C007
REDOX-SENSITIVE ACTIVATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE BY CATECHINS: ROLE OF HYDROXYL MOIETIES

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Objectives — Several rich sources of polyphenols have been shown to strongly increase the endothelial formation of nitric oxide (NO), a potent vasoprotecting factor, via the redox-sensitive activation of the PI3-kinase/Akt pathway leading to the phosphorylation of endothelial NO synthase. The purpose of the present study was to investigate the molecular mechanisms underlying the stimulatory effect of catechins on the endothelial formation of NO using different catechins (flavan-3-ols).

Methods — Vascular reactivity studies were performed using porcine coronary artery rings, which were suspended in organ chambers for the measurement of changes in isometric tension. All experiments were performed in the presence of indomethacin (an inhibitor of cyclooxygenases), and the combination of apamin and charybdotoxin (two inhibitors of endothelium-derived hyperpolarizing factor-mediated effects) to assess only the NO component of the relaxation. Cultures of porcine coronary artery endothelial cells (P1) were used to determine the phosphorylation level of Akt and endothelial NO synthase by Western blot analysis. Both natural and synthetic catechins were evaluated.

Results — (-)-Epigallocatechin-3-O-gallate (EGCg) induced potent endothelium-dependent relaxations in porcine coronary artery rings. The EGCg-induced relaxation was inhibited by MnTMPyP (a membrane permeant analogue of superoxide dismutase, SOD) whereas extracellular SOD had no effect, indicating a major role of the intracellular formation of ROS. Relaxations to EGCg were minimally affected by rotenone (an inhibitory of the mitochondrial respiratory chain), sulphenazol (an inhibitor of cytochrome P450), apocynin (an inhibitor of NADPH oxidase) or allopurinol (an inhibitor of xanthine oxidase). The replacement of all hydroxyl groups of EGCg by O-methyl groups resulted in the total loss of the relaxing activity whereas partial replacement decreased the relaxing activity.

Conclusions — EGCg caused endothelium-dependent relaxations of coronary arteries via the redox-sensitive formation of NO in endothelial cells. The stimulatory effect does not involve major intracellular sources of ROS including the mitochondrial respiratory chain, xanthine oxidase, NADPH oxidase and cytochrome P450 but is critically dependent on the presence of hydroxyl groups possibly leading to auto-oxidation of the polyphenol.

C008
ENDOTHELIAL NO SYNTHASE ACTIVATION BY A RED WINE PHENOLIC EXTRACT: ISOLATION OF ACTIVE COMPONENTS BY BIOGUIDED FRACTIONATION

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Objectives — Previous studies have shown that Red Wine Phenolic extracts induce nitric oxide (NO)-mediated vasoprotective effects, mainly by causing a redox-sensitive activation of endothelial NO synthase (eNOS). However, Red Wine Phenolic extracts are complex mixtures of a several hundreds of phenolic compounds. Therefore, the aim of the present study was to isolate active phenolic compounds using multi-step bioguided fractionation of the red wine extract.

Methods — Red Wine Phenolic Extract was submitted to a bioguided fractionation using chromatographic methods. The fractions obtained at each step were tested for their potential to induce the activation of eNOS in endothelial cells. Cultures of porcine coronary artery endothelial cells (P1) were used to determine the phosphorylation level of Akt and endothelial NO synthase by Western blot analysis. Identification of phenolic compounds in each active fraction was performed by MALDI-TOF and HPLC-MS techniques.

Results — The first step of fractionation on lipophilic Sephadex® yielded 9 fractions of which 4 of them significantly increased the phosphorylation level of Akt and eNOS in endothelial cells. The active fractions contained mainly procyanidins and some anthocyanins compounds. The fractionation of one of the active fractions by preparative reverse-phase HPLC yielded 11 sub-fractions; all of these sub-fractions significantly increased the phosphorylation level of Akt and eNOS. The analysis of the phenolic compounds indicated that these sub-fractions contained mixtures of procyanidin dimers or conjugated anthocyanins.

Conclusions — The red wine extract contains several types of phenolic compounds, which are able to enhance the activity of NO synthase in endothelial cells including procyanidins dimers and oligomers as well as several conjugated anthocyanins.

C009
PERTE DU GRADIENT TRANSMURAL DE LA FONCTION MITOCHONDRIALE ET ALTÉRATION DU COUPLAGE EXCITATION-CONTRACTION DANS L'INSUFFISANCE CARDIAQUE ISCHÉMIQUE

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L'insuffisance cardiaque (IC) est caractérisée par des altérations du métabolisme énergétique associées à une augmentation de la production de radicaux libres (RL). Les RL altèrent le couplage

excitation-contraction (CEC) des myocytes en interagissant avec la signalisation calcique et les protéines contractiles. Chez des rats ayant subit une ligature de l'artère coronaire gauche (PMI), nous avons déterminé si, au stade d'insuffisance cardiaque, la perte du gradient transmural de contractilité et l'altération de la signalisation Ca2+ étaient associées à une dysfonction mitochondriale régionalisée au sein de la paroi du ventricule gauche (VG).

