Human adaptation to ischemia by preconditioning or unstable angina: involvement of nuclear factor kappa B, but not hypoxia-inducible factor 1 alpha in the heart

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Abstract

Objective: Ischemic preconditioning reduces infarct size and improves hemodynamic function. Unstable angina may be a clinical analogue to ischemic preconditioning, and involve activation of gene programs. We hypothesized that preceding unstable angina and/or ischemic preconditioning activated genes regulated by nuclear factor kappa B (NFκB) or hypoxia-inducible factor 1 alpha in parallel to improved cardiac function. Methods: Patients undergoing coronary artery bypass grafting with stable or unstable angina were subjected to ischemic preconditioning or sham treatment (n = 10–11 in each group). Central hemodynamics were monitored. Left ventricular and atrial biopsies were harvested before cardioplegic arrest and after 25 min of reperfusion. Expression of heat shock protein 72 was evaluated by immunoblot, and activation of NFκB was detected by electrophoretic mobility shift assay. Real-time PCR was used to quantify expression of genes regulated by NFκB (inducible nitric oxide synthase, tumor necrosis factor-alpha, intercellular adhesion molecule 1) or by hypoxia-inducible factor 1 alpha (heme oxygenase-1, glucose transporter-1 and vascular endothelial growth factor-A). Results: Ischemic preconditioning improved postoperative cardiac index and left ventricular stroke work index in both stable and unstable groups on the first postoperative day. Expression of hypoxia-inducible factor 1 alpha regulated genes was not influenced by cardioplegia and reperfusion, ischemic preconditioning or unstable angina. Expression of the NFκB-regulated genes increased after cardioplegia and reperfusion, but this was not influenced by ischemic preconditioning in stable patients. Inducible nitric oxide synthase and tumor necrosis factor expression were reduced after ischemic preconditioning in patients with unstable angina. There were no significant differences in gene expression between stable and unstable patients before cardioplegia and ischemic preconditioning. NFκB translocation at reperfusion was reduced in stable preconditioned and unstable control patients compared to stable controls. Heat shock protein 72 expression increased after preconditioning of unstable patients. Conclusion: Cardiac function was improved by ischemic preconditioning in both stable and unstable patients. Unstable angina per se had no effect. NFκB-regulated genes were influenced by ischemic preconditioning, but hypoxia-inducible genes were not.

Keywords: Ischemic preconditioning; Gene expression; Human; Unstable angina

1. Introduction

Ischemic preconditioning reduces infarct size in experimental animals and improves hemodynamic recovery [1]. It provides a biphasic protection, an immediate protection during the first 2–3 h and a delayed protection coming after 24 h lasting for 2–3 days. Ischemic preconditioning is cardioprotective in humans and improves cardiovascular performance after open heart surgery [2–4]. The mechanisms underlying ischemic preconditioning are complex. Evidence suggests that the transcription factor nuclear factor kappa B (NFκB) may be important for evoking both immediate and delayed protection [5,6]. NFκB is considered to be a central regulator of innate and adaptive immunity with hundreds of target genes, some with proinflammatory effects and some promoting cell survival [7]. Among the NFκB-regulated genes are inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α) and intercellular adhesion molecule-1 (ICAM-1). More recently, the transcription factor hypoxia-inducible factor 1 alpha (HIF-1α) was found to play a role in protecting rodent...
Ischemia-reperfusion injury after cardioplegia induces an inflammatory response [14] which in animal experiments is reduced by ischemic preconditioning [15]. Some evidence suggests that adaptation to ischemia by unstable angina may be a clinical analogue to preconditioning [16]. We have previously shown that unstable angina prior to heart surgery provided cardioprotection [17]. Furthermore preoperative unstable angina caused a venous adaptation to surgical graft injury [18]. The purpose of the present study was to investigate whether expression of some genes shown to be involved in the processes of myocardial inflammation or cardioprotection in animals, and that are regulated by the transcription factors NFκB and HIF-1α, was influenced by ischemic preconditioning and/or unstable angina in patients undergoing coronary artery bypass grafting (CABG).

