TOPIC 03-1 – Oxydative stress, NO, aging

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0318

Enhanced anti-oxidant defence protects mitochondrial function against ischemia-reperfusion in oxidative muscles

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Purpose: Acute ischemia of the limb occurs frequently in humans and its prevalence will still grow with the general aging of the population. Ischemic insult and subsequent reperfusion seems to lead to different degrees of muscle injuries, depending on muscle metabolic phenotype. But the explanation of this difference remains unclear. We hypothesized that mitochondria play a main role in this difference.

Methods: 20 Wistar rats were divided in 2 groups: sham-operated group (C, n=11), and ischemia-reperfusion group (submitted to 3 hours of ischemia induced by infrarenal aortic clamping and followed by 2 hours of reperfusion; IR, n=9). Mitochondrial respiratory chain complexes I, II, III, and IV activities, reactive oxygen species (ROS) production by dihydroethidium staining, and anti-oxidant power (total glutathione) were determined in the superficial glycolytic gastrocnemius, and the oxidative soleus.

Results: In glycolytic muscle, IR impaired Vmax (complexes I, III, IV activities) as compared to C group (4.5±0.5 *versus* 6.6±0.4 µmol/min/g dry weight, respectively, -31.8%, p=0.004). The oxidative muscle was not altered. Similar results were observed for Vsucc (complexes II, III, IV activities). After IR, only in glycolytic muscle, ROS production increased (+122.5%, p=0.04), and total glutathione decreased (0.70±0.04 *versus* 0.52±0.06 µmol/L/g, in gastrocnemius C and IR group respectively, p=0.02). No change in ROS production or total glutathione was observed in soleus.

Conclusion: Enhanced anti-oxidant defence likely protects mitochondrial function, and explain that, when submitted to ischemia-reperfusion, oxidative muscles are most resistant than glycolytic muscles.

0169

Acute exposure to diesel particle matter impairs NO-mediated microvascular function

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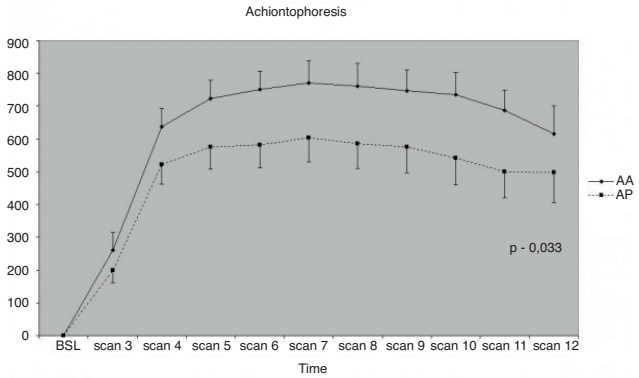
Background: Exposure to diesel Particle Matter (PM) was recently identified as an important cardiovascular risk factor. Whether diesel PM exerts acute specific deleterious effects on arterial stiffness, aortic wave reflection and endothelial function are not known.

Methods: We tested these hypotheses in a randomized, crossover study design in 13 healthy male. The effects of 2 hours exposure to diesel PM, as compared with normal air, on skin microvascular hyperemia to local heating and iontophoresis of acetylcholine (Ach) and sodium nitroprussiate (SNP), were examined using Laser Doppler Imager system. Before local heating, skin was pre-treated either by an iontophoresis of specific NO synthase inhibitor (L-NAME) or by saline solution (Control). Pulse wave velocity (PWV) and aortic augmentation index (AIx) were also evaluated. Diesel PM exposure was performed in computer-assisted inhalation room, controlling pollutants produced by motor engine.

Results: The PM<2.5 mean concentration was 10.01+-0.08µg/m³ on normal air and 127.9+-2.8µg/m³ on polluted air (p<0.001). Acute diesel PM exposure increased systolic BP (p<0.05) but had no effect on aortic wave reflection and pulse wave velocity. Compared to ambient air, diesel PM exposure reduced skin vasodilatation induced by Ach (p<0.05), but did not affect vasodilatation induced by SNP or local heating. However, NO-mediated vasodilatation, assessed by the skin thermal hyperemia difference between control and L-NAME sites; decreased from 2423±809% to 400±552% (p<0.05) after diesel PM exposure.

Conclusion: In healthy subjects, acute experimental Diesel PM exposure, at a level usually encountered during city's pollution peak, impairs microvascular endothelial mediated vasodilatation throughout a decrease in NO biovailibility.



