Anti-inflammatory Effects of Ischemic Preconditioning on Rat Small Bowel Allografts

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ABSTRACT

Introduction. Minimizing the inflammatory events that follow intestinal transplantation may influence immediate graft function and improve outcome. Ischemic preconditioning (IPc) has been shown to ameliorate early inflammatory responses, and it may also attenuate the potentially damaging inflammation after intestinal transplantation. Herein, we examine the influence of intestinal IPc on inflammatory indices (tissue expression of ICAM-1, CD11a, and CD44 and serum levels of the soluble ICAM-1, sICAM-1) after heterotopic intestinal transplantation.

Methods. Lewis rats received full-length preconditioned or non-preconditioned Brown Norway intestinal allografts in the absence of immunosuppression. Preconditioned grafts were subjected to 1 cycle of 10 minutes of ischemia-reperfusion. Preconditioned and non-preconditioned isografts acted as controls. Blood was collected on alternate days post-transplant, and graft tissue harvested on sacrifice. ICAM-1, CD44, and CD11a expression was determined by immunohistochemistry, and the area of staining was quantified using image analysis. Serum soluble ICAM-1 levels were determined using an R&D Systems Quantikine enzyme immunoassay.

Results. (1) IPc ameliorated serum levels of sICAM-1 until severe rejection (day 7) overcame this down-regulation when compared to non-preconditioned allografts (day 3: 34,404 vs 40,479 pg/mL; day 5: 52,441 vs 61,593 pg/mL; day 7: 75,114 vs 73,309 pg/mL; day 9: 72,872 vs 76,314 pg/mL, respectively). (2) ICAM-1 expression was significantly lower in preconditioned allografts (1.02 vs 2.01 mm²). (3) CD44 tissue levels were also found to be lower in preconditioned allografts (0.86 vs 1.13 mm²). (4) There was a significant relationship between tissue ICAM-1 expression and serum levels of soluble ICAM-1 (P < .02).

Conclusions. IPc improves inflammatory indices in the early stages following intestinal transplantation, and this might lead to a preserved cellular, architectural, and functional graft status. Furthermore, our results support the use of soluble ICAM-1 as a marker of endothelial activation, and thence of inflammation and developing rejection.
impacts on the outcome of clinical intestinal transplantation in the last decade. Nevertheless, recurrent rejection episodes, sepsis, and the inability to control or prevent chronic allograft rejection hamper long-term intestinal engraftment.

Cell adhesion molecules play key roles in recognition of alloantigens and activation and proliferation of allospecific T cells, and also in the recruitment, interaction, and migration of effector cells at inflammatory sites [4]. Similarly, increased levels of several of these molecules have been reported during rejection episodes in clinical transplantation [5–9] as well as in experimental intestinal transplantation [10–13].

Ischemic preconditioning (IPc) has been shown to reduce the severity of local and systemic deleterious effects following intestinal IRI in both transplant and nontransplant models [14–17]. The aim of this study was to investigate the effect of IPc on the expression of the adhesion molecules CD44, CD11a, and ICAM-1 in graft tissue, circulating levels of sICAM-1, and the relationship between tissue and serum levels of ICAM-1 in a rejection model of intestinal transplantation. This study could provide further insight into a potential valuable adjunct to mainstream therapies to improve long-term results of intestinal transplantation.

