Can Apparent Diffusion Coefficient Discriminate Ischemic From Nonischemic Livers? A Pilot Experimental Study


ABSTRACT

Purpose. Using magnetic resonance imaging, the apparent diffusion coefficient (ADC) is an indicator to assess cerebral ischemia. The aim of this porcine study was to evaluate whether ADC assessed hepatic ischemia during ex vivo hypothermic machine perfusion (HMP) as well as in vivo.

Methods. Ex vivo: ADC of normal versus warm ischemic (WI) livers was assessed during HMP and subsequent rewarming to mimic ischemia-reperfusion injury. As the preservation solution, we used either an acellular solution or diluted blood. WI was induced in the left lobe or in the whole liver and compared 2-hour WI and non-WI. In vivo: One liver was scanned with the left lobe vessels occluded for 2-hour WI and subsequently for 3 hour reperfusion to compare with the right lobe without WI. Aspartate aminotransferase (AST) in the perfusate and morphology were used as surrogates of WI.

Results. In all WI livers, AST reached high levels and histology showed severe injury. Ex vivo ADC during acellular perfusion showed negligible differences between the livers with versus without WI, namely, $0.75 \times 10^{-3}$ or $0.88 \times 10^{-3}$ mm$^2$/s during HMP. Ex vivo ADC using sanguineous perfusion showed $1.11 \times 10^{-3}$ or $0.83 \times 10^{-3}$ mm$^2$/s during HMP in regions with versus without WI, respectively, a difference that remained stable during the whole experiment. ADC in vivo decreased from the physiological level of $1.07 \times 10^{-3}$ mm$^2$/s to $0.75 \times 10^{-3}$ mm$^2$/s in the first 30 minutes of WI, whereas ADC in the non-WI liver remained constant.

Conclusion. ADC in vivo decreased during hepatic ischemia, as previously seen in cerebral ischemia. However, the effect of WI on ADC was less clear during ex vivo HMP.

The progress of liver transplantation is inspired by an insufficient number of heart-beating donors, which has led to the use of so-called “marginal” donors, such as non–heart-beating-donors (NHBD). But the main problem of livers originating from NHBD is primary nonfunction after implantation, which results from the warm ischemia (WI) injury to which these livers have been exposed prior to procurement.1 Trying to optimize organ preservation is one research avenue to avoid transplantation of nonviable organs. Liver hypothermic machine perfusion (HMP) is an alternative to standard clinical preservation by simple cold storage.2–4 However, there is still no objective, noninvasive, reliable test to assess WI injury and liver viability prior to transplantation.

Diffusion-weighted (DW) magnetic resonance imaging (MRI) is an imaging technique used to diagnose acute cerebral ischemia.5,6 This technique is based on a sensitivity to the random Brownian motion of water molecules in the extracellular space. Apparent diffusion coefficient (ADC), a parameter derived from DW MRI has been widely used as...
a regular indicator in studies of stroke. Low ADC values have been observed in the first hours after the onset in the ischemic region, because of the decreased extracellular space caused by cellular edema. ADC shows greater sensitivity compared with computed tomography for the early diagnosis of stroke. Other authors have suggested that ADC can be used to evaluate hepatic fibrosis and cancer. Therefore the aim of this pilot study was to evaluate whether ADC was a useful noninvasive marker to assess the viability of livers exposed to WI, by measuring ADC of control and WI (2 hours) livers ex vivo during HMP and subsequently after rewarmed perfusion. In a separate set of experiments, we also measured ADC of normal and WI livers in vivo.

MATERIALS AND METHODS

Animal Model

Inbred female Landrace pigs, 25 to 30 kg, were used in this study in accordance with the Belgian law on animal welfare. Pigs were fasted 24 hours before experiments with free access to water. General anesthesia during hepatectomy was maintained by isoflurane (1%) via endotracheal intubation accompanied by analgesia with intravenous fentanyl (4 μg/kg/h) and muscle relaxation by intravenous pancuronium (0.2 mg/kg/h). During the in vivo experiments, general anesthesia was maintained via continuous intravenous pentobarbital (5 mg/kg/h).

Study Design

Acellular perfusion ex vivo.