Graph 1 : Blood Flow under Normal Air or Filtered Air during the Ach administration, in Perfusion Unit

0302

Red wine polyphenols prevent the blunted EDHF-mediated relaxation induced by doxorubicin in the rat mesenteric artery

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Doxorubicin is a potent chemotherapeutic agent effective against many types of cancers. Unfortunately, its clinical use is restricted by its cardiotoxicity, which involves free radical formation. This study aims to determine whether doxorubicin affects vascular reactivity and, if so, to assess the preventive effect of red wine polyphenols (RWPs), a natural source of antioxidants. Male Wistar rats (12 weeks) were assigned to 4 groups; control, doxorubicin (15 mg/kg, i.p), RWPs (75 mg/kg/d in the drinking water for 22 weeks) and doxorubicin + RWPs. The reactivity of mesenteric artery rings was assessed in organ chambers, the vascular formation of reactive oxygen species (ROS) using dihydroethidine and the expression level of small and intermediate conductance calcium-activated potassium channels (SK_{Ca}, IK_{Ca}), connexin 40 (Cx40), endothelial NO synthase (eNOS), angiotensin II and AT1 receptors in mesenteric artery segments by immunohistochemistry. The EDHF-mediated relaxations to acetycholine were blunted in mesenteric artery rings of doxorubicin-treated rats in comparison to those of control rats whereas the NO-mediated relaxations were not affected. Impaired EDHF-mediated relaxations were associated with reduced expression of SK_{Ca}, IK_{Ca} and Cx40 in the arterial wall. Doxorubicin treatment increased the vascular formation of ROS and the expression of eNOS, angiotensin II and AT1 receptors. Intake of RWPs prevented the effects of doxorubicin on vascular reactivity, formation of ROS and expression of target proteins. Chronic treatment of rats with doxorubicin induced blunted EDHF-mediated relaxations which is due, at least in part, to a decreased expression of SK_{Ca}, IK_{Ca}, and Cx40 in the arterial wall. This is presumably the result of enhanced angiotensin II formation, which activates AT1 receptors leading to vascular oxidative stress. Intake of RWPs is able to prevent the doxorubicin-induced endothelial dysfunction most likely by preventing oxidative stress.

0388

Aronia juice, a polyphenol-rich berry juice, induces endothelium-dependent relaxations in porcine coronary arteries *via* the redox-sensitive activation of endothelial nitric oxide synthase

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Polyphenol-rich sources such as red wine strongly increase the formation of endothelial nitric oxide (NO), a potent vasoprotective factor, *via* the redox-sensitive activation of the Src/PI3-kinase/Akt pathway leading to activation of endothelial NO synthase (eNOS) by phosphorylation at Ser1177.

The present study examined the potency of Aronia juice, a polyphenol rich non-alcoholic natural product, to induce redox-sensitive relaxation and activation of eNOS. Vascular reactivity was assessed using porcine coronary artery rings in the presence of indomethacin and charybdotoxin plus apamin to prevent the formation of vasoactive prostanoids and inhibit endothelium-derived hyperpolarizing factor-mediated responses, respectively. The phosphorylation level of Src Tyr418, Akt Ser473 and eNOS Ser1177 was assessed in cultured coronary artery endothelial cells by Western blot analysis.

Aronia juice caused endothelium-dependent and NO-dependent relaxations in coronary artery rings. In cultured coronary artery endothelial cells, Aronia juice induced phosphorylation of Src, Akt and eNOS. Both the Aronia juice-induced relaxation and activation of eNOS were inhibited by membrane permeant analogues of superoxide dismutase (SOD) and catalase, MnTMPyP and PEG-catalase, respectively, but not by native SOD and catalase. Inhibitors of Src (PP2) and PI3-kinase (wortmannin) also inhibited both responses. Aronia juice induced the formation of reactive oxygen species as assessed by dihydroethidine staining, and of NO by electron paramagnetic resonance in endothelial cells.

Thus, the present findings indicate that Aronia juice is a potent inducer of endothelium-dependent relaxations of coronary arteries by increasing the endothelial formation of NO. This effect involves the redox-sensitive activation of the Src/PI3-kinase/Akt pathway leading to the phosphorylation of eNOS at Ser1177, an activator site.

0404

Redox-sensitive up-regulation of eNOS expression by Aronia melanocarpa juice in endothelial cells: Role of PI3-kinase/Akt, JNK, p38 MAPK, FoxO1, and FoxO3a

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The vascular protective effect of grape-derived polyphenols and tea catechins has been attributable, in part, to their action on blood vessels by stimulating the endothelial formation of nitric oxide (NO). This study examined whether Aronia melanocarpa juice (AMJ), a polyphenol rich natural product, up-regulates the expression of endothelial NO synthase (eNOS) and determined the underlying mechanism.

Endothelial cells were cultured from porcine coronary arteries. NO formation was assessed by electron paramagnetic resonance, the expression level of eNOS mRNA by RT-PCR and eNOS protein, p-Akt, p-JNK, p-p38 MAPK, p-ERK1/2, p-eNOS Ser1177, and p-FoxO1, p-FoxO3a, transcription factors regulating negatively eNOS expression, by Western blot analysis.

