Role of hydrogen peroxide in the vasomotor regulation in small veins

PhD thesis

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1. CLINICAL INTRODUCTION

Modern surgical techniques in the field of reconstructive plastic surgery to transfer tissues (skin, fat, muscle, bone) are flap-raising techniques or raising a microvascular flap. In such cases the key to tissue survival is the non-traumatic surgical technique and post-operative support of the blood flow. The success of the surgery depends greatly on availability of arterial inflow, but an adequate venous outflow is at least as important. The venous insufficiency, constriction and/or thrombosis of the affected vessel can lead to venous stasis and subsequent flap necrosis, which may lead to an unsuccessful surgery.

In reconstructive plastic surgery, ischemia reperfusion (I/R) damage partly due to the development of oxidative stress significantly affects muscle flap survival. However, in the course of I/R little information is available concerning the haemorheological parameters and the effects of oxidative stress, and in particular, the impact of these factors on the nutrition supply of venous microvessels.

Better understanding the physiological role of veins and the factors affecting venous function in pathological state, and developing regulatory processes in the background will greatly promote the development of surgical techniques and the development of microsurgical techniques, and it is important for developing perioperative treatments supporting flap circulation as well.

The tissue damage, the reperfusion and tissue infection are associated with oxidative stress. One such molecule is H_2O_2. However, it is interesting that the clinical application of H_2O_2 as an antiseptic agent during surgical procedures is a daily routine. Using H_2O_2 solution in rats and humans facilitates the detachment of the crust and shortens the healing time. However, typical bulla formation and ulceration appears when H_2O_2 is used when new epithelium becomes visible after the crust is peeled off, indicating the importance of the concentration-dependent effect of this molecule.

In reconstructive surgery, the transplanted tissue flap (skin, muscle, fat) are often treated with 0.75 to 3% H_2O_2 ($8x10^{-2}$ M) for the purpose of wound and tissue cleaning. H_2O_2 dissolves blood clots and removes dead skin cells and blood from both donor and recipient areas to prevent infection. Using H_2O_2 on transplanted tissue oxidative stress may develop during microsurgical procedures.

In the cardiovascular system in many disease state and in relation to the I/R the formation of ROS is increased, including the level of the most stable, not too reactive H_2O_2. H_2O_2 can be produced by a variety of cell types (activated leukocytes, macrophages, platelets, etc.). The

effect of H_2O_2 on the arterioles has been already characterized, but there is no information about the direct effect of H_2O_2 on the spontaneous tone of skeletal muscle venules.

The exogenous administration of H_2O_2 for the purpose of disinfecting surgical wounds is a widely used method. The change of the venous microcirculation in various pathological conditions (atherosclerosis, diabetes mellitus, hypertension, hyperhomocysteinaemia) assumes the role of the endogenous H_2O_2 in the tissue circulation.

2. HYPOTHESIS

1. Therefore, and based on the experiments previously carried out on arterioles we hypothesized that H_2O_2 has a significant vasomotor effect also in small veins.
2. The H_2O_2 influences the smooth muscle tone of small veins by activating endothelial and smooth muscle mechanisms.

3. OBJECTIVES

In isolated rat skeletal muscle small veins our objectives are to characterize:

1. the effect of increasing intraluminal pressure on the vessel diameter in active and passive (Ca^{2+}-free) circumstances
2. the changes of the myogenic response in the presence of catalase
3. the changes of the myogenic response in the presence of the non-selective COX_1 and COX_2-inhibitor indomethacin
4. the changes of the myogenic response in the presence of the selective TXA_2/PGH_2 blocker
5. the concentration dependent effect of H_2O_2 (10^{-6}-10^{-4} M) on the diameter of the isolated skeletal muscle venule, and characterizing the cellular mechanisms responsible for the vasomotor activity;
6. the concentration dependent effect of exogenous AA (10^{-6}-10^{-4} M) on the diameter of the isolated skeletal muscle venule, and characterizing the cellular mechanisms responsible for the vasomotor activity;
7. the effect of catalase on the pressure-, H_2O_2- or AA-induced vasomotor responses in skeletal muscle small veins

