In vivo visualization of an optical reporter gene expression transported by a cellular vector and locally activated by MRI guided HIFU in rat kidney

O. Eker[1], B. Quesson[1], N. Frulio[1], C. Deminiere[2], C. Moonen[1], F. Couillaud[1], and N. Grenier[1]

[1]Laboratory IMF, Bordeaux, France, [2]anatomopathology, CHU Pellegrin, Bordeaux, France

Introduction: Gene therapies require both an efficient targeting method and a precise spatial and temporal control of the transgene expression (1). In the present work, an exogenous gene is targeted to the rat kidney using genetically modified cells and its local expression is controlled by moderate hyperthermia induced in vivo by MRI controlled High Intensity Ultrasound (HIFU). In order to image gene expression in vivo using bioluminescence imaging (BLI), the firefly luciferase transgene was incorporated under the control of a thermosensitive promoter (human Hsp70B) (2).

Materials and Methods: Genetically modified glial C6 cell line (courtesy of Dr. C. Rome, IMF, Bordeaux) was stably modified using retroviral technologies to express the firefly luciferase gene under control of human heat-inducible promoter Heat shock protein, Hsp70B. Cells were cultivated routinely in DMEM medium and were characterized in vitro for luciferase activity related to heat shock for several heating conditions. Male Wistar rats (around 400g body weight) were sedated with Pentobarbital sodium (45 to 55 mg/kg) and left kidney was superficialized through the abdominal wall and positioned under the skin. The resistive indexes (RI) of the kidney were evaluated by Doppler examination before and after surgery. Four millions of genetically modified cells, initially heated in vitro at 45°C for 5 min, were resuspended, centrifuged, diluted in 500 µl of phosphate buffer saline 7 hrs after heating and slowly injected through the renal artery of the superficialized kidney using a 30-gauge needle. Rats were then injected intra peritoneal with luciferin (100mg/kg) and rapidly positioned under an optical imaging system (Berthold, NightOWL II LB 983, NC100, 1 min acquisition, 2 averages with 2x2 binning) to visualize luciferase expressing cell distribution in vivo before closing the surgical way. Rats had Paracetamol (orally, 200mg/kg) and Tolfedine (sub-cutaneous injection, 0,06mg/kg) to prevent pain, and were replaced in individual rearing cages. After two days, the animals were anaesthetized with Isofurane and positioned on a HIFU transducer operating at 1.5 MHz (focal point dimensions: 1×1×4 mm^3) and integrated in the MRI bed (1.5 T Achieva, Philips Medical Systems) (3). A segmented EPI sequence (TR= 342 ms, TE= 14,5 ms, EPI factor = 15, flip angle = 37°, matrix = 64×60, FOV= 54×40 mm^2, 5 slices, No averaging, 1 s temporal resolution) was used for dynamic and volumetric thermometry using the Proton Resonant Frequency Shift technique. Different temperature profiles were predefined and local temperature of the kidney was automatically regulated [REF] in several locations which were selected from initial BLI images of cells distribution acquired 2 days before. Six hours after in vivo heating, animals were sedated with Pentobarbital sodium and the kidney was exposed to acquire bioluminescence images in vivo (as specified above). Skin was sutured and the animals receive a pain releaser treatment. Rats were finally sacrificed two days later for histological control of the kidney (Hematoxilin -Eosine staining).

Results: The hemodynamic parameters of the kidney were not modified by the surgical procedure since the RI were not significantly modified (mean RI before and after the procedure were respectively 0,486±0,046 and 0,489±0,025; t-test, $p<0,05$). Figure 1 and 2 show luciferase activity in vitro within the genetically engineered C6 cells line related to the heating parameters. Optimal luciferase activity was obtained 7 hours after heating at 45°C during 8 min. When injected into the renal artery, preheated cells induced transient discoloration of the kidney (<2 min) in all cases (Figure 3a), corresponding to cell distribution as demonstrated by subsequent BLI (Figure 3b). In the 7 rats, 11 kidney regions were heated in vivo by MRI guided FUS (see table). The bioluminescence signal was quantified by the ratio (R) of signal intensity in the heated region and signal intensity in the non-heated region. During the heating procedure (Figures 3c and 3d), the standard deviation on temperature MRI was 1°C and animals body temperature remained stable (mean temperature increase: +0,25°±0,17°C). After in vivo heating, luciferase activity was detected using BLI in kidney regions corresponding to the heated regions (Figure 3e). Histological analysis of the kidneys, available in 9/11 heated regions, revealed regions of glomerular and tubular necrosis that did not exceed the size of the focal point (limited) in 6/9 cases and exceeded it (wide) in 1 case (see table).

