Sevoflurane but not propofol increases interstitial glycolysis metabolites availability during tourniquet-induced ischaemia–reperfusion

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Background. Ischaemia/reperfusion (I/R) is one of the main pathophysiological phenomena involved in the anaesthetic practice. The authors hypothesized that anaesthetic regimens can influence skeletal muscle tolerance to tourniquet-induced I/R that should be reflected by the interstitial metabolite levels of anaerobic glycolysis.

Methods. Microdialysis probes were implanted in three groups of 10 patients each receiving either sevoflurane (SEVO), propofol (PRO), or spinal (SA) anaesthesia (for induction and maintenance). SA group was considered as a control group. Interstitial fluid was obtained during tourniquet-induced I/R and was analysed for interstitial glucose, lactate, pyruvate, and glycerol.

Results. The microdialysis flow rate was 0.5 μl min⁻¹. Compared with the control group, the SEVO group had a higher level of both lactate and pyruvate and an increase in glucose during ischaemia. In contrast, the PRO group had a lower level of pyruvate, resulting in a significant higher increase (eight times from baseline) of the lactate pyruvate ratio. Glucose level remained low in this group. During reperfusion, lactate, pyruvate, and glucose levels remained at a significantly higher level in the SEVO group. In the PRO group, there was no difference in lactate, pyruvate, and glucose levels compared with the control group. The interstitial level of glycerol exhibits only few and comparable changes during I/R between the groups.

Conclusions. Our results indicate that there is a better availability of interstitial glycolysis metabolites (glucose, lactate, and pyruvate) in the skeletal muscle during ischaemia and reperfusion after sevoflurane exposure than after propofol, suggesting a potential preconditioning effect of sevoflurane on tourniquet-induced skeletal muscle I/R.

Br J Anaesth 2008; 100: 29–35

Keywords: anaesthetics i. v., propofol; anaesthetics volatile, sevoflurane; metabolism, glucose; metabolism, lactate; muscle skeletal, metabolism

Accepted for publication: September 1, 2007

Ischaemia–reperfusion (I/R) is one of the main pathophysiological phenomena seen in the anaesthetic practice (organ transplantation, coronary surgery, cardiopulmonary bypass, restoration of hypovolaemic shock, etc.), and that causes local and systemic inflammatory response.1–3

A particular situation of I/R injury is associated with the use of a tourniquet in orthopaedic surgery (to avoid intraoperative bleeding) or in emergency medicine (to arrest life-threatening extremity haemorrhage). Because of the side-effects due to I/R and to mechanical muscle and nerve injuries, the use of tourniquet still remains controversial.4–6 Muscle ischaemia is accompanied by hypoxic cellular challenge and anaerobic glycolysis, and reperfusion by neutrophil activation, formation of reactive oxygen species, and release of vasoactive factors.7 Thus, a variety of mechanisms or strategies have been proposed to
attenuate skeletal muscle injury, but none is yet available for clinical practice. Nevertheless, experimental and clinical data provide evidence for a protective role of volatile anaesthetics against myocardium I/R injury. Such an effect has been evoked for propofol too, but it remains controversial. However, there are no data on the physiological effect of such anaesthetics on the skeletal muscle subjected to tourniquet-induced I/R in humans.

Microdialysis is a valuable technique to monitor interstitial levels of metabolites (in particular, glycolysis metabolites) in human skeletal muscle during I/R, reflecting anaerobic glycolysis switch. Implantation of the dialysis probe after the induction of anaesthesia is painless, requiring only a few minutes and sampling of the fluid can be performed during the surgical procedure.

The aim of our study was therefore to evaluate the effect of various anaesthetic regimens on interstitial glycolysis metabolites in human skeletal muscle subjected to a tourniquet-related I/R for lower limb surgery.

