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High-frequency ultrasonic waves cause endothelial dysfunction on canine epicardial coronary arteries

Ondas ultra-sônicas de alta freqüência causam disfunção endotelial em artérias coronárias caninas epicárdicas

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Abstract

Objective: **Application of ultrasound energy by an endarterectomy probe can facilitate the removal of atheromatous plaque, but the effect of this procedure on surrounding vessel structure and function is still a matter of experimental investigations.**

Methods: **To determine whether ultrasound energy impairs the production of nitric oxide (NO) or damages vascular smooth muscle function of epicardial coronary arteries, isolated canine coronary artery segments were exposed to either high or low ultrasonic energy outputs for 15 seconds using an endarterectomy prototype device. After exposition, segments of coronary arteries were studied in organ chambers. For the pharmacological assays, the following drugs had been used: Adenosine diphosphate (ADP), acetylcholine (Ach) and sodium fluoride (NaF sodium) for the endothelium - dependent vasoreactivity study. Sodium nitroprusside (SNP) and isoproterenol (ISO) were used for the evaluation endothelium-independent relaxations. Following exposure to the ultrasonic endarterectomy probe, segments of epicardial coronary artery were studied in organ chambers.**

Results: **Application of high ultrasonic energy power (25 W) impaired endothelium-dependent relaxation to adenosine diphosphate (10-9 - 10-4 M), acetylcholine (10-9 - 10-4 M) and sodium fluoride (0.5 - 9,5 mM) in epicardial coronary**

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arteries. However, low ultrasound energy output (0-10 W) at the tip of the probe did not alter the endothelium-dependent relaxation (either maximal relaxation or EC50) to the same agonists. Vascular smooth muscle relaxation to isoproterenol or sodium nitroprusside was unaltered following exposure to either low or high ultrasonic energy outputs.

Conclusion: **These experiments demonstrate that ultrasonic energy alters endothelial function of epicardial coronary arteries at high power. However, ultrasound does not alter the ability of vascular smooth muscle of canine epicardial coronary arteries to relax.**

Descriptors: **Ultrasound, nitric oxide, coronary endarterectomy.**

Resumo

Objetivo: **Aplicação de energia por ultra-som pode facilitar a remoção da placa ateromatosa, mas o efeito desse procedimento em vasos próximos ainda é matéria de estudos experimentais.**

Métodos: **Para determinar se a energia ultra-sônica compromete a produção de óxido nítrico, segmentos de artérias coronárias caninas foram expostos a baixos (0-10 W) e altos (25 W) níveis de energia por 15 segundos, utilizando-se protótipo de aparelho para a realização de endarterectomia. Após exposição, segmentos das artérias coronarianas foram estudados em *organ chambers*. Para os ensaios farmacológicos foram utilizadas as seguintes drogas: difosfato de adenosina (ADP), acetilcolina (Ach) e fluoreto de sódio (NaF) para a avaliação do relaxamento dependente do endotélio. O nitroprussiato de sódio (NPS) e o isoproterenol foram utilizados para a avaliação do relaxamento independente do endotélio.**

Resultados: **A aplicação de alta energia ultra-sônica comprometeu o relaxamento dependente do endotélio induzido por ADP (10^{-9} - 10^{-4} M), Ach (10^{-9} - 10^{-4} M) e NaF (0,5 - 9,5 mM) em artérias coronarianas epicárdicas. Entretanto, baixos valores de energia ultra-sônica não alteraram o relaxamento dependente do endotélio (nem o relaxamento máximo e nem a EC_{50}) induzido pelos mesmos agonistas. O relaxamento da musculatura lisa vascular induzido por isoproterenol (10^{-9} - 10^{-5} M) ou NPS (10^{-9} - 10^{-6} M) não foi comprometido, tanto por baixos, quanto por altos níveis de energia ultra-sônica.**

Conclusão: **Os experimentos demonstram que altas energias ultra-sônicas alteram a função endotelial. Entretanto, o ultra-som não altera a habilidade de relaxamento da musculatura lisa vascular de artérias caninas epicárdicas.**

