female Wistar rats (8-10 weeks, 260-300g) was exteriorised and superfused in vivo with Tyrode’s solution (pH7.4, 37°C). Rats were either subjected to 30min mesenteric ischaemia via occlusion of the superior mesenteric artery followed by 2h reperfusion or were sham-operated. Intravital microscopy was used to quantify leukocyte rolling, rolling velocity, adhesion and emigration in mesenteric post-capillary venules at 15min intervals. After 2h reperfusion, blood, peritoneal lavage, and mesenteric tissue were collected. The proportions of blood and peritoneal RP1+ PMNs and ED1+ monocyte/macrophages were assessed by flow cytometry. Real-time qPCR, using SYBR Green, was performed to quantify tissue mRNA expression of chemokines (CCL2/MCP1, CCL3/MIP1α, CCL5/RANTES, CCL7/MCP3, CXCL1/KC, CXCL5/LIX, CXCL12/SDF1α, CX3CL1/fractalkine), and adhesion molecules (ICAM-1, VCAM-1, PECAM-1, JAM-A). Expression of mRNA was normalised to 18S rRNA and calculated as fold-difference relative to expression in sham-operated males. Gut necrosis was determined using p-nitroblue tetrazolium dye and expressed as percentage of total wet weight of a 4cm segment. Data is shown as mean±sem. Comparisons between 2 groups were made using unpaired Student’s t-test, or between multiple groups by 1-way ANOVA, or 2-way ANOVA followed by Bonferroni’s post-test.

Results: Mesenteric I/R stimulated enhanced leukocyte interactions with mesenteric venules in both sexes but whilst leukocyte rolling was significantly less in females as compared to males (23±2.7 and 52±2.6 cells/min at 2h, respectively; P<0.01,n=5-6), there was no sex-difference in rolling velocity (P>0.05). During reperfusion, leukocyte adhesion and emigration through venules was also significantly (P<0.01,n=5-6) lower in females compared to males. PMNs were the predominant cell type recruited in males whilst few PMNs were detected in the peritoneal wash of females. Moreover, I/R had no significant effect on the number of circulating PMNs in females (P>0.05, n=5) but elevated blood PMNs in males (P<0.01,n=6). In contrast, circulating monocytes were increased following I/R in females (from 2±0.6% to 9±0.5%, P<0.001) but not in males (1±0.4% to 2±0.3%, P>0.05). No change in peritoneal monocyte/macrophage numbers was evident in either sex. Gut necrosis was significantly less (P<0.001,n=5-6) in females compared to males (13±1.3% vs. 37±1.2%, respectively).

I/R had no effect on expression of chemokines CCL2, CXCL1 or CXCL5 in female mesenteric tissue but elevated (P<0.001, n=6) their expression in males (8±0.8, 11±0.9, and 28±3.1-fold respectively). In contrast, whilst expression of CCL3, CCL5, CCL7, CXCL12 and CX3CL1 were elevated in both sexes, this effect was significantly (P<0.05,n=5) greater in females compared to males (6±0.9, 23±1.3, 30±5.4, 37±2 and 30±1.9-fold in females compared with 3±0.4, 18±1.4, 4±0.7, 11±0.8 and 8±0.9-fold in males, respectively). I/R also had no effect on expression of ICAM-1, VCAM-1, PECAM-1 and JAM-A in females but elevated their expression (P<0.001, n=6) in males (1±0.4, 3±0.3, 6±0.5 and 4±0.4-fold, respectively). No
significant sex-differences in tissue chemokines or adhesion molecules were evident in sham-operated animals (n=3).

**Conclusion:** Mesenteric I/R-induced PMN recruitment and tissue injury is substantially dampened in female rats. This is associated with a lack of elevation of circulating PMNs, as well as reduced expression of key adhesion molecules that facilitate the interaction of leukocytes with the vasculature and support their extravasation into tissues. Furthermore, selective suppression of chemokines involved in PMN trafficking (CCL2, CXCL1, CXCL5) together with concurrent up-regulation of chemokines predominantly involved in monocyte/lymphocyte recruitment in females may explain the sex-difference in cell types that are mobilised and recruited during reperfusion and underlie profound sex-differences in the extent of I/R injury.

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