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Angiotensin IV improves subnormothermic machine perfusion preservation of rat liver graft

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ABSTRACT

This study aims to determine whether Angiotensin IV (Ang IV) addition to Celsior preservation solution could improve hepatic endothelium function and provide better liver protection during subnormothermic machine preservation (SMP).

Two experimental models were used: In the first part of the study, rings isolated from rat hepatic artery were preserved in Celsior solution (6 h, 20 °C) with and without Ang IV (10^{-9} M), then, endothelium–dependent relaxation (EDR) and the concentration of acetylcholine inducing half-maximal relaxation of pre-contracted rings (EC50) were measured. Also, in order to investigate the implication of nitric oxide (NO) on EDR, the rings of hepatic artery were incubated with L-NG-nitroarginine metyl ester (L-NAME). In the second part of the study, rat livers were subjected to SMP with oxygenated Celsior solution (6 h, 20 °C), supplemented or not with Ang IV (10^{-9} M) and then perfused (2 h, 37 °C) with Krebs Henseleit solution.

We found that Ang IV supplementation to Celsior solution decreased EC50 value and improved EDR of hepatic artery rings, 6h after sub-normothermic preservation. Interestingly, Ang IV amplified the vessel relaxation in a NO-dependent manner. Moreover, liver SMP with Ang IV reduced oxidative stress and cell injury and improved organ function. Ang IV activated pAkt, increased eNOS protein level and decreased apoptosis in the preserved liver grafts.

In conclusion, we showed that the use of Ang IV in Celsior solution for sub-normothermic graft preservation insured a better NO-dependent relaxation and improved liver functional recovery.

1. Introduction

Liver transplantation has been recognized as the most effective treatment of end-stage liver disease. However, the ischemia reperfusion injury (IRI) inherent to the transplantation procedure still compromises early and long-term outcomes [1]. The increasing demand for liver transplantation and the shortage of organs has led to extend the selection criteria to marginal grafts, eliciting thus an urgent need for improving the preservation techniques [2]. In the last decade, dynamic preservation has emerged as a better alternative to the conventional static cold storage [3–5]. In fact, several animal and clinical trials confirmed the reliability of hypothermic machine perfusion especially when it is performed at subnormothermic temperature [6–9]. Indeed, it was found that the use of subnormothermic machine perfusion (SMP) reduces sinusoidal cell apoptosis and improves hepatic function, leading to a better viability of the liver graft [6,10,11]. In addition, dynamic preservation methods offer the possibility to apply pharmacological maneuvers during the preservation phase, in an attempt to further prevent IRI.

Angiotensin IV (Ang IV) is a hexapeptide derived from the cleavage of angiotensin II, the main bioactive peptide of the renin angiotensin system (RAS). It has long been considered as an inactive catabolite of the RAS, but the discovery of high affinity specific binding sites distributed in a variety of tissues leaded to the emergence of numerous experimental works to establish its biological role [12–14]. Several studies highlighted the marked vasodilatory effect of Ang IV and its ability to activate endothelial nitric oxide synthase (eNOS) leading to NO release [15–19]. Also, it has been proven that Ang IV plays a protective role against cerebral IRI due to its capacity to dilate vessels, resulting in better redistribution of blood flow in the ischemic areas [20,21]. Recently, it has been shown that Ang IV has cardioprotective effect against IRI by inhibiting inflammation and apoptosis [13].

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Taken together, these observations point to AngIV as a potential candidate for pharmacological reconditioning of liver graft during the preservation phase. Therefore, the aim of this study was to evaluate the effect of Ang IV addition to Celsior preservation solution on endothelium-dependent relaxation (EDR) and machine perfusion preservation.

2. Materials and methods

To evaluate the relevance of Ang IV on tissue preservation, two studies were conducted in parallel with two different experimental models: the hepatic artery rings model to evaluate the EDR and the isolated perfused rat liver to assess the impact of SMP on the hepatic function recovery.

2.1. Animals

Adult male Sprague-Dawley rats, weighing 250–300 g were used. They were housed in a temperature (20–23 °C) and light (12 h light/dark cycles)-controlled room, and were fed with a standard laboratory chow with free access to water. All procedures were carried out in accordance with the European Union Regulations (Directive 2010/63/EU) for animal experiments, and approved by the French Agriculture Ministry (authorization n° A87638).

2.2. Study I: effect of Ang IV in Celsior solution on endothelium-dependent relaxation of hepatic artery rings

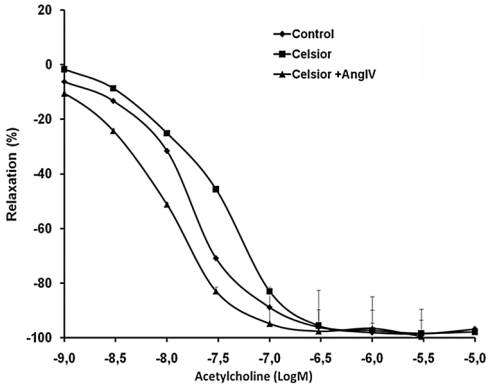
Tissue preparation procedure was performed as previously described [21,22]. Briefly, rats were anesthetized with isoflurane inhalation and then underwent laparotomy. The hepatic artery was exposed and stripped of adherent connective and adipose tissue. Special care was taken to avoid rubbing the intima surface of the vessels. Hepatic artery rings were stored in gassed Celsior solution (100% O_2, 20 °C) for 6 h (Celsior group, n = 9) or in Celsior solution containing 10^{-9} M of Ang IV (Celsior + Ang IV group, n = 11). Afterwards, the hepatic artery was cut into ring segments, of 2–3 mm in length, and suspended using two stainless steel holders in organ bath filled with gassed (95% O_2–5% CO_2, pH 7.4) Krebs bicarbonate buffer (KBB, VWR, France) solution at 37 °C. Each preparation was equilibrated under 0.75 g tension for 60 min and washed with fresh KBB solution at 20 min intervals. The hepatic artery rings from control group (n = 12) were suspended immediately in the organ bath without preservation. Mechanical activity of vessel was recorded by a force transducer fed to a personal computer including a data acquisition and processing system (PowerLab, AD Instruments).