4. MATERIALS, METHODS AND OUR PROTOKOLS

Male Wistar rats (~250 g) were housed separately and had free access to water and standard rat chow. Animals were anesthetized with intraperitoneally injected pentobarbital sodium (50 mg/kg) and small veins from the gracilis muscle were isolated. From the gracilis muscle we isolated the small gracilis vein with the help of microsurgery devices and an operation microscope. Vessels were transferred into an organ chamber containing standard Krebs solution (in mmol/L: NaCl 110, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.0, KH_2PO_4 1.0, glucose 5.5, and $NaHCO_3$ 24.0; equilibrated with 10% O_2, 5%CO_2, 85% N_2, at pH 7.4). Then vessels were cannulated at both sides and were continuously superfused with Krebs solution. Temperature was set to 37ºC and controlled by temperature controller. The vessels were incubated at constant intraluminal pressure (10 mmHg). The perfusate flow, which was zero, was monitored with a flowmeter. The inner diameter of the vessels was measured with a videomicroscope connected to a microangiometer and a recorder.

4.1 Spontaneous vasomotor responses

At the beginning of our experiments we incubated the small veins for 90 minutes. During incubation we did not apply any vasoactive agent. The vessels were superfused with Krebs solution only, and we measured the spontaneous vasomotor tone at 10 mmHg intraluminal pressure.

4.2 Measurement of the vasomotor responses in response to elevation of the intraluminal pressure

One characteristic of the vessel chamber-system is that it allows setting the intraluminal pressure arbitrarily. Based on previous studies and on our own experience we concluded that in small veins of Wistar rats, physiologically 6-10 mmHg intraluminal pressure is present, therefore, in the incubation period, and later during the incubation time of the administration of the inhibitors 10 mmHg intraluminal pressure was maintained.

After equilibration period at 10 mmHg, changes in the diameter of small veins were measured in response to elevations of the intraluminal pressure. The intraluminal pressure was changed stepwise between 1–12 mmHg (2 mmHg/3min). Vasoactive agents and enzyme inhibitors

were also used, and the changes of the diameter in response to the elevation of the intraluminal pressure were also taken in the presence of inhibitors.
Changes in vessel diameter due to the different vasoactive substances were compared to the myogenic tone developed as a result of gradually increased intraluminal pressure. In our experiments the vasomotor tone after the initial incubation was considered as the control condition, it evolved without the use of any vasoactive agent or inhibitor.

4.3 Examination of the signal transduction mechanisms of vasomotor responses

Vasomotor responses to increases in intraluminal pressure were tested in the presence of various inhibitors: indomethacin, SQ 29,548 and catalase were used. After registration of the myogenic tone as a control, small veins were incubated with the inhibitors in the previously described dosages and time. Then again vasomotor responses were examined after the elevation of the intraluminal pressure stepwise between 1 to 12 mmHg. Changes of the myogenic tone were recorded.

4.4 Examination of the direct vasoactive effect of arachidonic acid and H_2O_2

In various series of experiments we examined the direct vasoactive effects of arachidonic acid and H_2O_2. Concentration-dependent effect of exogenously applied arachidonic acid and increasing doses of H_2O_2 were investigated with and without the use of inhibitors (INDO, SQ) in the regulation of myogenic tone in small veins.

5. RESULTS

5.1 Development of myogenic tone of small veins

In the experiments, we used venules of the same size, from the initial 370 ± 12μm passive diameter the small veins was reduced during the incubation period to 260 ± 19μm active (basal) diameter. The observed diameter, which corresponds to the spontaneous myogenic tone, remained stable during the experiments. The resulting vascular tone is called active diameter or basal vascular tone. The phenomenon that the vessel constricts in the presence of an intraluminal pressure is called the active, pressure-induced myogenic response. We tested

how the myogenic tone was influenced by the level of the intraluminal pressure, that is, under what pressure would the myogenic response remain still intact. Presumably, a pressure of 20 mmHg only rarely occurs in these vessels in physiological conditions. From this result, we concluded that in the presence of myogenic tone at a pressure of 10 mmHg results in a stable, significant myogenic response. Active myogenic response was recorded on increase of the intraluminal pressure. The strongest myogenic response was seen in the pressure range of 6-10 mmHg.