2. Materials and methods

The study design was in agreement with the ethical standards laid down in the 1964 Declaration of Helsinki, and was accepted by the ethics committee of Tampere University Hospital, Finland. Informed consent was obtained from all patients.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Stable control, N=11</th>
<th>Stable preconditioned, N=11</th>
<th>Unstable control, N=11</th>
<th>Unstable preconditioned, N=10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68 ± 8</td>
<td>64 ± 7</td>
<td>70 ± 9</td>
<td>64 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>3/8</td>
<td>2/9</td>
<td>7/4</td>
<td>2/8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NYHA class</td>
<td>2.8 ± 0.6</td>
<td>2.8 ± 0.6</td>
<td>3.4 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cross-clamping time (min)</td>
<td>59 ± 13</td>
<td>63 ± 13</td>
<td>64 ± 17</td>
<td>68 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>110 ± 20</td>
<td>115 ± 23</td>
<td>111 ± 24</td>
<td>120 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Number of bypasses</td>
<td>3.3 ± 0.8</td>
<td>3.2 ± 0.6</td>
<td>3.0 ± 0.4</td>
<td>3.4 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>MI history (n)</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Inotropic support</td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urinary output (ml)</td>
<td>3146 ± 75</td>
<td>2806 ± 593</td>
<td>2855 ± 1041</td>
<td>2824 ± 672</td>
<td>NS</td>
</tr>
<tr>
<td>Renal function (creatinine)</td>
<td>88 ± 23</td>
<td>81 ± 17</td>
<td>100 ± 26</td>
<td>88 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>ICU stay (h)</td>
<td>78.1 ± 23.3</td>
<td>28.4 ± 17.1</td>
<td>45.9 ± 45.9</td>
<td>53.1 ± 18.3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Clinical data are presented as mean ± SD or number/group; NYHA: New York Heart Association; CPB: cardiopulmonary bypass; MI: myocardial infarction; ICU: intensive care unit.
CPB was conducted with non-pulsatile perfusion flow (2.2—2.4 l/min m²) and mild hypothermia (32°C), using membrane oxygenators with arterial line filtration. Blood from the pump reservoir was mixed with crystalloid solution in a ratio of 4:1, yielding a cardioplegia solution with 21 mmol/l potassium concentration in the initial dose and 9 mmol/l in subsequent doses. Combined antegrade and retrograde cold (6—9°C) cardioplegia were used. In antegrade delivery cardioplegia was administered at a pressure of 80 mmHg and in retrograde delivery at a pressure of 30—40 mmHg, with a minimum flow of 200 ml/min. The initial high-potassium cardioplegia was given 1.5 min antegrade, then 2.5 min retrogradely. Reinfusion of cardioplegia was administered for 1 min with retrograde delivery and to the vein grafts upon completion of each distal anastomosis. Warm cardioplegia (37°C) was given regradedly for 3 min before releasing the aortic cross-clamp.

2.4. Hemodynamic measurements

Central hemodynamics were measured using a Swan-Ganz catheter on the first postoperative day. Heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO) and right ventricular ejection fraction (RVEF) were monitored. Perioperatively, filling pressures were maintained at least at the preoperative levels. Derived variables such as cardiac index (CI), stroke index (SI), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI) and left ventricular stroke work index (LVSWI) were calculated using standard formulas. Measurements of CO were based on the thermodilution method and values represent an average of three consecutive measurements at end expiration. At the same time, urinary output, creatinine for monitoring basic renal function, the need for inotropic support and duration of stay in the intensive care unit (ICU) were noted.

2.5. Sample collection

Left ventricular biopsies (15—20 mg by a Tru-cut needle) and left atrial tissue (60—100 mg) were sampled before cross-clamping (before either preconditioning or sham treatment) and after 25 min of reperfusion (from patients at the Tampere University Hospital, Tampere, Finland). This procedure of taking biopsies is well established at the Tampere University Hospital, and no adverse effects are observed in the patients. Samples were collected under RNase-free conditions, snap-frozen in the operating room, and stored at −80°C. No adverse effects were seen from taking biopsies. The samples were processed at the Karolinska Institutet, Stockholm, Sweden and at the University of Oslo, Oslo, Norway.