Descritores: **Ultra-som. Óxido nítrico. Endarterectomia. Doença das coronárias.**

INTRODUCTION

Diffuse atherosclerotic coronary artery disease may not be amenable to standard methods of arterial grafting, and coronary artery endarterectomy may be necessary to remove atheromatous plaques that compromise arterial runoff [1,2]. Endarterectomy is a technically demanding procedure in which the surgeon removes the core of plaque to improve the luminal size and quality of the recipient vessel. Numerous methods have been used to facilitate endarterectomy including specially designed probes, carbon dioxide insufflation which dissects the plaque from the remained. Endareterectomy also entails the risks of increased morbidity and mortality [2,3].

Application of intraarterial ultrasound has been shown to be feasible for use in recanalization of calcified atherosclerotic occlusions *in vitro*, in animals and in humans [4,5]. The use of ultrasonic waves as an adjunct to manual endarterectomy can improve the effectiveness of the procedure by facilitating the removal of the atheromatous plaque completely from the arterial wall. However, very little is known about possible damaging effects of ultrasound energy application on epicardial coronary artery function.

The intima of the coronary artery produces an endothelium-derived relaxing factor (EDRF) [6]. The EDRF was identified as nitric oxide (NO) [7], which acts as an endogenous coronary nitrovasodilator [8,9], in addition to inhibiting platelet adhesion [10,11] and aggregation [12,13] in the blood vessel. Damaging effect of ultrasonic waves on the function of the coronary artery endothelium and vascular smooth muscle could impair the production of NO and increase the risk of coronary artery vasospasm and thrombosis. In the present investigation, we evaluated the effect of ultrasound energy on production of NO and vascular smooth muscle function of the epicardial coronary artery *in vitro*.

METHODS

Animal Preparation

Six heartworm-free mongrel dogs (n=6) (25-30 kg) of either sex were anesthetized with pentobarbital sodium (30 mg/kg intravenous injection; Fort Dodge Laboratories, Inc.,

Fort Dodge, Iowa), and exsanguinated via carotid arteries. The chest was opened, and the beating heart was harvested quickly and immersed in cool, oxygenated physiological salt solution of the following composition (mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.22, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, and glucose 11.1. The procedures and handling of the animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation.

***In Vitro* Experiments**

Vascular segments preparation:

The left circumflex coronary artery was carefully dissected free and placed in control solution. The artery was sectioned into eight rings (4 - 5 mm in length) with special care taken not to touch the intimal surface of the vascular segments. In four of these segments, in which vascular smooth muscle function was to be tested without the influence of the endothelium, the endothelium was removed by gently rubbing the intimal surface of the blood vessel with a pair of watchmakers' forceps. This procedure removes endothelium but does not affect ability of vascular smooth muscle to contract or relax [14,15]. In this manner, four pairs of coronary artery rings (with and without endothelium) from the same animal were studied in parallel in our eight-bath organ chamber system.

Ultrasonic Device and Experimental Groups

These experiments were performed using the KUE (King Ultrasonic Endarterectomy) device (Heart Tech of Minnesota, Minneapolis, MN) (Figure 1). The energy was transmitted to the vascular wall by a stainless steel endarterectomy probe at the time the probe was in contact with the intima of artery segments while they were immersed in physiological salt solution (Figure 2).