5.2 Effect of indomethacin on the myogenic tone of small veins

After the myogenic tone was examined vasomotor responses to increases in intraluminal pressure (1-12 mmHg stepwise elevation of the pressure) were tested and the mechanism of action was investigated. Under control conditions spontaneous myogenic tone developed in the small veins to an increase in intraluminal pressure. We examined how the presence of INDO affects the myogenic response. It can be seen that INDO significantly reduced the myogenic vascular tone that developed in response to intraluminal pressure. These results suggest that in the development of myogenic response, COX enzyme derived constrictor factors are involved.

5.3 Effect of SQ 29,548 on the myogenic tone of small veins

On the basis of our experiments with INDO we hypothesized that COX-derived constrictors, TXA_2/PGH_2 play a role in mediating the myogenic response. Therefore, we investigated the effect of the TXA_2/PGH_2 (TP) receptor antagonist SQ 29,548 on vasomotor response. Under control conditions the myogenic tone developed to elevation of intraluminal pressure that was significantly reduced in the presence of SQ.

5.4 Effect of catalase on the myogenic tone of small veins

The literature suggests that H_2O_2 may play a role in the regulation of vasomotor responses in microvessels. Thus we also assessed the effect of catalase (CAT), an enzyme metabolizing H_2O_2, on the myogenic tone of small veins. The myogenic tone was substantially and significantly reduced in the presence of catalase, similarly as in case of INDO or SQ 29,548 treatments.

5.5 Direct vasomotor effect of arachidonic acid on small veins

In these experiments we investigated the vasoactive responses to arachidonic acid in small veins. Increasing concentrations of arachidonic acid were administered extraluminally into the vessel chamber. Between the administrations of the different doses the vessel chamber was washed out with Krebs solution. Increasing concentration of arachidonic acid elicited a substantial vasoconstriction in small veins. The extent of the constriction response increased with increasing concentrations of arachidonic acid. Arachidonic acid-induced responses were examined after INDO and SQ 29,548 pretreatment in separate experiment sets. Our results show that both INDO and SQ 29,548 reduced, and INDO completely eliminated the arachidonic acid-induced constriction.

5.6 Direct vasomotor effect of H_2O_2 in small veins

Direct vasomotor effect of H_2O_2 in small veins was examined by extraluminally administered H_2O_2 at increasing concentrations. The applied concentrations correspond to the H_2O_2 concentrations physiologically present around of the microvessels. Increasing doses of H_2O_2 generated significant concentration-dependent constrictions. We hypothesized that constrictor prostaglandins play a role in the H_2O_2-induced constriction, thus we studied the H_2O_2-induced responses in the presence of INDO and SQ in separate experiments. The H_2O_2-induced vasoconstriction was decreased both after INDO and SQ pretreatments. Compared to the basal vascular tone, after administration of SQ significant vasodilation was present that refers to the increased level of the dilator factors after the inhibition of the powerful constrictor TXA_2/PGH_2.

