ABSTRACT

Preconditioning (PC) is a process where cells or tissues exposed to a sublethal stimulus are transiently protected from a subsequent normal lethal stress. Many forms of preconditioning have been investigated, such as ischemic, thermal, pharmacologic, or gas inhalation. The present study aimed to study the preconditioning effect of sevoflurane as a prophylaxis against ischemia reperfusion injury of the lower limb skeletal muscles after tourniquet deflation. The study was carried out on 40 adult patients. The patients were randomly assigned into two equal groups, each one including 20 patients. Group I: received sevoflurane anesthesia. Group II: received spinal anesthesia using heavy bupivacaine 15 mg. Biochemical parameters of muscle injury including serum CPK, AST, K, Ca, ABGs, lactic acid, IL-6 and TNF(alpha) were compared between both groups before induction of anaesthesia, and 5 min after deflation of the tourniquet.

Results: Both groups showed an increase in CPK and AST levels 5 minutes after tourniquet deflation but the increase was significantly higher in group II; CPK was 61.1+13.6 IU/L in the sevoflurane group versus 76.2+11.4 IU/L in the control(spinal) group. Also AST was 28.4+6.9 IU/L in the sevoflurane group versus 55.5+6.3IU/L in the control group. Both groups developed increase in serum K 5 min. after tourniquet deflation and the increase was significantly higher in the control group; serum K was 4.4+0.71 meq/L in the sevoflurane group vs. 5.1+0.4 meq/L in the control group. Both groups showed an increase in serum Ca level 5 minutes after tourniquet deflation but there was no significant difference between them. There were no significant differences in PaO2 or arterial pH between both groups, however the control group showed a significantly higher PaCO2 and a significantly lower arterial HCO3- values than in the sevoflurane group 5 min. after tourniquet deflation. Serum lactic acid, IL-6 and TNF(-alpha) significantly increased in both groups 5 min. after tourniquet deflation but the rate of increase was higher in the control group; serum lactic acid was 1.30+0.25 mg/dl in the sevoflurane group vs. 1.98+0.27 mg/dl in the control group, IL-6 was 50.4+12.6 pg/ml in the sevoflurane group vs. 66.7+9.9pg/ml in the control group, and TNF(-alpha) was 15.6+6.4 pg/ml in the sevoflurane group vs. 28.4+5.8 pg/ml in the control group 5 min. after tourniquet deflation.

In conclusion, the findings of the study has shown that sevoflurane has a preconditioning effect on human skeletal muscles as evidenced by a lower biochemical parameters of muscle injury.

Key words: Preconditioning - sevoflurane – ischemia/reperfusion injury- skeletal muscle.

INTRODUCTION

Preconditioning (PC) is a process where cells or tissues exposed to a sublethal stimulus are transiently protected from a subsequent normal lethal stress\(^{(3)}\). Many forms of preconditioning have been investigated, such as ischemic, thermal, pharmacologic, or gas inhalation (particularly in lung injury)\(^{(1-6)}\). Preconditioning can attenuate the subsequent prolonged or lethal tissue injury by increasing the cell tolerance to the stress. Organ PC was first recognized by Murray et al.in1986, who originally tried to create a larger area of myocardial infarction by performing several brief episodes of myocardial ischemia prior to protracted ischemia, but got paradoxical results\(^{(7)}\).

Several studies have addressed the way in which this form of protection occurs\(^{(1-6)}\). During the short preconditioning period of ischaemia, several trigger substances are released (adenosine, bradykinin, norepinephrine, opioids). By activation of membrane-bound receptors, these substances activate a
complex intracellular signalling cascade, which converges on mitochondrial end-effectors, including the ATP-sensitive potassium channel \( (K_{\text{ATP}}) \) and the mitochondrial permeability transition pore (MPTP). Activation of this pathway protects cardiomyocytes against both necrosis and apoptosis during a subsequent more prolonged ischaemic episode. The protection afforded by preconditioning lasts only two to three hours, but reappears 24 hours after the preconditioning stimulus. This delayed preconditioning requires synthesis of new proteins, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and heat shock proteins (HSP). Additionally, preconditioning is not confined to one organ, but can also limit infarct size in remote, non preconditioned organs (remote preconditioning). Knowledge of these mechanisms mediating ischaemic preconditioning is essential to understand which drugs are able to mimic preconditioning or interfere with preconditioning in patients at risk for myocardial ischaemia.