One liver was exposed to 2 hours of WI caused by ventricular fibrillation as described previously in a NHBD porcine model. One liver not exposed to WI served as the control. Livers then flushed with histidine tryptophan ketoglutarate solution (4°C) were procured and placed in a MRI-compatible cassette with connection of the portal vein (PV) and the hepatic artery (HA) to a liver transporter (Organ Recovery Systems, Zaventem, Belgium) for continuous HMP for 24 hours with kidney perfusion solution (KPS-1, Organ Recovery Systems, Chicago, Ill., USA). After HMP, livers flushed with Ringer’s solution were perfused with oxygenated Krebs-Henseleit Bicarbonate (KHB) solution (37°C) for 1 hour.

Sanguineous perfusion ex vivo.

One liver was procured after the left lobe underwent a 2 hour period of in situ WI by clamping the left PV and HA. After procurement, the liver was perfused with autologous pig blood diluted with AOIX solution (Imperial College BioIncubator, London, UK) at a hematocrit 9% during HMP for 1 hour and consequently rewarmed with oxygenated perfusion (ex vivo) or after reperfusion (in vivo) were stained using hemotoxylin-eosin.

Light microscopy.

Biopsies taken before cardiac arrest, after WI (for NHBD lobe), after initial flush, and after rewarmed perfusion (ex vivo) or after reperfusion (in vivo) were stained using hemotoxylin-eosin.

MRI

The main parameters for DWI ex vivo were time of repetition/time of echo (TR/TE) = 3000/70 ms and b-values = 0, 200, 500, 800 s/mm². The test was performed every 12 hours during HMP and every 30 minutes during rewarmed perfusion using the acellular medium and every hour during perfusion with diluted blood. The main parameters for DWI in vivo were TR/TE = 3000/73 ms, b-values = 0, 100, 250, 500, 750, 1000 s/mm². They were determined every hour from the time before occlusion until reperfusion. Perfusion imaging with the contrast agent gadolinium-diethylenetriaminepentaacetic acid was used (TR/TE = 3.4/1.5 ms) to visualize the homogeneity of the hepatic circulation before occlusion, during WI and during reperfusion with in vivo imaging.

Image analysis.

The liver parenchyma in DWI was manually delineated at a Linux workstation using dedicated software (Biopac; Novartis, Basel, Switzerland). The delineation for each b-value was merged for each liver to yield the average signal intensities, excluding the noise in the imaging. ADC was calculated by a least-squares solution.

RESULTS

Biochemical Parameters

During acellular HMP perfusion, the AST of the non-WI liver increased from 183 U/L to 281 U/L, peaking at 1173 U/L at the end of rewarmed perfusion; AST of the WI liver increased from 986 U/L to 1738 U/L during HMP peaking at 3654 U/L at the end of the rewarmed perfusion (Fig 1.1A). AST in diluted blood during sanguineous perfusion increased from 1035 U/L to 1785 U/L at the end of rewarmed perfusion; AST of the WI liver increased from 510 U/L during WI, and 3610 U/L at the end of reperfusion (Fig 1.2A). The AST in the in vivo experiment was 105 U/L before occlusion, increasing to 510 U/L during WI, and peaking at 1210 U/L at the end of reperfusion (Fig 1.3A).

Light Microscopy

The main characteristics after 2 hours of WI were congestion and vacuolization. After acellular and sanguineous rewarmed perfusion ex vivo, the architectural destruction was severe with obvious cell death, sinusoidal dilatation and enlarged Disse space (Fig 1.1B and 2B). The ischemic lobe after reperfusion in the in vivo experiment exhibited congestion, severe architectural destruction, cell death, and neutrophil infiltration (Fig 1.3B).

MRI

During 24 hours acellular HMP, ADC was 0.75 × 10⁻³ and 0.88 × 10⁻³ mm²/s in the WI and non-WI livers, respectively, increasing during rewarmed perfusion to 1.57 × 10⁻³ and 1.52 × 10⁻³ mm²/s, respectively. No clear difference was observed in ADC between WI and control livers (Fig 1.1C).