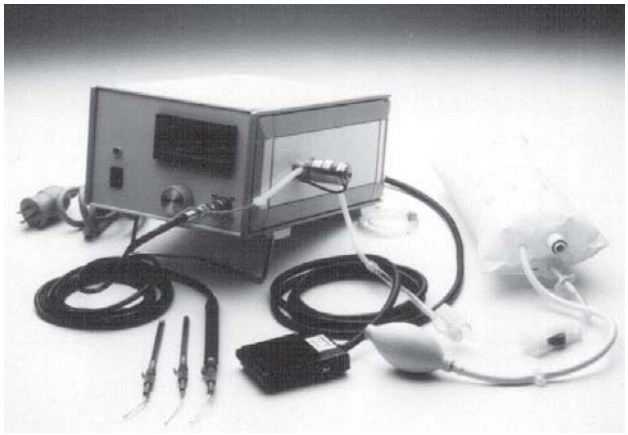

Fig. 1 - King Ultrasonic Endarterectomy (KUE) device

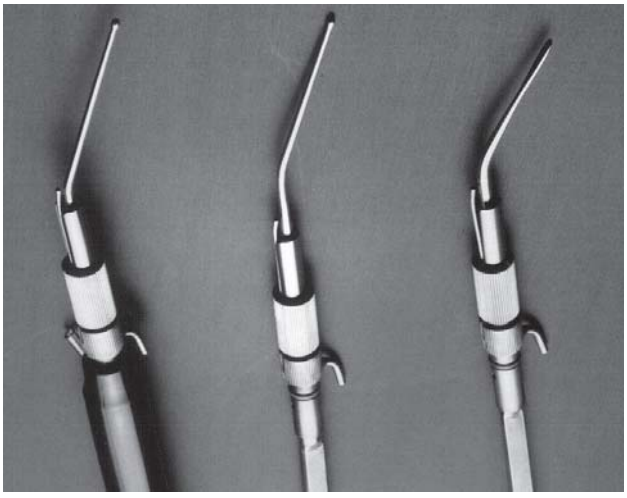

Fig. 2 - The endarterectomy probes of KUE device

Experiments were performed at probe-tip power outputs of 0-10 W or 25 W correspond to low and high energy levels of the device, respectively. The ultrasonic power that was actually delivered to the blood vessel wall was not directly measured.

After arterial rings were prepared and immersed in physiologic salt solution (as described earlier), they were exposed to ultrasonic energy by placing the tip of the probe into the lumen of the vessel. All the rings were treated one by one in the same manner, and then were suspended in organ chambers. The Lowest energy level of the device was used in one group, and highest energy was used in the other, in order to determine the effect of different energy outputs at the tip of the probe. Ultrasonic energy was delivered for 15 seconds. Two pairs of coronary artery segments were used in each group and one of these pairs was treated with the probe only by placing it into the lumen of the vessel without transmitting ultrasound energy, serving as a control of the influence of possible mechanical trauma of the probe itself.

Vascular reactivity study in organ chambers

Each ring was suspended by two stainless steel clips passed through its lumen. Vascular segments, with and without endothelium, were immersed in organ chambers (25 ml) filled with control solution maintained at 37°C and bubbled with 95% O_2 and 5% CO_2 (pH = 7.4). One clip was anchored to the bottom of the organ chamber, and the other was connected to a strain-gauge for measurement of isometric force (Statham UC 2, Gould, Cleveland, Ohio). The rings were placed at the optimal point of their length-tension relation by progressively stretching them until

contraction to potassium ions (20 mM), at each level of distension, was maximal [13,14]. After optimal tension was achieved, the coronary artery rings were allowed to equilibrate in control solution for 30-45 minutes before administration of drugs. In all experiments, the presence or absence of endothelium was confirmed by determining the response to acetylcholine (10^{-6} M) in rings contracted with potassium ions (20 mM) [6]. In all experiments, indomethacin (10^{-6} M) was added to the organ chambers 40 minutes prior to the administration of drugs to prevent synthesis of endogenous prostanoids and to warrant that relaxant effects were due NO action, as NO release is not inhibited by blockers of cyclooxygenase [6].

Drugs

The following drugs were used: acetylcholine chloride (Ach), adenosine diphosphate (ADP), indomethacin, isoproterenol hydrochloride, sodium fluoride (NaF), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and sodium nitroprusside (all from Sigma Chemical Company, St. Louis, Missouri). All drugs were prepared with distilled water except for indomethacin, which was dissolved in em Na_2CO_3 (10^{-5} M). The concentrations are expressed as final molar concentration in the organ chambers.