Methods

Patient population

The study was performed as a monocentric prospective randomized study in 30 patients undergoing knee surgery: total knee replacement (TKR) or anterior cruciate ligament repair (ACLR), requiring an intraoperative tourniquet for a predictable duration of more than 100 min. The study was approved by the institutional ethical committee (Comité de Protection des Personnes, Centre Hospitalier Universitaire, Nice, France), and written consent was obtained. Patients were excluded if they exhibited a diabetes mellitus, a known neuromuscular pathology, a severe cardiovascular or respiratory disease, or any contraindication to one of the three planned anaesthetic protocols. Patients were excluded from the study due to protocol violation or intraoperative requirement of neuromuscular blocking agent. Patients were randomly allocated (by a computer randomization scheme) to receive either propofol (PRO group), sevoflurane (SEVO group), or spinal anaesthesia (SA group).

Anaesthesia

All patients received hydroxyzine 100 mg orally 1 h before surgery as premedication. In the operating room, patients received routine monitoring (electrocardiograph, non-invasive arterial cuff, and pulse oximetry) and a femoral catheter was placed under local anaesthesia after nerve localization by neurostimulation. No injection in the catheter of the anaesthetic mixture was done at this time. All patients received a fluid preload of 500 ml of saline solution. In Group PRO, anaesthesia was induced by a target-controlled infusion (TCI) of propofol at a target plasma concentration of 4 \( \mu g \) ml\(^{-1}\) by a 30 s bolus.

In the SEVO group, anaesthesia was induced by inhalation of a sevoflurane 8% in oxygen 100% (vital capacity breath procedure). Anaesthesia was maintained with a delivered concentration of sevoflurane 2%. In both groups, a remifentanil TCI was started after loss of consciousness with a target effect-site concentration of 3 mg ml\(^{-1}\) for intubation and then adapted to haemodynamics throughout the surgical procedure. All patients in Groups SEVO and PRO were then intubated, without neuromuscular blocking agent as decided in the experimental protocol. The patients received morphine 0.1 mg kg\(^{-1}\) i.v. 30 min before the end of surgery followed by acetaminophen 1 g i.v. and were extubated in the operating room at the end of surgery. In Group SA, the patients were placed in lateral decubitus position (operative side underneath) and then received intrathecally 0.5% of hyperbaric bupivacaine 10 mg plus clonidine 30 \( \mu g \) (previously diluted in 1 ml of saline). At the patient’s request, midazolam 0.02 mg kg\(^{-1}\) was administered after inflating the tourniquet. Patients were maintained in lateral decubitus position for 20 min. For all groups, a pneumatic tourniquet was applied after the anaesthetic induction, as proximal as possible but not inflated. All patients received cefazolin 2 g as prophylaxis.

After the surgical dressing, pain was assessed with a visual analogue scale (VAS) on arrival then every hour for 4 h and six times daily thereafter. A first dose of ropivacaine 0.2%, 30 ml was injected through the femoral catheter immediately when the VAS was elevated more than 40, followed by a continuous infusion of ropivacaine 0.2%, 6 ml h\(^{-1}\). The delay of the first injection after the end of surgery was recorded. If the VAS remained above 40 after 30 min, a morphine infusion was started.

Microdialysis procedure

The microdialysis catheters used were CMA 60 (CMA Microdialysis, Solna, Sweden) 30 mm polyamide membrane length. After installation of the pneumatic tourniquet, the microdialysis probe was inserted (using guide cannulae) at an angle of 45° and after passing the muscle fascia (‘fascial click’ as the correct location control), at an angle of 20° into the vastus medialis of the quadriceps femoralis muscle, under aseptic conditions. The catheters were perfused by a CMA 106 pump (CMA Microdialysis). After the sequence of flushes (15 \( \mu l \) min\(^{-1}\) for 6 min followed by 1 \( \mu l \) min\(^{-1}\) for 10 min), the flow was settled at 0.5 \( \mu l \) min\(^{-1}\). Samples were collected on ice every 20 min and stored at −20°C. After the first sample (baseline), the pneumatic tourniquet was inflated at 250 mm Hg (after Esmarch ischaemia: leg elevation and exsanguination by a circumferential elastic bandage) and the surgery team started.
Samples were collected every 20 min until 2 h after deflation of tourniquet, then the catheter was removed. Samples were analysed for glucose, lactate, pyruvate, and glycerol on CMA 600 Microdialysis Analyzer (self-calibrating autoanalyser designed for microdialysis samples, using enzymatic fluorometric assays—CMA Microdialysis).