2.6. Subcellular protein extraction

After weighing the frozen left atrial samples, they were homogenized with mortar and pestle using liquid nitrogen. They were resuspended in lysis buffer with a final concentration of 200 mM mannitol, 70 mM sucrose, 1 mM EDTA, 10 mM Hepes pH 7.5, 50 mM NaF, 2 mM Na₂VO₄, 10 mM benzamidin, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin, 10 μM transepoxy succinyl-l-leucylamido (4-guanido)-butane, 30 mM β-glycerophosphate and 0.5 mM PMSF. Then the samples were centrifuged at 500 × g for 5 min. The pellet corresponds to the crude nuclear fraction and the supernatant contains the cytosolic, mitochondrial and microsomal fractions. The pellet was resuspended, and centrifuged at 5000 × g for 10 min in a buffer containing 10 mM Hepes pH 7.5, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 50 mM NaF, 2 mM Na₂VO₄, 10 mM benzamidin, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin, 10 μM transepoxy succinyl-l-leucylamido (4-guanido)-butane, 30 mM β-glycerophosphate, 1 mM DTT and 0.5 mM PMSF. The nuclear membrane was opened with Nonidet P-40 and samples were washed twice. Finally nuclear fractions were incubated for 30 min with a buffer containing 20 mM Hepes pH 7.5, 400 mM NaCl, 1 mM of each EDTA and EGTA plus the same protease inhibitors and they were centrifuged at 14,000 × g for 5 min. The resultant supernatant was the nuclear fraction. The original supernatant containing the cytosolic, mitochondrial and microsomal fractions was centrifuged at 100,000 × g for 10 min. The resultant supernatant was centrifuged at 100,000 × g for 2 h, and this supernatant corresponds to the cytosolic fraction. It was precipitated with 10 volumes of ice-cold methanol and redissolved in lysis buffer. All centrifugation steps were carried out at +4°C and samples were kept on ice between centrifugations. Subsequently, protein concentrations were measured with BCA-assy and cytosolic fractions were boiled with Laemml buffer. By the end, there were only 3—4 sample pairs per group that were found sufficient for subsequent immunoblot and electrophoretic mobility shift assay (EMSA) experiments.

2.7. Immunoblot

Twenty micrograms of cytosolic proteins were separated on a 7% polyacrylamide gel and were subsequently transferred to a nitrocellulose membrane (Amersham). Equal loading was checked with Ponceau-staining, and membranes were blocked with 5% milk in PBS-Tween. Membranes were incubated with 1:1000 mouse anti-HSP72 antibody (Stressgen) overnight, the day after incubated with goat anti-mouse secondary antibody (Pierce) diluted 1:1000, and developed using the ECL-kit (Amersham). Samples of three patients per group were used for immunoblots. Optical density of immunoblot bands was measured and the corresponding Ponceau-staining was used as loading control. Then expression values after reperfusion were divided by the expression values before cross-clamping and all resulting values were divided by one representative (mean) normalized value gained from a stable control patient. The resultant ratio corresponds to the change between the two time-points.

2.8. Electrophoretic mobility shift assay

EMSA was performed in nuclear protein fractions from atrial samples as previously described in detail [5,14]. Briefly, 8 μg of nuclear extracts were preincubated with binding buffer and 32P-labelled probe containing NFk8 binding site (Promega). DNA—protein complexes were electrophoresed on a 4% polyacrylamide gel. For supershift assays antibodies
against the p50 and p65 subunits of NFkB were used (Santa Cruz Biotechnology) and for competition assays unlabelled probes in 66-fold excess were applied. An unlabelled AP-1 probe (Promega) was used as a non-sense competition assay. Samples of four patients per group were used for EMSA. Calculations were carried out in the same way as for immunoblots.

2.9. RNA extraction

Total RNA was isolated from left ventricular biopsies using the RNeasy Mini Kit (QIAGEN Inc.) with an additional phenol–chloroform (Sigma) extraction step and in-column DNase treatment (QIAGEN). The quantity of RNA was calculated from adsorption at 260 nm with spectrophotometer. A260/280 ratio between 1.7 and 2.1 was accepted. Integrity of RNA was estimated on a 0.8% agarose gel. The extracts were stored at −80°C until usage.

2.10. cDNA synthesis

0.5 μg of RNA was reverse transcribed using random hexamers for priming (3 min at 70°C) followed by a modified First Strand cDNA Synthesis Protocol with Superscript II (Invitrogen) and RNasin (Promega) enzymes (10 min at 25°C, 50 min at 42°C and 4 min at 94°C).