The endogenous nucleoside adenosine is the first identified and probably most important trigger of classic preconditioning. Myocardial interstitial adenosine concentration increases rapidly during ischaemia\[^6\]. In 1991, it was discovered that adenosine A1 receptor stimulation during preconditioning ischaemia is essential for protection to occur and that intravenous administration of selective adenosine A1 receptor agonists instead of preconditioning ischaemia offers similar protection (pharmacological preconditioning)\[^9\]. Similarly, local intracoronary adenosine administration offers protection similar to ischaemic preconditioning in dog hearts\[^10\]. Later it was found both \textit{in vitro} and \textit{in vivo} that A3 receptor stimulation also contributes to ischaemic preconditioning\[^11\]. Several mechanisms have been discussed to explain the beneficial role of pharmacological preconditioning; however, the exact underlying mechanisms of protection are still unknown\[^12\]-\[^19\].

**Figure 1.** Simplified illustration of the mechanism of classical preconditioning.(1) 
\( (\text{PI3-kinase}) \) : phosphatidylinositol-3-kinase. \( (\text{PKC}) \) : protein kinase C 
\( (\text{PTK}) \) : protein tyrosine kinases. \( (\text{MAPKs}) \) : mitogen-activated-protein kinases 
\( K_{\text{ATP}} \) : ATP-sensitive potassium channel. \( \text{MPTP} \) : mitochondrial permeability transition pore.

Several studies have documented that volatile anesthetics including sevoflurane possess a pharmacological preconditioning effect in the heart, brain, kidney, lung, cultured rat smooth muscles and human endothelial cells. However, the effect of sevoflurane on human skeletal muscle ischemia reperfusion injury is not clear. The present study aimed to study the preconditioning effect of sevoflurane as a prophylaxis against ischemia reperfusion injury of the lower limb skeletal muscles after tourniquet deflation.

**MATERIAL AND METHOD**

The study was carried out in Menoufiya University Hospital on 40 adult patients aged 18-38 years undergoing lower limb operations involving the use of a pneumatic tourniquet. All patients were thoroughly evaluated and investigated to ensure that they were ASA I or II physical status.

The patients were randomly assigned into two equal groups, each one including 20 patients.

**Premedication:** All patients were premedicated with midazolam 0.1 mg/kg i.m. one hour before induction of anesthesia and were given IV propacetamol 1gm. as a preemptive analgesia. Ringer's solution was used to correct the deficits and for maintenance.

**Group I:** received IV thiopental sodium induction in a dose of 5-7 mg/kg + i.v. bolus 0.5 mg/Kg of atracurium. Intubation was done and anesthesia was maintained by using sevoflurane in an end tidal concentration of 2% in 50% oxygen + 50% air and additional doses of atracurium were given as appropriate. Fifteen minutes as a preconditioning time was allowed to pass between starting sevoflurane anesthesia and application of the tourniquet. Light anesthesia or moderate hypertension (> 20% of baseline) were treated with 20% increments of end tidal concentration of the inhaled sevoflurane. Sevoflurane anesthesia was continued till 5 minutes after deflation of the tourniquet.

**Group II:** received spinal anesthesia using heavy bupivacaine 15 mg and were given inspired O₂ concentration 50% +50% air through a ventouri mask. Similarly fifteen minutes as a preconditioning time was allowed to pass between starting spinal anesthesia and application of the tourniquet.

**RESULTS**

There was no significant difference between the two studied groups with regard to the patients' characteristics, type of surgery, duration of surgery or anesthesia or tourniquet time. MABP did not show significant differences between the two groups within the study period but the heart rate was significantly higher in group I. At 60 minutes after induction of anesthesia, body core temperature was lower in group I and this persisted till 10 minutes after tourniquet deflation. Oxygen saturation was comparable in both groups.