Statistical analysis

Since systemic anaesthetics were not used in this study for the SA group, it was analysed as the control group. Therefore, SEVO and PRO groups were analysed comparatively to this group. For data evaluation, the first sample over 20 min (‘baseline’) was defined as 100% for each curve. All subsequent data points are expressed as percentage over baseline. Data are expressed as mean (SD), or median (min–max) values, for each group. General data are analysed using non-parametric Kruskall–Wallis test or Fisher’s exact test if required. The intragroup data curves are compared using a \( t \)-test for paired data. To compare intergroup data, repeated measures ANOVA was primarily done with a step-by-step comparison using a Student’s \( t \)-test for unpaired series. A \( P \)-value of \( <0.05 \) was considered significant.

Results

Thirty patients were included in the study after obtaining informed consent. The microdialysis catheter had to be replaced for two patients because of inability to obtain a dialysate liquid from the first catheter after the flush (the removed catheter evidenced in each case a damage of the membrane). All other catheters were intact after removal. Demographic and general data (Table 1) are comparable between the three groups of patients, including medication and smoking (same number of smokers in each group). All patients had at least 30 min exposure to an anaesthetic agent before inflating the tourniquet.

Because of a shorter duration of surgery, six patients (three in the SA group, two in the SEVO group, and one in the PRO group) had a tourniquet duration <100 min resulting in only four microdialysis samples during the ischaemic period. Baseline values for each metabolite were comparable between the groups (Table 2).

During ischaemia (after inflating the tourniquet), interstitial lactate (Fig. 1) increased significantly in the three groups, as the result of the switch to the anaerobic glycolysis. Compared with the control group (SA group: +340% after 100 min of ischaemia), the level of lactate in the SEVO group was higher (+750% at the same time), Table 2

| Table 1 General data and anaesthetic procedures [median and (min–max) values] |
|-----------------|-----------------|-----------------|
| Age (yr)        | SEVO (n=10)     | PRO (n=10)      | SA (n=10)      |
|                 | 70 (22–82)      | 66 (20–85)      | 50 (31–84)     |
| Sex ratio       | 1.5             | 1               | 1              |
| Body mass index (kg m\(^{-2}\)) | 29 (19–31)      | 30 (19–32)      | 26 (23–30)     |
| Knee prosthesis (TKR)/ligamentoplasty (ACL) | 7/3             | 7/3             | 5/5            |
| Duration of surgery (min) | 50 (35–75)      | 50 (40–80)      | 50 (40–70)     |
| Dose of remifentanil before inflating the tourniquet (µg) | 403 (108–1200)  | 367 (100–534)  | 0              |
| Complete dose of remifentanil (µg) | 1145 (521–2550) | 1110 (586–2140)| 0              |
| Dose of propofol before inflating the tourniquet (mg) | 0               | 737 (342–1050) | 0              |
| Total dose of propofol (mg) | 0               | 1885 (1260–3720)| 0              |
| Time of exposure before inflating the tourniquet (min) | 54 (35–107)     | 40 (30–87)      | 61 (35–115)    |
| Total dose of ephedrine (mg) | 3 (0–30)        | 0 (0–30)        | 0 (0–18)       |
| Tourniquet duration (min) | 103 (80–126)    | 100 (80–130)    | 102 (80–135)   |
| Body temperature in recovery room (°C) | 36.3 (35.2–37.2)| 36.1 (34.5–36.6)| 36.0 (35.0–37.0) |