After full equilibration was completed, the rings were exposed to 10^{-6} M phenylephrine (Phe, Sigma–Aldrich) before addition of cumulative concentration (from 10^{-9} to 3.10^{-6} M) of acetylcholine (Ach, Sigma–Aldrich). Maximal relaxation (E_{max}) and the concentration of ACh inducing half-maximal relaxation of pre-contracted rings (EC_{50}) were determined from ACh induced relaxation curves. We also evaluated the involvement of Ang IV (10^{-9} M, Sigma–Aldrich) on the activation of endothelial nitric oxide synthase (eNOS), by treating the rings with L-NG-nitroarginine methyl ester (L-NAME 10^{-4} M, Sigma–Aldrich) before the addition of Phe.

2.3. Study II: effect of Ang IV in Celsior solution on sub-normothermic machine preservation of liver graft

2.3.1. Surgical procedure

Liver graft procurement was performed as previously described [22]. Briefly, rats were anesthetized with intraperitoneal administration of urethane (1.2 g/kg body weight) and the abdomen was opened by a transverse incision to expose the portal vein, the common bile duct and the inferior vena cava. The common bile duct was cannulated to collect bile outflow during normothermic reperfusion. The liver was then flushed (15 mL, 20 °C) *via* the abdominal aorta and portal vein with Ringer's lactate (control group, n = 6), Celsior (Celsior group, n = 6) or with Celsior supplemented with 10^{-9} M of Ang IV (Celsior + Ang IV group, n = 6). The infrahepatic vena cava was cannulated to collect the liver effluent.



	Control	Celsior	Celsior+AngIV
Emax(%)	98,1±06	99,1±0,1	99,5±0,5
E_{C50}(M)	(1,8±0,2)10^{-8}	(3,96±0,8)10^{-8}	(1,18±0,2)10^{-8} #

Fig. 1. Concentration-response curves for acetylcholine in hepatic artery pre-contracted with phenylephrine: maximal effect (Emax) and EC50 values. Control: hepatic artery rings reoxygenated *in vitro* without preservation. Celsior: hepatic artery rings reoxygenated *in vitro* after sub-normothermic preservation in Celsior solution. Celsior + Ang IV: hepatic artery rings reoxygenated *in vitro* after sub-normothermic preservation in Celsior + Ang IV (10^{-9} M) solution. *P < 0.05 *vs*. Control, #P < 0.05 *vs*. Celsior.

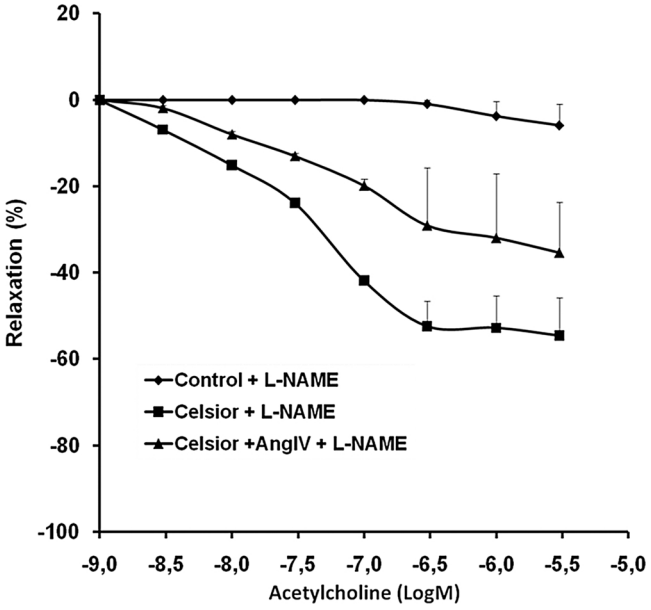


Fig. 2. Concentration-response curves for acetylcholine in hepatic artery pre-contracted with phenylephrine in presence of L-NAME. Control: hepatic artery rings pretreated with L-NAME and reoxygenated *in vitro* without preservation. Celsior: hepatic artery rings reoxygenated *in vitro* after sub-normothermic preservation in Celsior solution and pretreatment with L-NAME. Celsior + Ang IV: hepatic artery rings reoxygenated *in vitro* after sub-normothermic preservation in Celsior containing Ang IV (10^{-9} M) and pretreatment with L-NAME. *P < 0.05 *vs*. Control, #P < 0.05 *vs*. Celsior.

2.3.2. Sub-normothermic machine perfusion

Dynamic preservation procedure was performed as previously