Both groups showed an increase in CPK and AST levels 5 minutes after tourniquet deflation but the rate of increase was higher in group II. Also both groups developed increase in serum K 5 min. after tourniquet deflation and the increase was significantly higher in group II.
Both groups showed an increase in serum Ca level 5 minutes after tourniquet deflation but there was no significant difference between them (table III). Regarding ABG’s, there were no significant differences in PaO$_2$ or arterial pH between both groups, however group II showed a significantly higher PaCO$_2$ and a significantly lower Pa HCO$_3^-$ values than in group I 5 min. after tourniquet deflation (table III). Serum lactic acid, IL-6 and TNF (alpha) significantly increased in both groups 5 min. after tourniquet deflation but the rate of increase was higher in group I (table III).

**DISCUSSION**

Ischemia reperfusion injury is an area which received considerable attention in the past decade because of its direct clinical relevance. (1)

A number of drugs appear to mimic ischemic preconditioning. This phenomenon “pharmacological preconditioning” has been demonstrated with nicorandil, opioid receptor agonists and volatile anaesthetics(1,5).

A variety of mechanisms have been discussed to explain the beneficial role of pharmacological preconditioning; however, the exact underlying mechanisms of protection are still unknown(12-19).

Several studies have documented that volatile anesthetics including sevoflurane have a pharmacological preconditioning effect in the heart(20), brain(21), kidney(22), lung(23), cultured rat smooth muscles and human endothelial cells(24). However, the effect of sevoflurane on skeletal muscle ischemia reperfusion injury induced by the use of arterial tourniquet is not clear.

The findings of the present study suggest that sevoflurane has a pharmacological preconditioning effect against ischemia reperfusion injury in lower limb skeletal muscles as evidenced by a significantly lower biochemical markers of skeletal muscle injury in the form of lower values of serum CPK, AST, K, lactic acid, IL-6,TNF(alpha) and a less decrease in plasma HCO3 than in the control group.

Similar results were proved by Van Der Linden et al(20) who reported that sevoflurane preserved left ventricular function after coronary artery bypass grafting (CABG) with less biochemical evidence of myocardial damage.

Also Julier et al(22) has documented that sevoflurane decreased the biochemical markers of myocardial and renal dysfunction in CABG.

Zhang et al(25) have studied the preconditioning effect of sevoflurane on ischemic neurons in the rat brain; they reported that the area of apoptosis has decreased by 40% in the sevoflurane group compared with the control group.

Similarly, Pope et al(21) have documented that sevoflurane had sustained neuroprotective effect after cerebral ischemia/ reperfusion; the area of neuronal injury in the control group was 140-200% larger compared with the sevoflurane group during the first 3 days after inducing ischemic injury to one hemisphere in the rat brain.

Table I: Patient’s characteristics and perioperative data.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (sevoflurane)</th>
<th>Group II (spinal)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>28 +6.4</td>
<td>29+7.3</td>
<td>0.43</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>13/7</td>
<td>11/9</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.7 + 9.8</td>
<td>72.6+7.3</td>
<td>0.66</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.4 + 11.1</td>
<td>174.5 + 9.2</td>
<td>0.81</td>
</tr>
<tr>
<td>BMI (kg / m$^2$)</td>
<td>25.4 + 3.8</td>
<td>24.9 + 3.6</td>
<td>0.91</td>
</tr>
<tr>
<td>Types of surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee Arthroscopy</td>
<td>8</td>
<td>7</td>
<td>0.67</td>
</tr>
<tr>
<td>Medial menisectomy</td>
<td>4</td>
<td>5</td>
<td>0.75</td>
</tr>
<tr>
<td>Patelllectomy</td>
<td>2</td>
<td>2</td>
<td>0.97</td>
</tr>
<tr>
<td>Open reduction and internal fixation for Pott’s fracture</td>
<td>6</td>
<td>6</td>
<td>0.98</td>
</tr>
<tr>
<td>Duration of surgery, (min)</td>
<td>66 + 15</td>
<td>68+ 20</td>
<td>0.76</td>
</tr>
<tr>
<td>Duration of anesthesia, (min)</td>
<td>97 + 16</td>
<td>99 + 22</td>
<td>0.79</td>
</tr>
<tr>
<td>Tourniquet time (min)</td>
<td>78 + 12</td>
<td>77 + 13</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Table II: Comparison between both groups regarding haemodynamic data and temperature.

