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Urapidil enhances subcutaneous tissue oxygen tension during convective rewarming of mildly hypothermic rats

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Abstract

To investigate whether urapidil (α_1-adrenergic antagonist/5-HT_{1A} agonist) enhances subcutaneous tissue oxygen tension ($P_{sq}O_2$) during convective rewarming, we performed a prospective, randomized, placebo-controlled animal study. Mild hypothermia was achieved by surface cooling. Protocol A: before rewarming : i.v. bolus of 1.0 ml NaCl 0.9%/kg body weight; Protocol B: before rewarming: i.v. bolus of 5 mg urapidil/kg body weight.

Urapidil significantly reduced the rewarming time (placebo: 30.2 ± 2.9 min, urapidil: 24.2 ± 2.3 min, $P = 0.012$) and the $P_{sq}O_2$ during rewarming was significantly enhanced ($P = 0.023$, $AUC_{P_{sq}O_2\text{-placebo}}$ versus $AUC_{P_{sq}O_2\text{-urapidil}}$).

The α_1-adrenergic antagonist/5-HT_{1A} agonist urapidil accelerates convective rewarming and enhances $P_{sq}O_2$ during rewarming in mildly hypothermic rats. Obviously, urapidil therapy increased the shift of heat from the periphery to the core. It is known that 5-HT_{1A} receptor agonists reduce thermoregulatory thresholds to cold. Therefore, a reduction in oxygen consumption with an increased oxygen delivery to subcutaneous tissues by urapidil is a further possible mechanism.

Keywords: Body temperature regulation; Double-blind method; Animal; Hypothermia; Oxygen; Urapidil; Vasoconstriction

1. Introduction

Perioperative hypothermia is the source of numerous complications, including coagulopathy, morbid cardiac events, and a decreased resistance to surgical-wound infection (Sessler, 1997).

A shift in the oxygen dissociation curve of haemoglobin to the left at lower temperatures impairs oxygen delivery to hypothermic tissues (Danzl, 2001). Furthermore, hypothermia triggers wound infections by cutaneous vasoconstriction with consecutive reduction in oxygen delivery to tissues (Hopf et al., 1997; Wenisch et al., 1996). The subcutaneous perfusion is controlled by alpha receptors. In particular, the α_{1A}-adrenoceptor subtype in resistance arteries plays a predominant role in contractile responses to norepinephrine, which can be antagonized by either prazosin or urapidil (Jarajapu et al., 2001). Furthermore, it is known that activation of serotonin 1A (5-HT_{1A}) receptors with 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) inhibits cold-induced vasoconstriction leading to a dilation of the cutaneous vascular bed with an increased transfer of heat from the core to the periphery (Ootsuka and Blessing, 2003). Therefore, α_{1A}-adrenoceptor antagonists and 5-HT_{1A} receptor agonists could possibly inhibit cold-induced vasoconstriction with consecutive increase in oxygen delivery to the subcutis.

In the following study we tested the hypothesis that treatment with urapidil increases subcutaneous oxygen pressure ($P_{sq}O_2$) during convective rewarming of mildly hypothermic rats. Urapidil, an approved antihypertensive drug in Europe, is a selective α_1-adrenoceptor antagonist with high affinity for the α_{1A}-adrenoceptor subtype (Gross

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et al., 1988). Urapidil influences the CNS as well. Its antihypertensive central effects have been postulated to be mediated by an agonistic activity on serotonin 1A (5-HT_{1A}) receptors (Gross et al., 1987).

2. Materials and methods

2.1. Animal care

Experimental protocols were approved by the local Bioethical Committee and the procedures and animal comfort were controlled by the Veterinary Service of the University of Regensburg. Male Wistar-Kyoto rats, weighing 100–150 g upon arrival (Charles River Wiga, Sulzfeld, Germany), were housed with free access to water and standard rat chow in a humidity- and temperature-controlled room with 12-h light and dark cycles. Twenty animals with a body weight of 320–380 g were used for the experiments. All operations were done with the animals under general anaesthesia/monitored anaesthesia care with an experienced anaesthesiologist who was blind to the experimental groups of the animals.

2.2. Anaesthesia and general monitoring

As previously described, anaesthesia was induced by inhalation of sevoflurane 8.0 vol% (inspiratory oxygen concentration 30%, Sulla 303 V anaesthesia circuit system, Draeger, Lübeck, Germany) and maintained with 2.5–3.0 vol% under volume-controlled mechanical ventilation. (tidal volume 10 ml/kg, frequency 35 min, Animal Ventilator, REMA Labortechnik, Heidelberg, Germany) (Pawlik et al., 2005). The left or right hind paw vein was exposed and cannulated using a 24-gauge venous catheter. In order to maintain fluid balance we administered 4 ml/kg/h Ringer solution. Normothermia ($37 \pm 0.6\,^{\circ}C$) was maintained by positioning the animals supine on an electric heating plate until instillation of the LICOX device. Continuous monitoring was performed by ECG, oxygen saturation (SpO_2) and rectal temperature (T_r) (Siemens 9000C monitor, Erlangen, Germany).