| Table 2 Comparative baseline data for metabolites. Data are mean (SD) |
|-----------------|-----------------|-----------------|
| Lactate (mmol litre\(^{-1}\)) | 1.3 (1.7)       | 1.3 (1.1)       | 1.6 (1.3)     |
| Pyruvate (µmol litre\(^{-1}\)) | 32.6 (18.4)     | 34.6 (17)       | 37.4 (23)     |
| Glucose (mmol litre\(^{-1}\)) | 1.6 (1.4)       | 2.6 (1.6)       | 2.1 (1.2)     |
| Glycerol (µmol litre\(^{-1}\)) | 176.5 (94.9)    | 276.3 (189.6)   | 162.2 (88.5)  |
whereas it was not significantly different in the PRO group. Interstitial pyruvate (Fig. 2) did not differ from baseline whereas it increased in the SEVO group and decreased in the PRO group. Thus, the lactate pyruvate ratio (Fig. 3) increased in the three groups, in the same range as the control group for the SEVO group (+300% after 100 min of ischaemia) and significantly higher (+860%) for the PRO group. Interstitial glucose (Fig. 4) decreased significantly from baseline in the PRO group (−40% after 100 min of ischaemia), whereas it did not significantly differ from baseline values in the other groups. Interstitial glycerol (Fig. 5) increased significantly from baseline but in the same range for the three groups.

During reperfusion (after deflating the tourniquet), interstitial lactate (Fig. 1) decreased without reaching baseline level within 2 h of reperfusion in the control and SEVO groups. In contrast, in the PRO group, baseline was reached after 80 min of reperfusion. Interstitial pyruvate (Fig. 2) increased significantly after 40 min of reperfusion in all groups (from +420% to +690%). Unlike the PRO group, the level of interstitial pyruvate in the SEVO group was significantly higher than that in the control group. As a result, for all groups the lactate pyruvate ratio (Fig. 3) returned to baseline during the first hour of reperfusion. In the same time, interstitial glucose (Fig. 4) increased significantly in all groups during after 40 min of reperfusion. Interstitial glycerol (Fig. 5) returns to baseline values within the first reperfusion hour, without significant differences between the groups.
Pain level (VAS) (Fig. 6) and morphine consumption were comparable between the groups: the median 24 h postoperative period amount of morphine was 0 (0–18), 0 (0–53), and 0 (0–19) mg in the SEVO, PRO, and SA groups, respectively (P=NS), and the number of patients requiring morphine (four in each group). The delay of the first requirement of analgesic femoral catheter injection was longer in the SA group [91 (15–420) min] than in the SEVO [35 (25–70) min] or PRO groups [54 (22–124) min] without reaching statistical significance.

Discussion

Despite the publication of several clinical studies on the preconditioning effect of anaesthetic drugs on myocardium muscle I/R,12–14 16 there is no clinical study published about the modulation of I/R injury by anaesthetics on skeletal muscle in humans. Our results indicate that sevoflurane use as induction and maintenance anaesthetics is associated with a better availability of anaerobic glycolysis metabolites of the skeletal muscle during tourniquet-induced I/R (compared with patients who underwent spinal anaesthesia). In contrast, propofol did not provide such a protective effect but impairs physiological markers of anaerobic glycolysis during tourniquet-induced I/R. By reducing I/R-related tissue injuries, preconditioning with sevoflurane can attenuate postoperative complications such as tourniquet-related nerve palsies.4

Microdialysis is a well-described technique, allowing to monitor interstitial levels of metabolites in humans.20 21 In particular, analysis of lactate and other potential markers of glycolysis obtained by microdialysis has been previously used to study changes of energy metabolism in human skeletal muscle.7 18 The use of a low flow perfusate at 0.5 μl min⁻¹ reinforces the reliability of the results.22 Moreover the percutaneous insertion is a safe, fast, and simple procedure.7 Therefore, microdialysis represents an attractive and powerful technique to analyse metabolic changes in a continuous manner in the skeletal muscle in the operating room.