6. DISCUSSION, PHYSIOLOGICAL AND CLINICAL SIGNIFICANCE

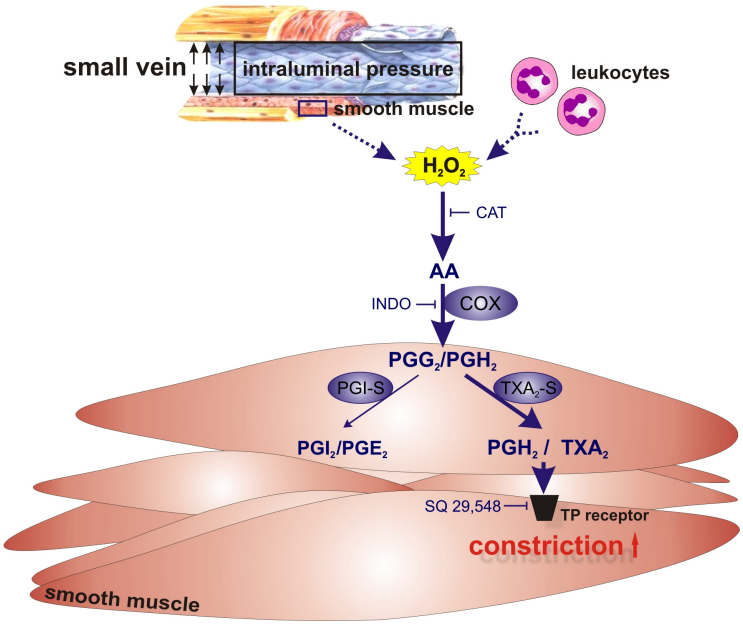
Based on these results, considerable pressure-sensitive myogenic response can be detected in isolated rat skeletal muscle small veins. The myogenic response is mainly mediated by constrictor prostaglandins, and by H_2O_2. H_2O_2 and also arachidonic acid itself influences the diameter of the small veins. Thus, our results suggest that pressure and ROS are able to significantly change the diameter of the small veins thereby affecting the function of the microcirculation.

In the small veins considerable pressure-sensitive myogenic response has developed, which has been studied only by few investigators. Similarly to the arterioles, in small veins the myogenic mechanism is an important mechanism to regulate local blood flow. Myogenic vasomotor function of small veins regulates rheological functions as well, and in general, regulates the venous circulation. Our data suggest that constrictor prostaglandins, TXA_2 and PGH_2 produced by COX enzymes are responsible for the myogenic response. In the present experiments, in isolated skeletal muscle small veins we proved the role of oxidative stress and ROS in myogenic tone regulation.

In animal I/R model it was demonstrated that leukocyte levels are elevated in the venous circulation. It is also known that activated leukocytes produce H_2O_2, which can reach micromolar tissue levels. H_2O_2 plays a significant vasomotor role in small veins, leading to constriction. The H_2O_2-mediated constriction responses are also mediated by constrictor prostaglandins.

H_2O_2 plays a role in the development of myogenic tone also by increasing the production of constrictor prostaglandins. In various diseases affecting the cardiovascular system and during local oxidative mechanisms the produced H_2O_2 is able to mediate such vasomotor effects that are important for the entire circulatory system. Previous studies have suggested that H_2O_2 produced by other cells (e.g. leucocytes) has an effect on vascular tone. Our results demonstrate that H_2O_2 has a constrictor effect in skeletal muscle small veins, which supports the idea that during inflammation H_2O_2 released from cellular components can play an important role in the regulation of microcirculation.

Proposed intracellular mechanisms responsible for intraluminal pressure-induced myogenic responses and H_2O_2-induced responses in small skeletal muscle veins



In response to intraluminal pressure or H_2O_2 the synthesis of the constrictor prostaglandins (PGH_2/TxA_2) from arachidonic acid increases eliciting constrictions. The physiological role and importance can be envisioned as follows: H_2O_2 released due to increases in intraluminal pressure, from activated leukocytes, macrophages or from exogenous sources - via upregulation of arachidonic acid (AA) pathway, and thus production of constrictor prostanoids - can modulate the vasomotor tone of small venous vessels, affect rheological parameters and promote platelet aggregation, thereby substantially influencing venous and capillary microcirculation.