2.11. Real-time PCR

Real-time PCR was carried out using the 5′–3′ nuclease activity of the Taq DNA polymerase enzyme based on the release and subsequent direct detection of a fluorescent reporter dye of probes. Primers and probes were designed using the Primer Express software and custom made (PE Applied Biosystems) on the basis of published human cDNA sequences. All primers and probes were designed to span exon–exon junctions, where appropriate (Table 2). To evaluate choice of endogenous control gene we performed a pilot study using the Taqman Human Endogenous Control Plate (PE Applied Biosystems) and thereafter 18S rRNA was selected as the most stable in our conditions (data not shown). Furthermore, 18S rRNA was used to verify the efficacy of reverse transcription and to normalize for any loading variations of cDNA.

Reactions took place in a MicroAmp Optical 96-well plates (PE Applied Biosystems) using 2 μL of cDNA, 12.5 μL of Universal Master Mix (PE Applied Biosystems), primers at a final concentration of 900 nM and probes at a final concentration of 200 nM. The total reaction volume was 25 μL and the samples were run in duplicates. The PCR reaction had a standard amplification scheme: one cycle of 2 min at 50°C (AmpEraser UNG activation), one cycle of 10 min at 95°C (Gold AmpliTaq activation, AmpEraser UNG inactivation), followed by 40 cycles of denaturation for 15 s at 95°C and annealing/extension for 1 min at 60°C. ABI 7700 Sequence Detection System (PE Applied Biosystems) was used to run PCR reactions.

2.12. Relative quantitation of mRNA

Gene expression analysis was performed on samples from nine patients in each group. Relative quantification of gene expression was carried out following the relative standard curve method (User Bulletin #2; PE Applied Biosystems). Briefly, first, threshold cycle values of target genes and endogenous controls were averaged (duplicates). The target gene cDNA was normalized to endogenous control cDNA and then to a normalized calibrator, which was a pair of samples taken from stable control patients. Gene expression after reperfusion was then divided by gene expression before cross-clamping to see the relative change between the two time-points.

2.13. Statistics

Student’s t-test (two-tailed) for independent groups was used for continuous variables. Chi square test was employed for categorical data for comparisons between two groups. One-way ANOVA was applied to compare single time-points of hemodynamics between groups. Expression data (immunoblot, electrophoretic mobility shift assay, real-time PCR) are presented as relative to a ratio of expression after declamping/expression before cross-clamping in relation to the stable control group. Assuming a non-Gaussian distribution of data, the non-parametric Mann–Whitney U test was used to evaluate normalized expression data for inter-group comparisons. Wilcoxon matched pairs test was applied to compare the effect of cardioplegia and reperfusion per se within each group. A simple regression analysis between time of cardioplegic arrest and gene expression was performed.

For all parameters within each group (stable or unstable) the preconditioned group is compared with its control. Furthermore within each group (stable or unstable) the control patients or the preconditioned patients are compared to the corresponding patients in the other group. There is always a problem with multiple comparisons; in the present study two comparisons are sometimes performed and then a
A p value of 0.025 is needed when a Bonferroni correction is done. Values are presented as mean ± SD. Differences were considered significant when p < 0.05.

3. Results

3.1. Outcome of surgery

There were no perioperative myocardial infarctions or postoperative deaths. There was one case of late cardiac tamponade in the stable control group, one stroke in stable preconditioned patients, no complication in the unstable control group, and one case of late cardiac tamponade and one deep sternal infection in unstable, preconditioned patients.

There was no difference between groups in urinary output and basic renal function on the first postoperative day (Table 1). Preconditioning reduced the need for inotropic support in stable patients, but not in unstable ones. Similarly, the stay in ICU was significantly reduced by preconditioning in stable, but not in unstable patients (Table 1).

3.2. Cardiovascular performance

There were no differences between groups in basal, preoperative hemodynamics (data not shown). On the first postoperative day differences between groups were found in four parameters: HR, MAP, CI, and L VSWI (Fig. 1). Postoperatively, HR was slower in unstable control patients than in the stable ones, however, preconditioning increased HR in unstable patients. MAP was lower in unstable control patients compared to stable controls, but it was higher in unstable preconditioned patients compared to control patients with unstable angina (Fig. 1). Postoperative CI tended to be lower in unstable control patients than in the stable control group. Preconditioning increased CI both in stable and unstable patients. L VSWI was increased by preconditioning in both stable and unstable patients (Fig. 1).

