A Clinical Trial of Cyclical Ischaemic Preconditioning to Modulate the Adaptive Immune Response in Human Limb Ischaemia Reperfusion Injury.

Abstract

Introduction

Reperfusion injury (RI) has potentially significant local and systemic consequences; ischaemic preconditioning (IP) modulates RI and the innate immune response. This prospective, randomised, controlled trial examines RI and IP on patient lymphocyte populations and function following elective surgery.

Methods

The study was approved by the Galway Regional Hospitals Ethics Committee. Participants were recruited from consecutive healthy adults undergoing anterior cruciate ligament repair. 25 patients were consented and randomised to preconditioned (n=12) or non-preconditioned (n=13) groups.

Preoperatively the limb was exsanguinated and in the preconditioned groups a tourniquet was inflated. Inflation was maintained for 5 minutes and then released for 5 minutes to allow for reperfusion. Three cycles of preconditioning were performed. The operative tourniquet was applied for the duration of the procedure. Venous blood was obtained at baseline and post-operatively at 4 and 24 hours after reperfusion.

Whole heparinised blood was processed within one hour. Antibody staining was carried out for flow cytometry. Samples were stained for expression of CD3, CD4 &CD8. The ratio of CD4 to CD8 T cells was calculated. Whole blood samples were stained for CD45RO, CD45RA, CD62L& CD95.

Heparinised whole venous blood was collected from healthy volunteers. Peripheral blood mononuclear cells were isolated and incubated for with either RPMI, healthy control serum or serum from study patients. Lymphocyte stimulators PMA and Ionomycin were added with brefeldin A. Cells were surface stained monoclonal antibodies (CD3, CD4, CD8, CD69). The relevant anti-cytokine antibody (INF, IL-2, IL-4, IL-10) was added. The percentages of lymphocytes expressing CD4 or CD8 were calculated and the numbers expressing each cytokine measured by flow cytometry to determine if post-operative serum from IP patients would alter the numbers of helper and cytotoxic lymphocytes present or differential cytokine production. The experiment was repeated in the presence of a recombinant human CTLA-4/Fc chimera to assess the antigenic effect of patient serum on volunteer PBMCs. Serum samples were also collected from patients and ELISA for IL-2 and IL-4 was carried out.

Data analysis used Kruskal Wallis tests and Squared Ranks Variance tests or Dwass Steel-Chritchlow-Fligner method. Differences were assessed using unpaired Student t or Mann Whitney U Significance value P value of <0.05

Results

Mean Perioperative T Cell Populations.

(A) Reduced CD4 expression at 4hrs and 24hrs post-operatively versus baseline in preconditioned patients. Increased CD4 expression in non-preconditioned patients T cells. (B) Increased CD8 expression in non-preconditioned patients at 24 hours versus baseline §. (C) Increased ratio in non-preconditioned patients at 4hrs and 24hrs post-operatively respectively.

Mean T Cell Populations from PBMC culture experiments

At baseline there was no difference in the percentage of CD45RO+ T cells between groups. At 24 hours there were significantly greater numbers of helper cells expressing CD45RO amongst non-preconditioned patients compared to pre-op levels. Cells expressing CD45RO dropped in the preconditioned group at 4 hours and by 24 hours returned to near pre-op levels. While non-preconditioned patients had more CD45RO+ cells at 24 hours the difference compared to preconditioned patients was not significant. At 4 hours, preconditioned patients had a decrease in the number of CD45RO+ lymphocytes; non-preconditioned patients had a similar increase. At 24 hours significant differences could be seen between groups as non-preconditioned patients dropped their expressing cell numbers and preconditioned patients had a significant increase.

There were no differences in baseline CD95 expression. The preconditioned group had an initial drop in helper cell numbers expressing CD95; however, this was then followed by a significant increase at 24 hours compared to 4 hours. At 24 hours differences in cell numbers expressing CD95 between groups had widened to reach significance. Both groups had an insignificant drop in CD95 expression on cytotoxic cells at 4 hours. At 24 hours both groups had a significant rise in CD95 numbers compared to baseline. No differences were detected in numbers of T cells expressing CD45RA or CD62L.

Intracellular cytokines expression in volunteer T cell populations cultured with post-reperfusion patient serum.

There were significant differences INFγ productions after co-culture with post-reperfusion serum, from both study groups. Serum obtained from non-preconditioned patients increased the IL-2 producing cells after co-culture compared to control or preconditioned serum. Cytotoxic cells failed to show such changes between groups.

Serum from both study groups reduced the number of T helper cells producing IL-4. Serum from reperfused patients reduced the numbers of cytotoxic cells producing IL-4 (non significant).

Analysis of Serum Cytokine Levels.

There were no significant changes in IL-2 concentrations peri-operatively. There was a significant difference between preconditioned and non-preconditioned patients at 24hrs post-reperfusion. Analysis of IL-4 levels showed no significant changes in mean cytokine concentrations across the experiment.

Discussion

IP induces T cell modulation in limb RI through reduced activation, pro-inflammatory cytokine production and secretion by CD4 cells while preventing CD4/CD8 derangements. IP prevents lymphocyte directed immune dysfunction in surgical (RI).