METHODS
Surgery, Experimental Groups, and Sample Collection
Fully allogeneic heterotopic intestinal transplantation (HITx) without immunosuppression were performed between Brown Norway donors and Lewis recipient male rats weighing 300 g using standard microvascular technique [18]. For this, the intestinal graft was harvested on a vascular pedicle comprising the portal vein and the superior mesenteric artery on an aortic cuff. The vasculature was immersed in Marshall saline at room temperature until processing and parafin embedding. Formalin-fixed, paraffin-embedded graft tissue (5 μm) was transferred to positively charged Superfrost slides (Fisher Scientific Inc) and dried overnight at 37°C. Slides were stained using an indirect immunoperoxidase technique. CD44, CD11a, and ICAM-1 expression was detected using a 1/50 dilution of primary mouse anti-rat CD44 (AbD Serotec, Oxford, UK; catalogue number MCA774GA), CD11a (AbD Serotec; catalogue number MCA773GA), or ICAM-1 (CD54, AbD Serotec; catalogue number MCA773GA) monoclonal antibodies followed by a biotinylated anti-murine IgG secondary antibodies (Vectorstain Elite mouse kit; catalogue no. PK6102). Sections were then incubated with 3,3′-diaminobenzidine (DAB; Vector Laboratories, Inc, Burlingame, United States; DAB Catalogue no. SK4100), and counterstained with hematoxylin (Gills Haematoxylin III Surgipath product no. 01541 Gill III) prior to assessment.

Evaluation of Staining and Statistical Analysis
Counterstained tissue sections were photographed, and a minimum of 140 visual fields were blindly evaluated for each adhesion molecule by 3 independent observers. Expression (ie, intensity of staining) was semiquantitatively assessed using image analysis (“Image J”; National Institutes of Health, United States). For this, images obtained using ×10 objective were converted to gray scale prior to adjusting the threshold to the level of positive staining by removing all background staining. Following this, the positively stained area of pixels was multiplied by 0.54 conversion factor in order to obtain a final area of staining in square micrometers (μm²). Results were expressed as mean ± standard error of the mean (SEM) of at least 4 animals per group.

All data were analyzed using the analysis of variance. Given that variables followed a normal distribution, parametric tests were used. Differences between the groups were compared using Windows SPSS version 10.0 statistical package and were considered to be of statistical significance when P < .05.

RESULTS
IPc Reduces ICAM-1 Expression
IPc significantly reduced ICAM-1 expression in isografts (treated = 0.41 μm² [SEM = 0.13] vs untreated = 0.63 μm² [SEM = 0.29]) and allografts (treated = 1.02 μm² [SEM = 0.08] vs untreated = 2.01 μm² [SEM = 0.26]; P < .001; Fig 1). The effect of IPc on the expression of ICAM-1 in isografts may represent protection from IRI, whereas the improved inflammatory status in allografts may translate into a less intense host immune response and consequently decreased risk of rejection and improved functional outcome.
Herein we report that intestinal IPc significantly down-regulates the tissue expression of CD44 (by 24%) and ICAM-1 (by 50%) and transiently reduces increases in circulating sICAM levels that are induced following intestinal allografting in the absence of immunosuppression. Intestinal IPc influences the effects of IRI by attenuating deleterious systemic inflammatory responses [19–21] and controls tissue damage induced by proinflammatory mediators via the reduction of oxidative stress [15,22–27], improving microvascular dynamics [28,29], promoting cytoprotection [30,31], and down-regulating the expression of adhesion molecules [32], humoral responses [33], and the genetic response to injury [27,34,35].

Evidence suggests that the inflammatory mediators that are induced by innate mechanisms in the early stages after intestinal transplantation are further amplified by the subsequent adaptive response [36,37]. It has also been suggested that CD8+ T cells may be the link between innate and adaptive immune responses [38]. In this context, an association between ICAM-1 overexpression and increased apoptosis and levels of infiltrating CD8+ T cells and allograft rejection has been reported in an experimental model of intestinal transplantation [39].

Furthermore, high levels of sICAM-1 have been reported in transplant recipients [40,41]. This phenomenon might relate to the subsequent development of transplant-associated vasculopathy [42] and allograft rejection [43–45].

In summary, this study provides further evidence of the multifaceted anti-inflammatory drive of IPc upon allografted tissue and supports its future consideration as an adjunct to mainstay therapies.

**REFERENCES**


ANTI-INFLAMMATORY EFFECTS OF ISCHEMIC PRECONDITIONING