7. NEW OWN OBSERVATIONS

1. In isolated small muscle veins myogenic response was established in response to the increases in intraluminal pressure (1-12 mmHg), confirming the previous results of Dörnyei, Racz, and Koller et al.
2. According to our results, the myogenic response is partially mediated by constrictor prostaglandins since COX inhibition (indomethacin) significantly reduced the myogenic tone. Since inhibition of the TP receptor significantly reduced the spontaneous myogenic tone, we concluded that the myogenic response is mediated by TXA_2/PGH_2. Addition of catalase significantly reduced the pressure-induced myogenic tone, so among the mediators H_2O_2 has a high impact. In summary, we believe that the intraluminal pressure-induced wall stretch leads to the release of H_2O_2 in skeletal muscle venules, which activates the arachidonic acid metabolism, leading to the production of constrictor prostaglandins (TXA_2/PGH_2) and thus constriction.

3. H_2O_2 itself may have an effect on the diameter of isolated skeletal muscle venules. Increasing concentrations of H_2O_2 (10^{-9}-10^{-5} M) elicited substantial, concentration dependent decreases in the diameter of small veins. The mechanism is mediated by endothelial and smooth muscle derived TXA_2/PGH_2, because COX inhibition (indomethacin) and TP receptor blockade (SQ 29,548) significantly reduced H_2O_2-induced constrictions.
4. Administration of arachidonic acid (10^{-7}- 10^{-4} M) also established a concentration-dependent decrease in diameter, which was significantly inhibited by the COX inhibitor indomethacin and the TXA_2 receptor inhibitor SQ 29,548. Accordingly, we conclude that in small veins the constrictor effect of arachidonic acid is mediated by constrictor prostaglandins, namely TXA_2/PGH_2.

Our results are the first in the literature, revealing details regarding the pressure-induced myogenic tone of the small veins and the molecular mechanisms responsible for H_2O_2-induced vasomotor response.

H_2O_2 production and the consequent effect of constrictor prostaglandins can lead to a reduction in venous microcirculation with increased thrombus formation. Thus H_2O_2 can have significant effects on the microcirculation of the transplanted tissue flap and on its survival. It is likely that these results will help to develop pharmaceutical means to reduce tissue injury due to oxidative stress by local antioxidant therapy and/or application of a COX inhibitor, or using TXA_2/PGH_2 receptor blockers. New therapeutic options in the future could prevent major complications of venous microcirculation and increase tissue survival during plastic surgery.

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10. LIST OF OWN PUBLICATIONS

10.1 PUBLICATIONS IN CONNECTION WITH THE THESIS

B. Debreczeni, Z. Veresh, E. Gara, A. Marki, A. Racz, R. Matics, J. Hamar and A. Koller Hydrogen peroxide via thromboxane A_2 receptors mediates myogenic response of small skeletal muscle veins in rats. Clinical Hemorheology and Microcirculation. 54 (2013) 393–407 DOI 10.3233/CH-131709
Impact Factor: 3.398

Veresh Z, **Debreczeni B**, Hamar J, Kaminski PM, Wolin MS, Koller A.
Asymmetric dimethylarginine reduces nitric oxide donor-mediated dilation of arterioles by activating the vascular renin-angiotensin system and reactive oxygen species. Journal of Vascular Research. 2012;49(4):363-72. doi: 10.1159/000337485. Epub 2012 May 30**.**
Impact Factor: 2.651

10.2 OTHER PUBLICATIONS NOT RELATED TO THE THESIS

Tamas R, Nemeth N, Brath E, Sasvari M, Nyakas C, **Debreczeni B**, Miko I, Furka I.
Hemorheological, morphological, and oxidative changes during ischemia-reperfusion of latissimus dorsi muscle flaps in a canine model. Microsurgery. 2010 May;30(4):282-8. doi: 10.1002/micr.20699
Impact factor: 1.555

CUMULATIVE IMPACT FACTOR: 7,604

10.3 OTHER PUBLICATIONS

Hamar J, Solymár M, Tanai E, Cseplo P, Springo Z, Berta G, **Debreceni B**, Koller A
Bioassay-comparison of the antioxidant efficacy of hydrogen sulfide and superoxide dismutase in isolated arteries and veins. Acta Physiologica Hungarica Dec 1, 2012
Impact Factor: 0.821