To minimize the influence of anaesthesia on the rewarming experiments, general anaesthesia with sevoflurane with controlled ventilation was completed after placement of the LICOX device and changed to monitored anaesthesia care. Thiopental was titrated to abolish the provoked righting reflex during surface cooling and rewarming while allowing spontaneous breathing (Gustafsson et al., 1996).

2.3. Subcutaneous tissue oxygen tension ($P_{sq}O_2$)

$P_{sq}O_2$ was assessed by means of a LICOX CMP™ monitoring device (GMS, Kiel-Mielkendorf, Germany; http://www.agmt.de/english/mitglieder/gms/kprofil.htm) including a flexible, minimally invasive microsensoric partial pressure of oxygen catheter. The catheter was placed subcutaneously during general anaesthesia in the right thigh via a 20-gauge catheter, fixed with sterile bandages in order to ensure a stable position and protected from environmental influences with a thin cotton towel.

2.4. Surface cooling

After installation of the LICOX device, mild hypothermia was induced with ice packs placed on the neck, abdomen, and both upper extremities and blowing by an electric fan under monitored anaesthesia care with thiopental. Cooling was stopped at a rectal temperature of $35.0\,^{\circ}C$. A waiting period of 15-min was completed to reach a steady state. Subsequently, before the rewarming treatment with placebo or urapidil, baseline values were recorded (T_r, SpO_2, $P_{sq}O_2$).

2.5. Rewarming experiments with placebo or urapidil

The study was a randomized, double-blind comparison of placebo (NaCl 0.9%) and urapidil of 20 mildly hypothermic animals (10 animals per group). Since rodents are generally less sensitive to drugs than humans based on body weight, we therefore used five times the human intravenous dose (5 mg/kg body weight) (Klaassen, 2001).

Rewarming was performed with a conventional convective air warming device (Warm Touch®, Tyco Healthcare, Germany, step 2: 38–40 °C) until normothermia (T_r: $37.0\,^{\circ}C$) was achieved. The animals were allocated for the experiments on two randomly assigned days according to the following two experimental protocols:

Protocol A: before rewarming: i.v. bolus of 1.0 ml NaCl 0.9% kg body weight;

Protocol B: before rewarming: i.v. bolus of 5 mg urapidil/kg body weight.

2.6. Electron microscopy

Four cutaneous tissue blocks of the thigh with the LICOX device were taken from each animal after reaching normothermia. The tissues were fixed in situ 1 h after irradiation with Karnovsky buffer (2% formaldehyde, 2.5% glutaraldehyde in cacodylate buffer) at 4 °C for 1 h. The tissues were then washed with cacodylate buffer (6×5 min). After fixation with osmium for 1 h (1% osmium tetroxide in cacodylate buffer) tissues were washed again with cacodylate buffer (6×5 min). Following dehydration in ethanol, the tissues were embedded in propylene oxide (EMbed 812). After polymerization (60 °C, 48 h) the coverslips were removed with liquid nitrogen. For orientation, semi thin sections (1.0 μm) were stained with toluidine blue. Ultrathin sections (70 nm) were mounted on copper grids and stained with uranyl acetate and lead citrate. Per tissue block, 20 test fields were semiquantitatively (score: (1) no swelling; (2) moderate swelling; (3) extreme swelling; (4) loss of structure) evaluated for the morphology of every structure of interest (endothelial cells, myocytes, mitochondria) by a pathologist who was blind to

the experimental groups of the animals, using a CEM 902 transmission electron microscope (Zeiss, Jena, Germany).

2.7. Data analysis

Area under the curves (AUC, trapezoidal rule) of the $P_{sq}O_2$ courses ($AUC_{P_{sq}O_2\text{-placebo}}$ and $AUC_{P_{sq}O_2\text{-urapidil}}$) of both treatment groups were calculated with Kinetica 2000 (www.innaphase.com). For statistical analysis statistical software package SPSS version 12.0 was used. Continuous data were compared with Student *t*-tests for independent groups. Kruskall–Wallis tests were used to compare ordinal data. The results are presented as means ± SD. All *P* values are two sided. $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Baseline