The hypothesis of our study was to determine whether volatile anaesthetics, in particular sevoflurane, would reproduce on skeletal muscle, the preconditioning effect demonstrated in the myocardium muscle.8 23 We also studied propofol, since it has a similar structure to phenol-based scavengers, such as the endogenous antioxidant vitamin E, and has been shown to be protective against myocardial injury induced by I/R.24 Because of the possible role of opioids as a preconditioning drug,25 it was important to administer the same drug (i.e. remifentanil) for both groups under general anaesthesia. We used patients who underwent spinal anaesthesia as the control group, to avoid any systemic anaesthetic drug. In this group, the use of small doses of midazolam was not a significant factor for modifying I/R metabolites.26

The baseline level of interstitial metabolites from our study and two previous studies on tourniquet I/R in human skeletal muscle are broadly comparable, despite some lower values possibly related to the lack of extrapolation of our data to zero flow, as reported by others.7 18 During ischaemia, the concomitant increase of lactate in the three groups is in accordance with anaerobic glycolysis data (with higher values in the SEVO group). In the same time, in the SA group, there is a decrease in the mean interstitial glucose level.5 Comparatively with control group, higher values in the SEVO group suggest that the glucose availability for glycolysis is better preserved with sevoflurane. Observed differences of interstitial lactate and glucose between the groups may be related to a specific anaesthetic regulation of the glycogen metabolism. Indeed, higher production of lactate in anaerobic conditions requires a higher availability of glucose, which is in part dependent on glycogen metabolism. Since the glycogen metabolism is regulated by protein kinase A (PKA) at different levels (glycogen synthetase, phosphorylase) and the activation of PKA by volatile anaesthetics was recently demonstrated,27 this mechanism may be involved in the better availability of the glucose in the SEVO group. As a consequence of the better glucose availability, the level of pyruvate is higher in the SEVO group. This may be also the consequence of a possible enhancement of glucose transport by sevoflurane.28 Thus, the lactate pyruvate ratio increased largely during the ischaemic period in the PRO group, and only slightly increased in the SA group and in the SEVO group. Since the lactate pyruvate ratio is

![Fig 6 VAS median values on arrival in recovery room, discharge from recovery room, and postoperative day 1. There is no significant difference between the groups.](https://academic.oup.com/bja/article-abstract/100/1/29/387428 by guest on 19 March 2019)
recognized as a good indicator of the anaerobic glycolysis, these results indicate that there is a more efficient anaerobic glycolysis after sevoflurane exposure because of higher availability of energetic substratum, that is pyruvate, allowing higher production of lactate and therefore higher mitochondrial ATP.\textsuperscript{29,30} Comparatively with the control group, propofol did not exhibit similar properties.\textsuperscript{31}

During reperfusion, the lactate level remained higher in the SEVO group vs the two other groups. The pyruvate values increased and then remained higher than baseline for the three groups (and comparatively even higher in the SEVO group). The interstitial glucose increased significantly (because of blood supply) in the three groups as shown by others, but significantly more in the SEVO group.

Our results indicate that there are some differences in the time-course of glycolysis metabolism markers during skeletal muscle I/R, depending on which anaesthetic drug is used. Metabolic response of the SA group (control group) is in accordance with the published data.\textsuperscript{7,18} Comparatively, the data of the SEVO group indicate that sevoflurane optimized glycolysis during tourniquet-induced ischaemia. Indeed, lactate is now recognized as a ‘central player in cellular, regional and whole body metabolism’.\textsuperscript{30} Thus, higher production of lactate may be related to a preservation of the function of the Na\textsuperscript{+}–K\textsuperscript{+}–ATPase pump, because it can use glycolytic ATP.\textsuperscript{32} In the same time, in the PRO group there is decreased energetic substrate availability. On the basis of these data, a preconditioning effect of sevoflurane exposure on skeletal muscle before tourniquet-induced I/R can be suggested.