3.3. Heat shock protein 72

To check for the presence of the cardioprotective factor HSP72, we used the cytosolic fractions of left atrial samples analyzed by immunoblot. Three sample pairs per group were run and the results shown as ratio between after and before cardioplegic arrest are shown in Fig. 2 (baseline values are given in Table 3). When the baseline values of HSP72 expression of all unstable patients were compared with all stable, the expression was higher (p = 0.016). Preconditioning increased the expression of HSP72 in unstable patients, but did not influence the expression in stable patients (Fig. 2).

3.4. Nuclear factor kappa B

Nuclear translocation and DNA-binding activity of NFκB was investigated with EMSA from nuclear protein fractions...
For number of experiments please see text. Data are presented as mean ± SD. No important difference is detected between any groups.

### Table 3

Pre-cardioplegic expression data in patients with or without preceding unstable angina scheduled for coronary artery bypass surgery randomized for either ischemic preconditioning or sham treatment.

<table>
<thead>
<tr>
<th></th>
<th>Stable control</th>
<th>Stable preconditioned</th>
<th>Unstable control</th>
<th>Unstable preconditioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP72</td>
<td>6.8 ± 4.8</td>
<td>7.5 ± 5.1</td>
<td>9.6 ± 6.5</td>
<td>8.8 ± 6.7</td>
</tr>
<tr>
<td>NFκB</td>
<td>3.3 ± 1.0</td>
<td>3.1 ± 0.9</td>
<td>3.5 ± 1.3</td>
<td>3.2 ± 1.6</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.7 ± 0.5</td>
<td>0.9 ± 1.1</td>
<td>1.4 ± 1.5</td>
<td>1.2 ± 2.1</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>0.46 ± 0.23</td>
<td>0.54 ± 0.31</td>
<td>1.54 ± 1.73</td>
<td>0.63 ± 0.75</td>
</tr>
<tr>
<td>INOS</td>
<td>1.60 ± 1.25</td>
<td>1.81 ± 1.95</td>
<td>1.76 ± 2.15</td>
<td>1.26 ± 0.93</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>0.89 ± 0.68</td>
<td>0.60 ± 0.15</td>
<td>0.78 ± 0.44</td>
<td>0.63 ± 0.42</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>2.04 ± 1.54</td>
<td>2.58 ± 2.68</td>
<td>1.87 ± 2.2</td>
<td>1.31 ± 1.27</td>
</tr>
<tr>
<td>HMOX-1</td>
<td>0.42 ± 0.27</td>
<td>0.35 ± 0.21</td>
<td>0.57 ± 0.27</td>
<td>1.26 ± 2.73</td>
</tr>
<tr>
<td>GLUT-1</td>
<td>3.19 ± 1.75</td>
<td>3.00 ± 1.86</td>
<td>3.95 ± 2.77</td>
<td>2.36 ± 1.78</td>
</tr>
</tbody>
</table>

For number of experiments please see text. Data are presented as mean ± SD. No important difference is detected between any groups.

### 3.5. Gene expression

Genes regulated mainly by NFκB and HIF-1α were investigated with real-time PCR from left ventricular samples (Fig. 4, Table 3). Gene expression of HIF-1α was not altered by cardioplegia-reperfusion, ischemic preconditioning or unstable angina (Fig. 4). There was no important difference in the gene expression of the HIF-1α-regulated genes HOX-1, GLUT-1 and VEGF-A after post-cardioplegic reperfusion, and no difference between groups (Fig. 4). Relative expression of TNF-α (Fig. 5) increased by cardioplegia and reperfusion in all groups ($p < 0.05$, respectively, shown as fold-induction of baseline). TNF-α gene expression was not significantly influenced by preconditioning in stable patients, but ischemic preconditioning reduced expression of TNF-α in unstable patients ($p = 0.031$). Expression of ICAM-1 (Fig. 5)
Fig. 4. Gene expression of hypoxia-inducible factor 1 alpha (HIF-1α), vascular endothelial growth factor-A (VEGF-A), heme oxygenase-1 (HOX-1) and glucose transporter-1 (GLUT-1) in left ventricular biopsies from patients undergoing coronary artery bypass surgery with aortic cross-clamping and cardiopulmonary bypass. Gene expression after reperfusion was related to the corresponding gene expression before cross-clamping. Expression of these genes did not change by reperfusion, unstable angina and/or ischemic preconditioning and there was no difference between groups. Stable controls (SC), stable preconditioned patients (SP), unstable controls (UC) and unstable preconditioned patients (UP) are shown. Values are in mean ± SD of nine observations in each group.