Data analysis

Results are expressed as mean ± SEM. In all experiments, n refers to the number of animals from which blood vessels were taken. In segments contracted with $PGF_{2\alpha}$, relaxation responses are expressed as percent changes from the contracted levels. Statistical evaluation of data was performed using the two-way ANOVA and Bonferroni post-test for multiple comparisons. Differences were considered to be statistically significant when p was less than 0.05.

RESULTS

Endothelium-dependent relaxation

Adenosine diphosphate

ADP (10^{-9} - 10^{-4} M) induced concentration-dependent relaxation in control and all experimental coronary artery segments with endothelium which had been contracted with $PGF_{2\alpha}$. ADP caused a slight decrease in tension in coronary artery segments without endothelium. Exposure to low intensity ultrasound energy output (0-10 W) did not alter the maximal relaxation or sensitivity to ADP-mediated relaxation in coronary artery segments with or without endothelium. However, exposure to high intensity ultrasound energy output (25 W) impaired the maximal relaxation and sensitivity to ADP-mediated relaxations in coronary artery segments with endothelium (Figure 3, $p < 0.05$, n = 6 in each group).

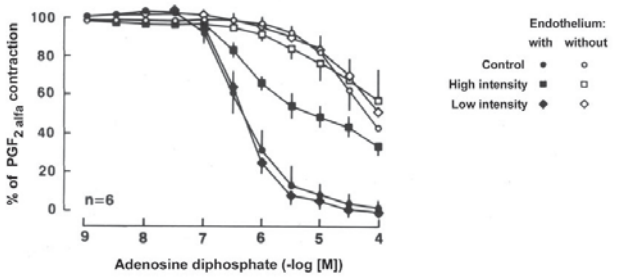

Fig. 3 - Endothelium-dependent relaxation to adenosine diphosphate (ADP) in the canine coronary artery. Canine coronary arteries were exposed to either low or high intensity ultrasonic energy and contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When the $PGF_{2\alpha}$ contraction was stable, arteries were exposed to increasing concentrations of ADP. Data are presented as means ± SEM, n = 6

Acetylcholine

Ach (10^{-9} - 10^{-4} M) induced comparable, concentration-dependent relaxation in control and all experimental coronary artery segments with endothelium which had been contracted with $PGF_{2\alpha}$. Ach produced no significant change in tension in coronary artery segments without endothelium. Exposure to low intensity ultrasound energy output (0-10 W) did not alter the maximal relaxation or sensitivity to Ach-induced relaxation in coronary artery segments with endothelium. However, as with ADP, relaxation was impaired in coronary artery segments with endothelium exposed to high intensity ultrasound energy output (25 W) (Figure 4, $p < 0.05$, n = 6 in each group).

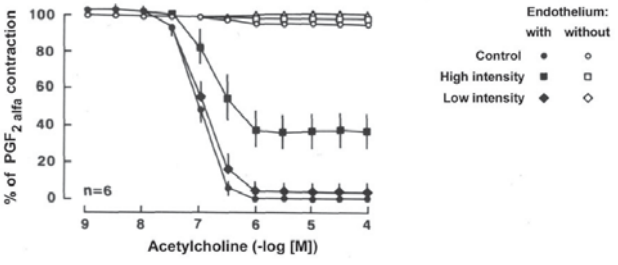

Fig. 4 - Endothelium-dependent relaxation to acetylcholine (Ach) in the canine coronary artery. Canine coronary arteries were exposed to either low or high intensity ultrasonic energy and contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When the $PGF_{2\alpha}$ contraction was stable, arteries were exposed to increasing concentrations of Ach. Data are presented as means ± SEM, n = 6

Sodium fluoride

NaF (0.5 – 9.5 mM) induced concentration dependent relaxation in canine coronary arteries with endothelium which had been contracted with $PGF_{2\alpha}$. However, exposure to NaF increased tension in coronary arteries without endothelium. In coronary artery segments with endothelium exposed to low intensity ultrasound energy (0-10 W), NaF-induced concentration-dependent relaxations were comparable to control arteries treated only mechanically. In contrast, in coronary artery segments with endothelium exposed to high intensity ultrasound energy output (25 W), NaF-induced concentration dependent relaxations were significantly less than relaxations in arteries exposed to low ultrasonic energy or control rings (Figure 5, $p < 0.05$, $n = 6$ in each group).