AMJ time-dependently increased eNOS mRNA level up to 9-fold after 8 h and protein level up to 2.3-fold after 15 h, leading to an 1.5-fold increased formation of NO. The stimulatory effect of AMJ on eNOS mRNA and protein expression was inhibited by membrane permeant analogues of superoxide dismutase (SOD) and catalase, MnTMPyP and PEG-catalase, respectively, and by LY294002 (PI3-kinase inhibitor), SP600125 (JNK inhibitor), and SB203580 (p38 MAPK inhibitor), but not by PD098059 (ERK1/2 inhibitor). AMJ induced the phosphorylation of Akt, JNK, p38 MAPK, and ERK1/2. Except for ERK1/2, these phosphorylations were prevented by MnTMPyP and PEG-catalase. AMJ induced the phosphorylation of FoxO1 and FoxO3a, which leads to their inactivation, and this effect was inhibited by MnTMPyP, PEG-catalase, LY294002, SP600125, and SB203580.

The present findings indicate that AMJ up-regulates the expression of eNOS leading to an increased formation of NO in endothelial cells. The stimulatory effect of AMJ is a redox-sensitive event involving PI3-kinase/Akt, JNK, p38 MAPK pathways and the subsequent inactivation of transcription factors FoxO1 and FoxO3a by phosphorylation thereby preventing their negative regulation of eNOS expression.

0377

The Free Oxygen Radicals Test (FORT) to assess circulating oxidative stress in patients with acute myocardial infarction

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Background and aim: Reactive oxygen species (ROS) play an important role in the pathogenesis of many diseases including cardiovascular diseases. Several methods have been developed for the direct or indirect measurement of oxygen free radical and its by-products. The current study was designed to validate the new Free Oxygen Radicals Test (FORT) and to investigate the potential relationships between ROS and clinical or biological factors in male patients with acute myocardial infarction (AMI).

Methods: We analysed FORT values in samples from 66 patients with AMI.

Results: FORT values ranged from 324 to 1198 FORT units, with a median value of 581 (494-754) FORT units. In univariate analysis, FORT values were positively related only to LVEF <40% (p=0.005), levels of CRP (r=0.438, p<0.001) and peak CK (r=0.274, p=0.028). Multiple linear regression analysis showed that CRP (p=0.023), LVEF <40% (p<0.001) and the presence of diabetes (p=0.039) were independent predictors of serum FORT values. This statistical model can explain 45% of the variance in FORT values (R^2=0.45) (Table).

Conclusions: The FORT is a simple tool to assess circulating ROS in routine clinical practice. Oxidative conditions such as inflammation and diabetes are the major determinants of FORT values in patients with AMI.

	Population totale	
	Coefficient β	**p**
Age	+0,133	0,301
β– -Blocker on admission	-0,134	0,265
LVEF<40%	+0,421	<0,001
Peak Creatinine kinase	+0,172	0,177
CRP	+0,265	0,023
ST-Elevation MI	-0,033	0,785
Neutrophils Count	+0,071	0,589
Prior MI	-0,197	0,119
Diabetes	+0,232	0,039
R^2	0,45	*

0307

Association of plasma oxidative stress with leukocyte length in early postmyocardial infarction period

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Introduction: Asymmetric dimethylarginine (ADMA), is an endogenous competitive inhibitor of NO synthase (NOS), whereas in the closely related compound symmetric diméthylarginine (SDMA) does not inhibit NOS. ADMA leads to a decreased NO bioavailability, increasing oxidative stress and endothelial dysfunction. Recent data suggest that leukocyte telomere length (LTL) may be a possible reliable marker for cardiovascular pathologies such as acute myocardial infarction (MI). The aim of our study was to identify the possible association of plasma ADMA and LTL as biomarkers of oxidative stress in early postmyocardial infarction period.

Methods: Blood samples from 33 consecutive patients hospitalized <24 hours after symptom onset for acute MI and admitted to the coronary care unit of from Dijon University Hospital were taken. Serum levels of L-arginine and SDMA were determined using high-performance liquid chromatography. For ADMA, ELISA kits were used. LTL was evaluated by extracting leukocyte DNA from venous blood samples and performing real time PCR. The L-arginine/ADMA ratio was used as a biomarker of endothelial dysfunction.

Results: LTL was significantly reduced in patients with the lowest L-arginine/ADMA ratio (1.15 vs 1.27 ratio T/S-1, p=0.005). A trend for a positive correlation between L-arginine/ADMA ratio, but not with SDMA, and LTL was noted (r=+0.339, p=0.053). Moreover, positive associations were found between serum levels of SDMA and age (r=+0.468, p=0.006), homocystéine (r=+0.462, p=0.012) and a negative association with creatinine clearance (r=-0.603, p<0.001).