Fig. 5. Gene expression of tumor necrosis factor-alpha (TNF-α), intercellular adhesion molecule 1 (ICAM-1) and inducible nitric oxide synthase (iNOS) in left ventricular biopsies from patients undergoing coronary artery bypass surgery with aortic cross-clamping and cardiopulmonary bypass. Gene expression after reperfusion was related to the corresponding gene expression before cross-clamping. TNF-α and ICAM-1 expressions were increased by cardioplegia and reperfusion (p < 0.05 except in unstable controls, respectively). Gene expression of iNOS was not upregulated. TNF-α and INOS expressions were reduced in unstable preconditioned patients versus unstable controls, yet ICAM-1 showed the opposite change between the same two groups. Stable groups did not show differences in gene expression of TNF-α, INOS and ICAM-1 by preconditioning. Stable controls (SC), stable preconditioned patients (SP), unstable controls (UC) and unstable preconditioned patients (UP). Values are in mean ± SD of nine observations in each group.
was increased by cardioplegia and reperfusion \( (p < 0.05 \) for all groups except for unstable control patients). Preconditioning did not influence cardiac ICAM-1 expression during reperfusion in stable patients \( (p = 0.12 \) ), but it increased ICAM-1 expression in unstable patients \( (p = 0.001 \) ). Gene expression of iNOS was not influenced by cardioplegia and reperfusion \( (p = 0.10 \) ), but it was reduced after ischemic preconditioning of unstable patients compared to unstable controls. In stable patients preconditioning had no effect on iNOS expression \( (p = 0.05 \) ). There was no correlation between gene expression and time for cross-clamping or cardiopulmonary bypass \( (p = 0.05 \) ).

4. Discussion

In the present study we investigated some molecular changes accompanying cardioprotection induced by ischemic preconditioning and preceding unstable angina in CABG patients. This investigation was not primarily designed to investigate long-term clinical outcome. In depth analysis of hemodynamics has been extensively studied before [2,4,16,17,19]. In the present study ischemic preconditioning improved cardiac function evaluated by CI and LSVWI, confirming findings by others \( (\text{ref.} \) above). In patients with stable, but not unstable angina, ischemic preconditioning reduced the need for inotropic support and reduced the stay in ICU. The same model of preconditioning was previously shown to reduce release of cardiac troponin T postoperatively \( [2—4] \). In a prior study \( [17] \) it was found that unstable angina within 3 days before CABG improved postoperative contractile function evaluated as CI for up to 6 h, whereas LSVWI was not significantly improved \( [17] \). However, we did not find any improved cardiovascular performance on the first postoperative day in patients with unstable angina. Possibly the protection afforded by preoperative unstable angina has a limited cardioprotective ability and it is outpowered by the ‘true’ ischemic preconditioning. The low number of patients as well as hemodynamic evaluation at a single postoperative time-point may explain why no protection was found in the present study by unstable angina. Moreover, evidence for stunned myocardium (reversible loss of ventricular function in the absence of cell death) has been observed in patients with unstable angina \( [20] \). Thus, unstable angina may potentially be both protective and detrimental, presumably influenced by the time frame since onset of angina \( [20] \) and the intensity of the ischemic episode.