Both groups had a similar body weight at the beginning of the experiments (placebo: 343 ± 21 g; urapidil: 349 ± 28 g; ns, $P = 0.67$). The cooling time to achieve mild hypothermia before treatment was 21.5 ± 3.9 min in the placebo group and 24.8 ± 2.8 min in the urapidil group (ns, $P = 0.41$). The thiopental requirement for monitored anaesthesia care during cooling and rewarming was similar in both groups (placebo: 48.9 ± 4.1 mg/kg; urapidil: 52.8 ± 5.1 mg/kg; ns, $P = 0.72$). Rectal temperatures after cooling were comparable and nearly constant before rewarming treatment with placebo or urapidil (placebo: 34.46 ± 0.1 °C, urapidil: 34.29 ± 0.1 °C; $P = 0.26$). The subcutaneous tissue oxygen tension ($P_{sq}O_2$) after cooling was similar in both groups (placebo: 27.8 ± 13 mmHg; urapidil: 26.9 ± 11 mmHg; ns, $P = 0.36$). Thus, baseline characteristics before starting the rewarming therapy after randomized administration of placebo or urapidil were similar in both groups.

3.2. Rewarming time

Administration of urapidil significantly decreased the rewarming time (placebo: 30.2 ± 2.9 min, urapidil: 24.2 ± 2.3 min, $P = 0.012$) until normothermia (37.0 °C) was achieved (Fig. 1).

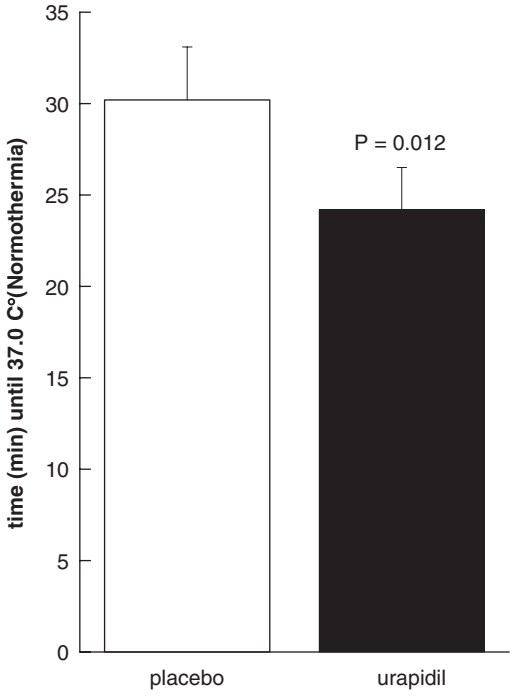

Fig. 1. Urapidil significantly decreased the rewarming time.

3.3. Subcutaneous tissue oxygen tension ($P_{sq}O_2$)

Urapidil treatment together with rewarming showed an acute increase in $P_{sq}O_2$. Within 5 min after urapidil the tpo2 rose from 27 to 53 mmHg whereas the placebo group showed a slow response and needed 10 min ($P<0.001$) to reach its plateau of about 48 mmHg (Fig. 2).

$P_{sq}O_2$ in the urapidil group was significantly higher throughout the rewarming experiment compared with the placebo group (Fig. 2, $P = 0.023$ between $AUC_{P_{sq}O_2\text{-placebo}}$ and $AUC_{P_{sq}O_2\text{-urapidil}}$).

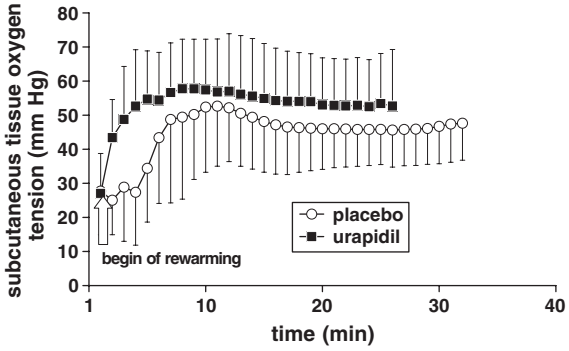

Fig. 2. Rewarming with urapidil is associated with an acute and higher increase in $P_{sq}O_2$ compared with placebo. The $AUC_{P_{sq}O_2\text{-urapidil}}$ is significantly higher compared with the $AUC_{P_{sq}O_2\text{-placebo}}$.

3.4. Electron microscopy

The subcutaneous increase in oxygen pressure in the urapidil treatment group was not associated with swelling of subcutaneous endothelial cells, myocytes or their mitochondria compared with the placebo group (example of normal structures presented in Fig. 3).

4. Discussion

The main finding of this randomized, placebo-controlled study is the immediate increase and significantly higher level of subcutaneous tissue oxygen tension during convective rewarming with urapidil.