Temperature	Duration	Number of heated regions	Presence of light	mean R	Lesions in histology		
					No	Limited	Wide
43° C	2 min.	1	0/1	na.	na.	na.	na.
43° C	5 min.	3	1/3	4.9	1	1	na.
45° C	2 min.	2	2/2	3.5±1.4	0	2	0
45° C	5 min.	5	5/5	4.2±0.7	1	3	1

Conclusion: Genetically engineered cells were successfully targeted to the rat kidney. After 2 days, they could be activated in vivo by a moderate and controlled heating using MRI guided FUS. This technique provides a precise local control of the reporter gene expression in rat kidney. The reporter expression can be detected and followed in vivo using BLI. Further optimization of the choice of the heating parameters should allow the activation of detectable transgene expression with limited tissue alteration.

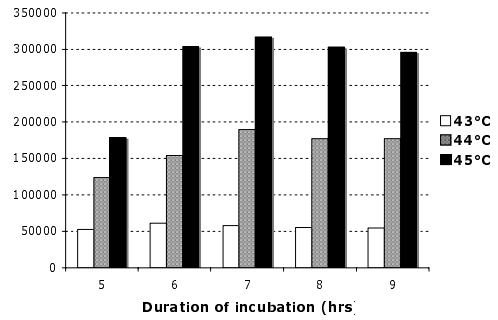

Figure 1 : *In vitro luciferase activity according to different temperature levels and duration of incubation.*

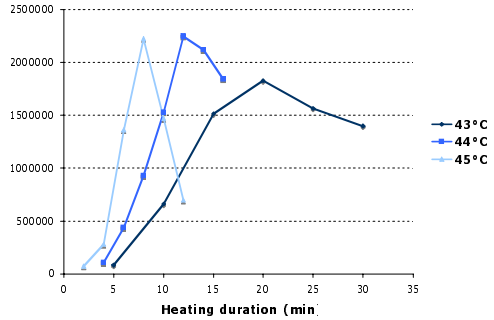

Figure 2: *In vitro luciferase activity related to different temperature levels and duration of heating.*

References :

(1) Rome C. et al, Eur Radiol 2007; 17:305-319.

(2) Guilhon E. et al, Mol Imaging 2003; 2:11-17.

(3) Salomir R. et al, Magn Reson Med 2000; 43:342-347.

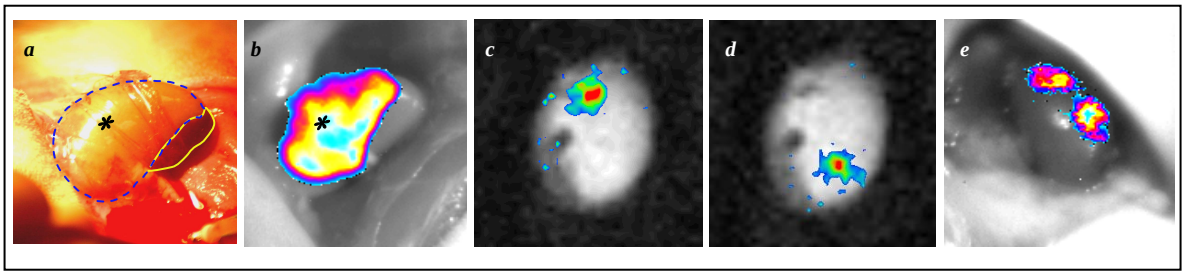

Figure 3 : *Transient renal discoloration (a) and signal in BLI (b) after intra-renal injection of preheated modified cells. MRI temperature maps (c and d) during heating procedure and signal at the heated regions in BLI ,6 hours after the heating procedure.*