4.1. Nuclear factor kappa B

Involvement of NFkB in both ischemia-reperfusion injury and protective pathways has previously been observed \( [5—7] \). In the present study expression of the primarily NFkB-regulated genes TNF- \( \alpha \) and ICAM-1 was increased by cardioprotection and reperfusion, which is in accordance with previous findings \( [14,21] \). Expression of neither TNF- \( \alpha \) nor ICAM-1 was influenced by ischemic preconditioning in stable patients. However, ischemic preconditioning reduced nuclear translocation of NFkB during reperfusion in stable patients. Reduced NFkB translocation during reperfusion \( [5] \) and reduced expression of NFkB-regulated genes \( [15] \) have previously been found after ischemic preconditioning in rat hearts. The fact that reduced NFkB transactivation was not accompanied by changes in gene expression in the present study may be due to the short time frame between the two samples \( (\text{after only 25 min of reperfusion}) \). In contrast, preconditioning of unstable patients decreased gene expression of TNF- \( \alpha \) and iNOS. Since this was not associated with a reduced NFkB translocation, other transcriptional regulators may be involved.

4.2. Heat shock protein 72 and nitric oxide

HSP72 is a molecular chaperone and it is known to reduce NFkB translocation and DNA-binding \( [7] \). We have previously found that patients with unstable angina have an increased atrial expression of HSP72 \( [22] \). In support of this, the patients with unstable angina had a higher baseline HSP72 expression than the stable patients. The change of HSP72 expression after, compared with before, cardioprotective treatment however was higher in preconditioned patients with unstable angina compared to unstable patients without ischemic preconditioning. Patients with unstable angina and no preconditioning had no increase in HSP72 compared to stable patients without preconditioning. We are at loss to explain why the unstable patients, who had a high baseline HSP72 expression, increased relatively more than the stable patients with lower expression. However, the increased HSP72 expression in the unstable patients may explain the loss of NFkB translocation during post-cardioprotective reperfusion in these patients.

Nitric oxide has been implicated both as a trigger and as a mediator of preconditioning \( [1] \). In the present study we found downregulation of iNOS in unstable, preconditioned patients after 25 min of reperfusion compared to unstable controls. Despite the fact that patients with unstable angina were pretreated with intravenous nitroglycerin, their cardiovascular performance did not improve compared to stable controls, which is in contrast with an earlier report \( [23] \). This may be due to the nitroglycerin treatment regimen applied \( (\text{continuous in our study vs intermittent in the previous}) \), which may influence NFkB activation as well as NO action.

4.3. Hypoxia-inducible factor 1a

HIF-1 \( \alpha \) plays an essential role in physiological responses elicited by chronic hypoxia or cardioprotection evoked by intermittent hypoxia \( [8] \). It has been speculated that HIF-1 \( \alpha \) through its target genes (erythropoietin, VEGF-A, HOX-1) may play a role in ischemic as well as hypoxic preconditioning \( [8—10] \). However, the hypoxia-inducible genes were unaffected by both ischemic preconditioning and unstable angina. This is either a true finding or represents a limitation of the study. The first possibility is indirectly supported by the fact that lactate (a product of a HIF-1 target gene lactate dehydrogenase) production was not increased by preconditioning in CABG patients \( [24] \). However, a recent study in mice indicates a crucial dependence of the acute phase of ischemic preconditioning on HIF-1 \( \alpha \), as HIF-1 \( \alpha \) heterozygous knockout mice did not benefit from the postischemic
functional protection and infarct-sparing effect of preconditioning [25]. As HIF-1α and a few of its target genes remained unchanged by ischemic preconditioning or/and unstable angina, at this point it is not possible to tell whether HIF-1α plays a role in the protection afforded by ischemic preconditioning in humans.

4.4. Limitations of the present study

The small groups, especially for protein experiments and the randomization of patients for ischemic preconditioning or sham treatment, may hide hemodynamic differences as mentioned above. Some of the non-significant differences in gene expression may theoretically be caused by the low number of patients, because absence of evidence may not necessarily be evidence of absence. Furthermore, the time frame of taking biopsies was restricted to the clinically and ethically acceptable period, before weaning off bypass and decannulation. The biopsy site was standardized, but heterogeneity of pathology in the biopsy specimen cannot be excluded. Thus, possible larger changes of both protein and gene expression at a later stage of reperfusion may have been missed.

4.5. Implications

The goal of preconditioning research is to find molecular targets that can be specifically stimulated or blocked to bring a protective state when needed. Our findings corroborated some results from animal studies and provided molecular insight into human myocardial pathophysiology.

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References