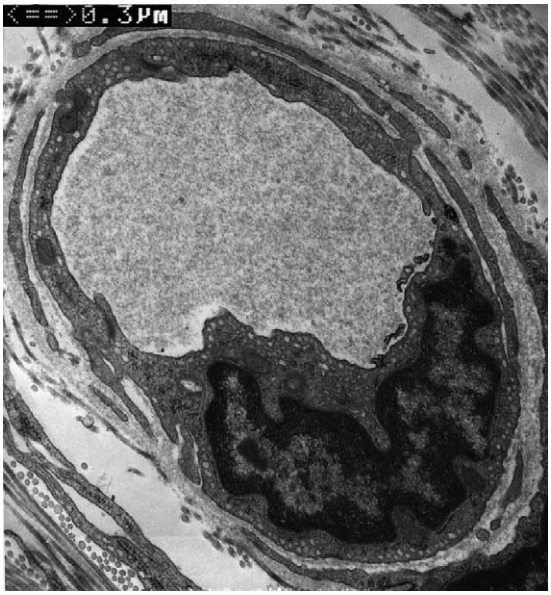

Fig. 3. Rewarming with urapidil showed no reperfusion injury compared with placebo. Depicted is an example of an electron micrograph of a subcutaneous capillary after rewarming under urapidil with normal endothelial cells, myocytes and mitochondria. Reference bar for magnification is 0.3 µm.

The conscious sedation with thiopental did not influence the results since the thiopental consumption was similar in both treatment groups. In addition, oxygen can be ruled out as an effector, since both groups breathed room air. No serious adverse reactions were monitored. All animals survived in very good clinical condition. Electron microscopy showed no reperfusion injuries on subcutaneous endothelial cells, myocytes or mitochondria after urapidil treatment compared with placebo therapy.

Despite not having measured vasotonus in subcutaneous vessels or subcutaneous blood flows in our experiments, there is evidence that vasodilation with urapidil during rewarming may have influenced the significant subcutaneous oxygen increase compared with placebo treatment. The subcutaneous perfusion is controlled by α_1-adrenoceptors. Jarajapu et al. (2001) have shown that vasoconstriction in subcutaneous resistance arteries is predominantly mediated by the α_{1A}-adrenoceptor subtype. Urapidil is a selective α_1-adrenoceptor antagonist with high affinity for the α_{1A}-adrenoceptor subtype and a central 5-HT_{1A} agonist (Gross et al., 1987, 1988). The significantly reduced time course of rewarming with urapidil versus placebo may be a further indicator that vasodilation increased the shift of heat from the periphery to the core.

We cannot rule out an additional reducing effect of urapidil on metabolism with consecutive reduction of oxygen consumption and increased oxygen delivery to subcutaneous tissues. Besides the α_1-adrenoceptor antagonistic activity, urapidil activates serotonin 1A (5-HT_{1A}) receptors in the central nervous system (CNS) (Mandal et al., 1989). It is known that 5-HT_{1A} receptor agonists mediate hypothermia (Millan et al., 1993). Furthermore, it has been shown, that urapidil suppresses cold-induced shivering in humans (Ittner et al., 1996) and reduces the thermoregulatory thresholds for cold (Fritz et al., 2002).

There is no general agreement regarding the influence of pharmacological vasodilation on transfer of heat between the periphery and the core. Clough et al. (1996) found that peripheral-to-core heat transfer was unimpeded during general anaesthesia in young volunteers, whether subjects were vasodilated or vasoconstricted. Furthermore, phentolamine, an unspecific α_1-adrenoceptor antagonist, inhibits cold-induced vasoconstriction in elderly awake volunteers but not in young awake volunteers (Frank et al., 1996). On the other hand, Vassilieff et al. (1994) showed that vasodilation induced by the calcium channel blocker nifedipine several hours before induction of anaesthesia minimizes redistribution hypothermia. In contrast, redistribution hypothermia was aggravated by administration of the same drug immediately before induction of anaesthesia. Obviously, the vasodilator nifedipine acted by decreasing the core-to-peripheral tissue temperature gradient. One important distinction between these studies may have influenced the results: i.e. anaesthesia. The volunteers in the Clough study were fully anaesthetized, whereas they received no anaesthesia in the studies by Frank and Vassilieff.

The clinical relevance of our results has to be determined by further studies. Since we only investigated subcutaneous oxygen pressure in healthy tissues but not oxygen tension in surgical wounds, we have no predictive data for a reduced development of wound infections and improvement of healing. Whether rewarming of mildly hypothermic volunteers or patients could be accelerated by urapidil treatment also needs to be determined as well. It is clear that our dose of 5 mg/kg body weight for rats should not be used in humans since such a dose is about five times that of the initial human dose used in hypertensive emergencies (Hirschl et al., 1997).

In conclusion, urapidil enhanced $P_{sq}O_2$ during convective rewarming in mildly hypothermic rats and this effect was accompanied by an accelerated rewarming time. No reperfusion injuries could be observed.

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