During sanguineous perfusion in HMP, ADC was 1.11 × 10⁻³ and 0.83 × 10⁻³ mm²/s in the WI and non-WI region, respectively, increasing on the same slope when the tem-
Temperature was raised, reaching a plateau of $1.63 \times 10^{-3}$ and $1.40 \times 10^{-3}$ mm$^2$/s, respectively, after the first hour of rewarmed perfusion. Here also there was no clear difference in ADC observed between WI and control (Fig 1.2C).

In the in vivo assessment, the baseline ADC of $1.07 \times 10^{-3}$ mm$^2$/s decreased to $0.75 \times 10^{-3}$ mm$^2$/s during the first 30 minutes of WI, remaining stable (in the range of $0.70$ to $0.80 \times 10^{-3}$ mm$^2$/s) during the following 90 minutes of WI and the reperfusion phase. In the control, non-WI liver ADC remained constant (Fig 1.3C).

**DISCUSSION**

Due to the organ shortage, transplant teams are considering the use of “marginal” or “expanded criteria” donors including NHBD. Among the strategies to avoid transplantation of nonviable livers, HMP is considered to have the potential to optimize liver preservation. Nevertheless, there is a need to develop tools to assess the quality and viability of ischemic or marginal grafts during organ preservation ex vivo. ADC has been used clinically as a noninvasive indicator of cerebral ischemia. Its sensitivity in the early stage of stroke raised our interest to study whether it could be a useful indicator of liver ischemia and in particular, whether it could help to discriminate between WI and non-WI livers during HMP ex vivo.

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We studied livers exposed to 2 hours of WI. Based on our previous findings, these grafts are considered nonviable,10...
as was confirmed in the current study, in which all livers exposed to 2-hour WI showed severe morphological alterations and high transaminase release.

In the first experiment using acellular preservation solutions, no difference was observed in ADC values between WI and control livers. A possible reason for this phenomenon was the continuous machine perfusion, which causes sinusoidal dilatation, artificially maintaining the extracellular space. Another possible reason is the absence of inflammatory cells, which presents inflammation and cannot exactly simulate the ischemia-reperfusion injury. A steep increase of ADC noted during rewarmed perfusion was thought to confirm the theoretical effect of temperature on ADC. Indeed the Brownian mobility of water molecules increases with higher temperatures.

The absence of white blood cell infiltration in the acellular perfusion led us to design a second experiment using sanguineous perfusion. Here a negligible difference of ADC was also detected between the WI and non-WI regions. We speculated that the reasons included the effect of continuous machine perfusion and the insufficient number of inflammatory cells, which were only present in diluted blood and not recruited from the periphery.

The *ex vivo* results led us to consequently design the *in vivo* experiment. As expected and similar to what had been seen in stroke, ADC discriminated between WI and non-WI regions. Of note, ADC dropped immediately after the start of WI, remaining stable after the first 30 minutes, a finding similar to available studies on cerebral ischemia.12 The unexpected finding in our study was the stability of ADC during ischemia reperfusion, although the AST continued to rise. We speculate that the ischemia-reperfusion injury during the 3 hours of observation did not induce observable alterations of the extracellular space. A further decrease in ADC may occur when a longer period of follow-up is studied. Nevertheless, this study documented the sensitivity of ADC in hepatic ischemia *in vivo* as compared to ADC in the ischemic liver.

Why does ADC lose its sensitivity to discriminate WI from non-WI livers *ex vivo* compared to *in vivo*? The congestion and coagulation in the ischemic liver *in vivo* reduces the mobility of water molecules in the extracellular space and subsequently decreases ADC. The neutrophils *in vivo* can provoke extensive inflammatory activity during WI and ischemia reperfusion. Absence or reduced quantities of these cells during *ex vivo* machine perfusion may account for the difference observed between the *ex vivo* and *in vivo* situations. Additionally, the effect of machine perfusion on sinusoidal dilatation may also intervene.

In conclusion, ADC in MRI was a sensitive indicator of *in vivo* hepatic ischemia. ADC decreased rapidly after the onset of ischemia, similar to what had been observed in cerebral ischemia. However, this technique—in its current development—cannot be used *ex vivo* to discriminate ischemic from normal livers during HMP.

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REFERENCES