Conclusion: Our study showed that in patients with acute MI, ADMA may be a useful biomarker of cardiovascular diseases and that reduced LTL was associated with lower serum ADMA levels. Further studies are now needed in order to explore the exact relationship between L-arginine metabolism pathways and mechanisms of telomere shortening.

0069

Cardiovascular sensitivity to acute oxidative stress after prolonged exposure to low concentrations of carbon monoxide: a key role for iNOS expression

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Introduction: Carbon monoxide (CO) is an ubiquitous environmental pollutant, which has been correlated with mortality related to cardiovascular diseases. We have previously reported that prolonged exposure to low CO level was responsible for phenotypical remodelling of the heart, implying exacerbated oxidative stress. In cardiovascular pathologies associated with redox status alterations, an inducible expression of iNOS is regularly mentioned as playing a key role. Therefore, the aim of the present study was to investigate whether iNOS expression is involved in the higher vulnerability of CO-exposed rats' cardiovascular system.

Materials and Methods: Wistar rats were exposed to simulated urban CO pollution (30/100 ppm) for 4 weeks. Vascular and cardiac expressions of iNOS were evaluated by western immunoblot and histological immunostaining. Then, cardiomyocyte function was evaluated on isolated cells after an anoxia-reoxygenation (A/R) sequence performed with or without S-methyl-isothiourea (SMT, 0.5µM) a specific iNOS inhibitor. Finally, vascular function was evaluated on isolated aortic rings before and after an oxidative stress (H_2O_2, 200µM, 20 min) performed in presence or not of SMT.

Results and discussion: iNOS expression was found to be upregulated in vascular-endothelium and cardiac tissues of CO exposed rats. In our model, iNOS overexpression was associated with exacerbated impairments of cardiomyocytes' function following A/R, and of endothelium-dependent vasodilation. Since, its inhibition by SMT, resulting in a lower synthesis of NO, prevents those damages, it seems that iNOS-dependent NO overproduction could be crucial in these phenomenon. In conclusion, this study highlights that prolonged exposure to CO at level found in urban environment is responsible for an upregulation of iNOS expression in cardiovascular system. This expression of iNOS appeared to be highly implicated in the increased sensitivity of cardiovascular system to oxidative stress.

0096

Implication of NOX-induced oxidative stress in transient left ventricular dysfunction observed after prolonged strenuous exercise

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Introduction: Mechanisms responsible for the transient left ventricular (LV) dysfunction following a prolonged strenuous exercise (PSE) are not well understood. We hypothesised that NADPH oxidase enzyme (Nox)-induced oxidative stress was involved in LV dysfunction after PSE.

Materials and methods: Rats were randomised into four groups: control (CTL), exercised (i.e. 4h continuous running on treadmill, PSE) with or without 3 days apocynin treatment in drinking water, a specific inhibitor of Nox. In each group, we assessed LV function *ex-vivo* (i.e. LV developed pressure, dP/dt_{max}, dP/dt_{min}, myocardial perfusion) with the isolated perfused Langendorff heart model. We assessed also plasmatic cardiac troponin I (cTnI) with immunochemiluminescence, connexin 43 (Cx43) dephosphorylation with Western blot, GSH/GSSG ratio and MDA tissue concentration with spectrophotometry.

Results: After PSE, LV dysfunction was characterised by a decrease in contractility (dP/dt_{max} in $mmHg.s^{-1}$: CTL, 4110±289 vs. PSE, 2917±197, P <0.05) and relaxation (dP/dt_{min} in $mmHg.s^{-1}$: CTL, -2307±170 vs. PSE, -1645±110, P <0.05) associated with cellular (higher plasmatic cTnI release) and functional (decreased myocardial perfusion, Cx43 dephosphorylation and decreased GSH/GSSG ratio) alterations. Apocynin treatment prevented LV dysfunction (dP/dt_{max} in $mmHg.s^{-1:}$ PSE APO, 3407±134 vs. PSE, 2917±197 and dP/dt_{min} in $mmHg.s^{-1:}$ PSE APO, -1923±96 vs. PSE, -1645±110, P <0.05) as well as previously described alterations. However, no increase in MDA was observed after PSE in all groups.

Conclusion: Our results underlined the implication of Nox-induced oxidative stress in the transient LV dysfunction observed after PSE. Indeed we showed that Nox inhibition with apocynin prevented the decrease in myocardial function *ex-vivo* and prevented the cellular oxidation. However, we showed no myocardial lipid peroxidation in all groups. Taken together, our findings support the implication of a signaling oxidative stress from Nox enzyme in transient LV dysfunction after PSE.