<table>
<thead>
<tr>
<th></th>
<th>Before deflation of the tourniquet</th>
<th>after deflation of the tourniquet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>MABP (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>81.5+5.5</td>
<td>80.6+4.7</td>
</tr>
<tr>
<td>Group II</td>
<td>80.6+3.7</td>
<td>79.9+5.1</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.17</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Heart rate (beat/minute)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>75.6+8.2</td>
<td>84.5+7.4</td>
</tr>
<tr>
<td>Group II</td>
<td>76.1+5.9</td>
<td>75.6+6.4</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>O2 Saturation (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>97.8+0.66</td>
<td>98+0.60</td>
</tr>
<tr>
<td>Group II</td>
<td>97.6+0.71</td>
<td>97.9+0.68</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.22</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Temperature (ºC)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.2+0.0</td>
<td>37.2+0.0</td>
</tr>
<tr>
<td>Group II</td>
<td>37.1+0.0</td>
<td>37+0.1</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.26</td>
<td>0.11</td>
</tr>
</tbody>
</table>

- **MABP (mmHg)**: Mean arterial blood pressure
- **Heart rate (beat/minute)**
- **O2 Saturation (%)**: Oxygen saturation percentage
- **Temperature (ºC)**
Table III: Comparison between both groups regarding laboratory data.

<table>
<thead>
<tr>
<th></th>
<th>Before induction of anesthesia</th>
<th>5 minutes after tourniquet deflation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CPK (IU/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>58.3±14.2</td>
<td>61.1±13.6</td>
</tr>
<tr>
<td>Group II</td>
<td>56.7±12.7</td>
<td>76.2±11.4</td>
</tr>
<tr>
<td>P</td>
<td>0.45</td>
<td>0.002 *</td>
</tr>
<tr>
<td><strong>AST (IU/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>26.9±7.3</td>
<td>28.4±6.9</td>
</tr>
<tr>
<td>Group II</td>
<td>27.2±6.4</td>
<td>55.5±6.3</td>
</tr>
<tr>
<td>P</td>
<td>0.66</td>
<td>0.001 *</td>
</tr>
<tr>
<td><strong>Serum K (meq/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>4.1±0.65</td>
<td>4.4±0.71</td>
</tr>
<tr>
<td>Group II</td>
<td>4.2±0.6</td>
<td>5.1±0.4</td>
</tr>
<tr>
<td>P</td>
<td>0.74</td>
<td>0.001 *</td>
</tr>
<tr>
<td><strong>Serum Ca (mg/dl)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>9.1±0.39</td>
<td>9.9±0.6</td>
</tr>
<tr>
<td>Group II</td>
<td>9.2±0.5</td>
<td>10.1±0.6</td>
</tr>
<tr>
<td>P</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Pa02 (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>151.4±4.8</td>
<td>149.4±5.4</td>
</tr>
<tr>
<td>Group II</td>
<td>149.7±5.4</td>
<td>148.8±6.1</td>
</tr>
<tr>
<td>P</td>
<td>0.75</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Pa CO2 (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>37.7±2.1</td>
<td>41.7±2.0</td>
</tr>
<tr>
<td>Group II</td>
<td>37.5±1.3</td>
<td>44.8±1.1</td>
</tr>
<tr>
<td>P</td>
<td>0.91</td>
<td>0.002 *</td>
</tr>
<tr>
<td><strong>Pa HCO3- (meq/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>23.9±1.0</td>
<td>22.1±1.4</td>
</tr>
<tr>
<td>Group II</td>
<td>24.1±1.1</td>
<td>19.8±1.2</td>
</tr>
<tr>
<td>P</td>
<td>0.56</td>
<td>0.002 *</td>
</tr>
<tr>
<td><strong>Arterial Ph</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>7.4±0.01</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>7.4±0.01</td>
<td>7.37±0.01</td>
</tr>
<tr>
<td>P</td>
<td>0.88</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Serum lactic acid (mg/dl)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>1.28±0.26</td>
<td>1.30±0.25</td>
</tr>
<tr>
<td>Group II</td>
<td>1.27±0.25</td>
<td>1.98±0.27</td>
</tr>
<tr>
<td>P</td>
<td>0.35</td>
<td>0.003 *</td>
</tr>
<tr>
<td><strong>Serum interleukin 6 (IL-6) in (pg/ml).</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>7.1±3.5</td>
<td>50.4±12.6</td>
</tr>
<tr>
<td>Group II</td>
<td>6.9±4.6</td>
<td>66.7±9.9</td>
</tr>
<tr>
<td>P</td>
<td>0.43</td>
<td>0.002 *</td>
</tr>
<tr>
<td><strong>Serum TNF (-alpha) in (pg/ml).</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5.2±3.2</td>
<td>15.6±6.4</td>
</tr>
<tr>
<td>Group II</td>
<td>5.5±2.9</td>
<td>28.4±5.8</td>
</tr>
<tr>
<td>P</td>
<td>0.33</td>
<td>0.001 *</td>
</tr>
</tbody>
</table>