Les propriétés métaboliques ont été évaluées en mesurant l'autofluorescence du NADH (microscopie multiphotonique), et les activités de la citrate synthase (CS) et de la cytochrome-c oxydase (COX) de cardiomyocytes isolés du sous-endocarde (ENDO) et du sous-épicarde (EPI) du VG de rats PMI ou contrôles (sham). Parallèlement, nous avons mesuré les activités de la superoxyde dismutase (SOD) et de la catalase ainsi que la production mitochondriale de RL (MitoSOX) en microscopie confocale. Le raccourcissement cellulaire, la sensibilité au Ca2+ des myofilaments, le transitoire Ca2+, ainsi que les sparks Ca2+ ont été mesurés en absence ou en présence d'un antioxydant (N-acetyl cysteine NAC : 20 mM).

Chez les shams, l'utilisation du NADH au cours d'une stimulation électrique est plus importante dans l'ENDO que dans l'EPI et s'accompagne d'activités CS et COX plus élevées. Ce gradient transmural de capacité oxydative disparait au cours de l'IC en raison d'altérations localisées uniquement dans l'ENDO. Ces perturbations métaboliques sont associées à une diminution des défenses antioxydantes et à une élévation de la production de RL dans l'ENDO. Le NAC améliore les propriétés contractiles, la fuite diastolique de Ca2+ du réticulum sarcoplasmique (baisse de la fréquence des sparks spontanés) et réduit le nombre de transitoires Ca2+ ectopiques pro-arythmogéniques dans l'ENDO.

En conclusion, la perte du gradient transmural de contractilité au cours de l'IC est partiellement due à une altération régionalisée de la fonction mitochondriale. De plus, la production exacerbée de RL associée aux troubles métaboliques participe à la genèse d'événements arythmiques dans la région sous-endocardique.

C010

DESIGN AND PRELIMINARY EVALUATION OF A COOLING-HEATING DEVICE TO ASSESS MICROVASCULAR FUNCTION IN HUMAN SKIN

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Objective — In order to investigate skin vasomotor response to local cooling in humans, we made a new local cooling/heating laser Doppler flowmetry probe. Using this probe, we tested the skin response to local cooling, down to 15 °C, and its reproducibility.

Methods — The cooling/heating probe is made of paired optic fibres and a Peltier element as a thermoelectric cooler (TEC). The polarity of the voltage influences the direction of the heat flow, allowing either heating or cooling. We performed local cooling on the forearm of 11 healthy volunteers, from 33 °C to 24 °C, and then to 15 °C, twice, and repeated the measurement seven days later. Skin blood flow was simultaneously recorded at two control sites (5 cm and contralateral). We also assessed the effect of a prolonged (30 minute) 15 °C local cooling.

Results — Local cooling decreased cutaneous vascular conductance (CVC) between 33 °C, 24° and 15 °C ($p<0.05$, Friedman test), whereas we observed no -or mild- effect on control sites. Short term reproducibility of the CVC was very good at 24 and 15 °C (intra-subject CV were 13 and 8%, respectively). Seven day reproducibility was good when expressed as a percentage of baseline. Local cooling was well tolerated by all the volunteers.

Conclusion — This prototype was safe and able to perform local cooling, leading to a temperature-dependant, local, reproducible vasoconstriction. This new tool could be of great interest to assess skin microvascular function in diseases such as Raynaud's phenomenon.

C011

EXPRESSION AND REPRODUCIBILITY OF SKIN MICROVASCULAR REACTIVITY ASSESSED WITH LASER DOPPLER FLOWMETRY

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Objective — The study of microvascular function can be routinely performed in humans using laser Doppler flowmetry (LDF) of the skin. Post-occlusive reactive hyperemia (PORH) and local thermal hyperemia (LTH) are used as tools to investigate endothelial and neurovascular microvascular functions. Sodium nitroprusside (SNP) iontophoresis is a commonly used technique to assess non endothelium-dependent skin microvascular function. However, there is no consensus over their reproducibility and variation over time, with room temperature, prandial status, and stress. Our objective was to test the reproducibility of those techniques on the finger and on the forearm, and their physiological variations.

Methods — We performed local heating up to 44 °C, 5 min postocclusive hyperemia and SNP iontophoresis in fourteen healthy volunteers, at day 0, day 7 and month 6. We also tested the impact of a standard meal, of variations of room temperature, and of a psychological stress (Stroop test). Cutaneous blood flow was recorded with LDF probes. Data are expressed as cutaneous vascular conductance (CVC) in mV/mmHg, as % of maximal vasodilation and as a function of baseline. Reproducibility data are expressed as intra-subject coefficient of variation (CV) and intraclass correlation coefficients (ICC).

Results — One-week reproducibility of PORH and LTH on the finger pad is very good when expressed as CVC raw values (CV=12.6 to 23.7%, ICC>0.71). On the dorsum of the finger and the forearm, reproducibility was better when expressed as a% of maximal vasodilation (CV=11.9 to 26.4%, ICC>0.55). SNP iontophoresis did not lead to a vasodilation on the finger pad. On the dorsum and the forearm, its reproducibility was poor. Room temperature had a significant influence LTH and PORH hyperemia parameters, especially on the finger pad.

Conclusion — Reproducibility was very good for both PORH and LTH, but the way of expressing data plays a key role. Reproducibility of SNP iontophoresis was poor. Room temperature (especially when elevated) influenced both PORH and LTH responses on the finger pad, highlighting the need for temperature-controlled conditions while performing these tests.