This study provides supplementary data to further understand the mechanism of such preconditioning effect. The hypothesis for volatile anaesthetic-induced myocardial protection implies multiple endogenous pathways such as mitochondrial K\textsubscript{ATP} channels, protein kinases, and G protein-coupled receptors.\textsuperscript{8} Thus, during the ischaemic period, one of the suggested physiological phenomena resulting from these mechanisms is an increasing oxygen supply by coronary vasodilatation.\textsuperscript{33} However, tourniquet-induced ischaemia provided a complete disruption of blood supply, so such mechanisms cannot be involved during skeletal muscle ischaemia. Conversely, vasodilatation may be in part involved during reperfusion, related to higher values of energetic metabolites available in the sevoflurane group. Sevoflurane has been shown to preserve ATP synthesis in cardiac mitochondria during early reperfusion \textit{in vivo}.\textsuperscript{34} Higher interstitial glycolysis substratum levels resulting from sevoflurane exposure (as shown in our study) may participate in the preservation of ATP synthesis in the skeletal muscle. Further studies using genomic tools could highlight the underlying molecular mechanisms of such findings.\textsuperscript{35}

Glycerol data were not very informative in our study. Glycerol is supposed to reflect cellular damage because of the degradation of membrane phospholipids.\textsuperscript{30} Rather than I/R damage, glycerol release can be due to direct tissue injury related to tourniquet pressure. In our study, the pressure level at 250 mm Hg was lower than that reported in the previous studies,\textsuperscript{7} possibly explaining the slight increase in glycerol values. Moreover, direct cell damage after insertion of the probe can modify local baseline glycerol levels.\textsuperscript{36} This study had some limitations. First, we did not collect blood samples, as done in the previous study.\textsuperscript{7} However, several studies demonstrated that microdialysis gives very different results compared with systemic values and is more accurate to describe tissue metabolic changes.\textsuperscript{37,38} Thus, we believe that plasma values would not provide additional specific information. Another methodological issue is the mode of administration of anaesthetic drugs. We have to use carefully the term ‘preconditioning’, which implies a sequence including exposure, washout, and then ischaemia.\textsuperscript{29} Nevertheless, a pre-emptive use of sevoflurane seems to improve energy metabolism substrates of the skeletal muscle during I/R. A previous study reported a preconditioning effect of sevoflurane on myocardial I/R administered as pre-emptive drug.\textsuperscript{13} A third issue is that the clinical relevance of a potential preconditioning effect of anaesthetic drugs on skeletal muscle needs to be further studied. Since the size of the studied population is limited, we did not show a clinical improvement (in terms of pain or analgesic requirement) related to a potential preconditioning effect. Studies focused on pharmacodynamic evaluation (postoperative rehabilitation, muscle weakness) related to physiological data are needed.

**Clinical implications**

The ability to study physiological phenomena in a clinical setting using microdialysis is very promising to better understand the consequences of techniques and therapeutics in anaesthesiology. Our preliminary results will allow to profile optimized anaesthetic regimens, in order to improve the physiological (and therefore clinical) outcome of patients with prolonged peripheral ischaemia and severe underlying diseases (for instance in vascular surgery).

In conclusion, our results indicate that sevoflurane used as a pre-emptive drug before skeletal muscle tourniquet-induced I/R optimizes interstitial release of energy metabolism substrates. In contrast, propofol seems to impair the energy metabolism response of skeletal muscle to tourniquet-induced I/R. These findings may have some importance for the choice of anaesthetics in patients undergoing limb surgery with a tourniquet. However, further clinical studies are required to allow us to propose a volatile agent as primary choice for anaesthesia under I/R setting in peripheral muscle.

**Funding**

Direction de la Recherche clinique—Centre Hospitalier Universitaire de Nice (Governmental funds).
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