Hu et al\(^{(26)}\) approved that sevoflurane preconditioning against neutrophil-induced contractile dysfunction in isolated rat hearts. Riess et al\(^{(27)}\) have reported that a supra-clinical concentration (4MAC sevoflurane) led to a better cardioprotection than a lower concentration (2MAC) as shown by postischemic function and infarct size in an isolated guinea pig heart model.
Transferring these results into clinical practice, however is limited by the marked cardiac depressant side effects of such high concentration. Whether anesthetic preconditioning is dose dependent in vivo and within the range of clinically applicable concentrations has not yet been fully elucidated(28).

Also matching with our results, was the research done by El Azab et al(29) who studied the effect of sevoflurane on the secretion of TNF-(alpha) during and after CABG versus a control group that received total intravenous anesthesia with midazolam-sufentanyl. TNF-(alpha) concentration was lower in the sevoflurane group 25(21-30) versus 37(28-79) pg/ml in the control group(p<0.05) and they concluded that this may reduce cardiac morbidity and the length of stay in the ICU.

Few studies have failed to approve a preconditioning effect for sevoflurane as the study done by Piriou et al(30) who reported that sevoflurane had no significant preconditioning effect; where the mean infarct size in the rabbit myocardium was 54±18% of the risk area in untreated controls and 40±19% in the sevoflurane group(p>0.05, ns.).

Also, another study done by Zvara et al(31) has decided that sevoflurane does not protect the spinal cord after an ischemic-reperfusion injury in the rat. They reported that histologic evaluation of the spinal cord showed severe neurologic damage similarly in both sevoflurane and control groups.

Ischemia reperfusion injury is a complex process involving the generation and release of inflammatory cytokines, the accumulation and infiltration of neutrophils and cell death(16).

The clinical morbidity associated with these post-ischemic tissue reactions has often gone unappreciated postoperatively in limbs that have been immobilized and concealed by casts or dressings. This lack of appreciation is diminishing with the current emphasis on early motion following stable skeletal fixation, arthroplasty, and so on. The effect of sublethal ischemic injury to cells are not only unpleasant for the patient, but could theoretically be significant with respect to postoperative narcotic requirements, tolerance of early motion therapy, wound healing and resistance to infection. It therefore seems appropriate to take all reasonable measures to minimize even reversible muscle injury rather than to merely avoid frank necrosis of tissue(22).

In conclusion, our study has proved that sevoflurane had a preconditioning effect on human skeletal muscles and recommends its use in operations where there is high risk of skeletal muscle injury as operations with expected long tourniquet time.

REFERENCES


