Luciferin bioavailability in mice during in-vivo imaging

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Introduction

Reporter genes are widely used for monitoring gene expression in *in vitro* and *in vivo* assays. This technology enables non-invasive visualization of gene expression in intact animals. Bioluminescent models based on luciferase gene expression have become useful tools for evaluating cancer treatment efficiencies and the role of receptors in invasion and proliferation.

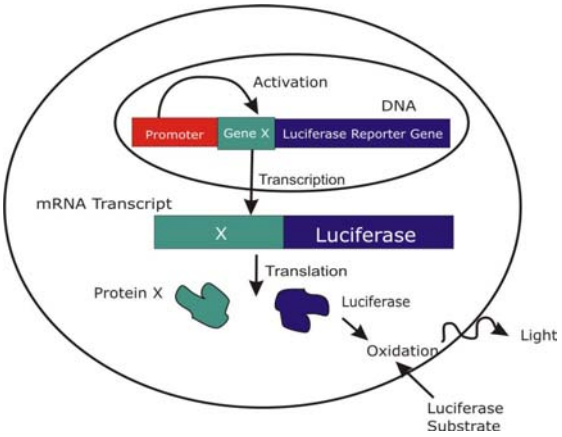

Figure 1: Principle of Reporter Gene Assays. The promoter activates the transcription of gene x and the close luciferase reporter in the cell nucleus. After translation the luciferase enzyme will catalyse the reaction of Luciferin (luciferase substrate) and ATP to oxyluciferin and light.

The light resulting from the bioluminescent oxidation of luciferin in the presence of ATP, magnesium and oxygen can be easily detected and quantified with a cooled charge-coupled device (CCD) camera, like the NightOWL LB 981 NC 100 system from BERTHOLD TECHNOLOGIES.

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The advantages of a luciferase assay are the high sensitivity, the absence of luciferase activity inside most of the cell types, the wide dynamic range, rapidity and low costs. The most versatile and common reporter gene is the luciferase of the North American firefly *Photinus pyralis* with the highest quantum efficency of the light reaction. The protein requires no posttranslational modification for enzyme activity. At the concentrations used for bioluminescence imaging, D-luciferin is non-toxic and non-immunogenic, and so serial imaging examinations can be performed with the same mouse. D-luciferin crosses cell membranes and penetrates the intact blood-brain barrier in addition to placental barriers after injection in mice, allowing this reporter protein to be imaged in any anatomic site. As the technique does not harm the animals, multiple sequential imaging studies in the same animal are possible.

Experimental Procedure

Usually luciferase expression is analyzed after intraperitoneal luciferin injection. Concentrations of D-Luciferin used for *in vivo* imaging are between 120 mg/kg up to 225 mg/kg bodyweight in mice. Since the magnitude of bioluminescence measured *in vivo* varies with time after luciferin injection, as well as with dose, time after injection and dose of D-luciferin have to kept constant in the sequential imaging experiences with the same animals (Burgos et al.). Only under constant conditions comparison of the quantitative results are possible.

To determine the optimal luciferase activity detection time, time course experiments were performed. Serial pictures were taken at different time intervals following luciferin injection (125 mg/kg bodyweight) roughly every 2 min. (Fig. 2). Maximal bioluminescent signal was obtained about 10 min after injection and remained stable during 10 more minutes.

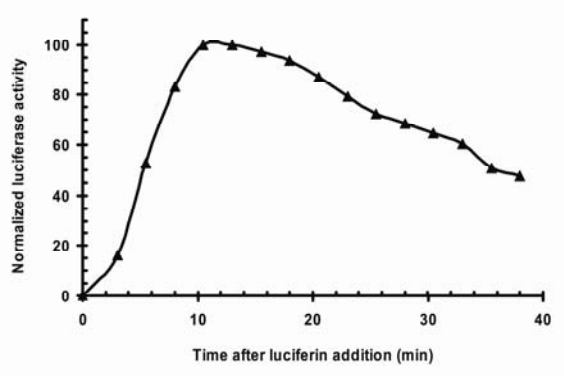


Figure 2: Time course of in vivo light emission from luciferin-treated LSCL^{+}N cells. Data were obtained after subcutaneous implantation of LSCL^{+}N cells in female Swiss nude mice. Mice were anesthetized and luciferin was ip injected. After 2 min imaging with CCD camera, data were extracted using WinLight software (BERTHOLD TECHNOLOGIES). The curve represents the normalized light units at each point measured by taking maximum value as 100.

To perform further studies with several mice simultaneously, NightOWL LB 981 NC 100 system (BERTHOLD TECHNOLOGIES) was coupled with an anaesthesia system from TEM (Bordeaux) that allow mice imaging in combination with a temperature controlled mice holder in the inner of the instrument (Fig.3).