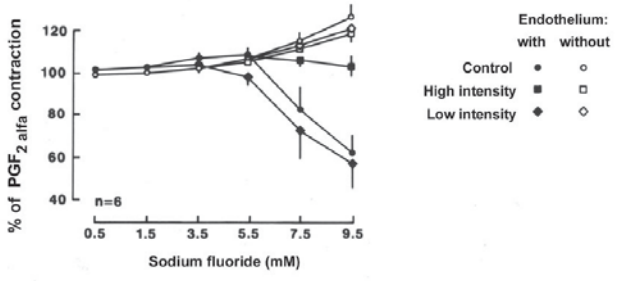

Fig. 5 - Endothelium-dependent relaxation to sodium fluoride (NaF) in the canine coronary artery. Canine coronary arteries were exposed to either low or high intensity ultrasonic energy and contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When the $PGF_{2\alpha}$ contraction was stable, arteries were exposed to increasing concentrations of NaF. Data are presented as means ± SEM, n = 6

Endothelium-independent relaxation

Cyclic GMP-mediated

Sodium nitroprusside (10^{-9} - 10^{-6} M) induced comparable concentration-dependent relaxation in control and experimental coronary artery segments without endothelium. Either low or high intensity ultrasound energy output did not alter the maximal relaxation induced by sodium nitroprusside or change the sensitivity of the vascular smooth muscle to the compound (Figure 6, $p < 0.05$, $n = 6$ in each group).

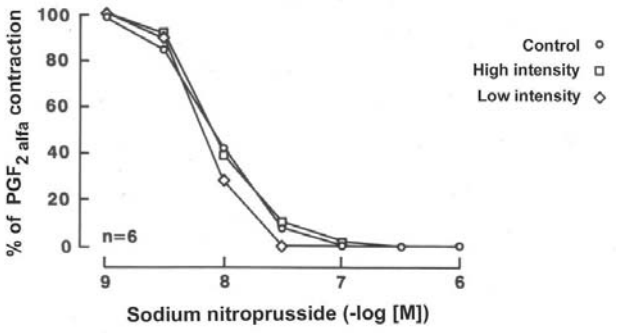

Fig. 6 - Relaxation to sodium nitroprusside in the canine coronary artery. Canine coronary arteries were exposed to either low or high intensity ultrasonic energy and contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When the $PGF_{2\alpha}$ contraction was stable, arteries were exposed to increasing concentrations of sodium nitroprusside. Data are presented as means ± SEM, n = 6

Cyclic AMP-mediated

Isoproterenol (10^{-9} - 10^{-6} M) induced comparable concentration dependent relaxation in control and experimental coronary artery segments without endothelium. Either low or high intensity ultrasound energy output did not alter the maximal relaxation induced by isoproterenol or change the sensitivity of the vascular smooth muscle to the compound, as it was with sodium nitropruside (Figure 7, $p < 0.05$, $n = 6$ in each group).

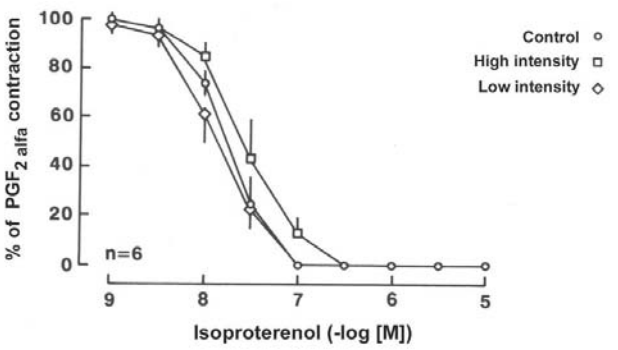

Fig. 7 - Relaxation to isoproterenol in the canine coronary artery. Canine coronary arteries were exposed to either low or high intensity ultrasonic energy and contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When the $PGF_{2\alpha}$ contraction was stable, arteries were exposed to increasing concentrations of isoproterenol. Data are presented as means ± SEM, n = 6

DISCUSSION

In patients with diffuse coronary artery disease, effective revascularization is impossible without improving arterial runoff. Coronary endarterectomy has been used to remove the obstructing plaque in occluded arteries with diffuse disease and enables them to be grafted [1,2,16]. However, endarterectomy is a technically challenging procedure and entails the risks of increased morbidity and mortality [1,3,16].

In this endarterectomy procedure, the surgeon elevates, dissects out and removes the core of the plaque, restoring a normal lumen. Techniques for distal coronary endarterectomy have included mechanical or manual methods as well as carbon dioxide gas and laser for dissection of the plaque [1,16,17]. Recently, application of intraarterial ultrasound has been shown to be feasible for use in recanalization of calcified atherosclerotic occlusions *in vitro*, in animals and in humans [4,18]. One of the important features of any device or technique used to treat obstructive arterial disease is its effect on arterial vasomotor behavior [18]. The use of ultrasonic waves as an adjunct to surgical endarterectomy can improve the effectiveness of the procedure by facilitating the removal of atheromatous plaque from the arterial wall. In this experiment the probe was placed into the vessel lumen. However, clinically the probe tip would be inserted between intima and the middle layer of the artery allowing the ultrasonic oscillations of the probe to help with the endarterectomy. Ultrasonic oscillations could allow the surgeon to perform the endarterectomy with less damage to the artery wall than present methods, facilitating the endarterectomy and reducing the risk of perforating the artery, especially in the presence of marked calcinosis.

It is well known that in the physical property analyses of one determined material it must be evaluated its magnitude and intensity. Thus, one third experimental group with application of the ultrasonic energy of low intensity for an extended time could be carried out. This detail was considered in the experiment design. The presented model was adopted based on the fact that energies applications for drawn out time it is not one practical usual in cardiac surgeries, which electric energies of low intensity and short duration is a kind of rule.

It has been shown that ultrasonic energy is capable of causing arterial relaxation and this relaxation is endothelium dependent and mediated by NO [19]. Controversially, in another study ultrasound-mediated relaxations have been shown to be endothelium independent and unlikely due to release of NO or other endothelium-derived substances (e.g., prostacyclin) [18]. These studies demonstrate the direct effect of ultrasound on the vessel wall. In our experiment, we studied the effect of ultrasound on the ability of the vessel to relax, and we showed that 25 W of ultrasonic energy impairs endothelium function but not smooth muscle function in canine epicardial coronary artery when the vessel was exposed to ultrasonic waves for 15 seconds.

Nowadays ultrasonic scalpels are commercially available and have already described advantages. The ultrasonic scalpel facilitates thoracoscopic internal mammary artery harvest and is expected to minimize hyperthermic damage of this artery [20,21].

Investigators recently demonstrated increased free blood flow from radial artery free grafts harvested using ultrasonic technology. Also, experimental canine internal artery sonication induces vasorelaxation almost completely by time-dependent endothelial nitric oxide and prostacyclin release, which appears unrelated to tissue heating or endothelial architectural disruption [22].

The left internal mammary artery dissection in a skeletonized fashion with an ultrasonic scalpel does not produce endothelial structural damage in it being similar to the one dissected with conventional methods. This permits its safe use, allowing us to benefit from the numerous advantages of arterial grafts usage in modern era coronary surgery [23].

Sternal perfusion increases soon after coronary bypass surgery, probably as a consequence of the healing process, but the source of perfusion for the harvest side remains unclear. Harvesting of internal thoracic arteries with an ultrasonic scalpel has no advantageous effects on postoperative sternal perfusion [24].

CONCLUSION

Results of the present study demonstrate that ultrasonic energy, delivered via a special endarterectomy probe causes energy dependent impairment in NO release from canine epicardial coronary artery. However, ultrasound energy does not alter endothelium-independent relaxation in canine coronary artery regardless of which energy level is used.

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