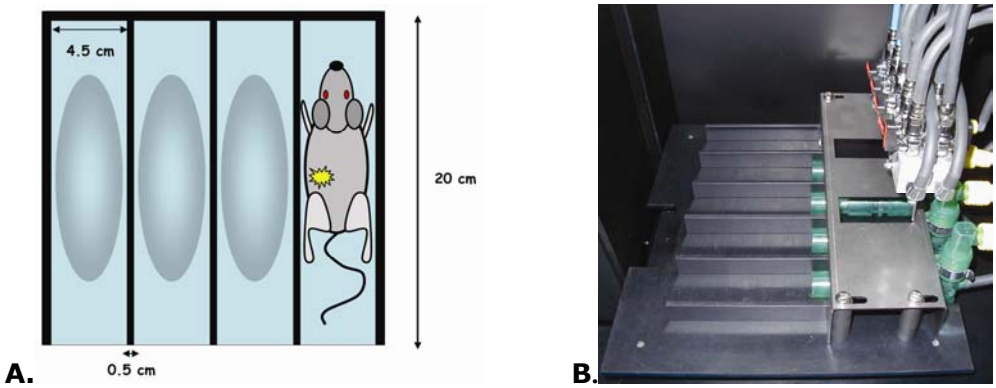


Figure 3: NightOWL NC 100 mice holder. Schematic view (A) of the mice holder was made by INSERM U 540, Montpellier, France and the realization of the final product was achieved by BERTHOLD TECHNOLOGIES engineers (B).

This anesthesia system allowed us to perform kinetic of luciferine distribution within 1 to 4 mice simultaneously. For this purpose, we used the sequence acquisition mode of WinLight software to follow the apparition of the bioluminescent signal as shown in figure 4.

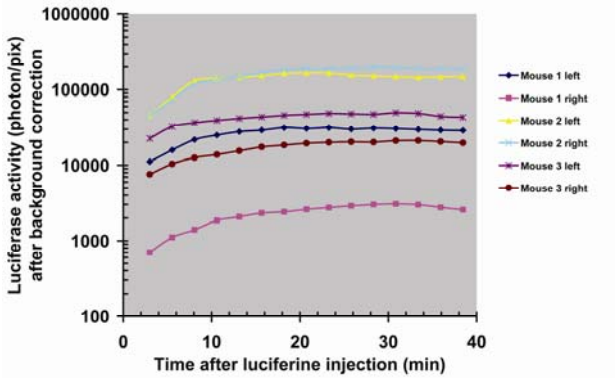


Figure 4: Time course of in vivo light emission from luciferin-treated mice. Data were obtained after subcutaneous implantation of bioluminescent cells in female Swiss nude mice (two implantations per mice: left flank and right flank). Mice were anesthetized and luciferin was subcutaneously injected. Mice were imaged using acquisition mode within a 2 min period and data were extracted using WinLight software (BERTHOLD TECHNOLOGIES).

Most of the time kinetic was similar but we have noted some variation mostly due to luciferin distribution that depends on injection (either intraperitoneal or subcutaneous injection). Figure 5 showed three different kinetic often obtained.

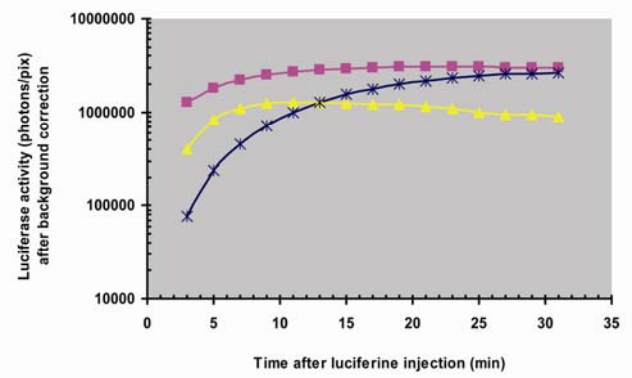


Figure 5: Three different time courses of in vivo light emission from luciferin-treated mice. Data were obtained after subcutaneous implantation of bioluminescent cells nude mice. Mice were anesthetized and luciferin was subcutaneously injected. Mice were imaged using acquisition mode within a 2 min period and data were extracted using WinLight software (BERTHOLD TECHNOLOGIES).

Those three patterns were very different. The yellow curve in which the maximal bioluminescent signal was obtained about 10 min after injection and remained stable during 10 more minutes and when decreased, was the more often obtained. But sometimes, the signal remained stable during more than 30 minutes (red curve) or it took more than 30 minutes to reach the maximum luciferase activity (blue curve). This experiment point out the necessity to perform time courses using the sequence acquisition mode to reduce signal variability of *in vivo* measurements. The bioavailability of luciferin within the animal could be different from an experiment to another. To avoid this problem, we have used constitutive luciferase expressing cells as an internal standard. Mice were subcutaneously double grafted (right and left flanks) as shown in figure 7.

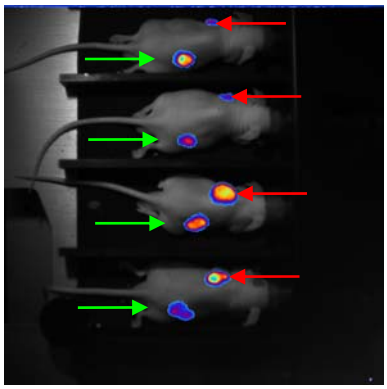
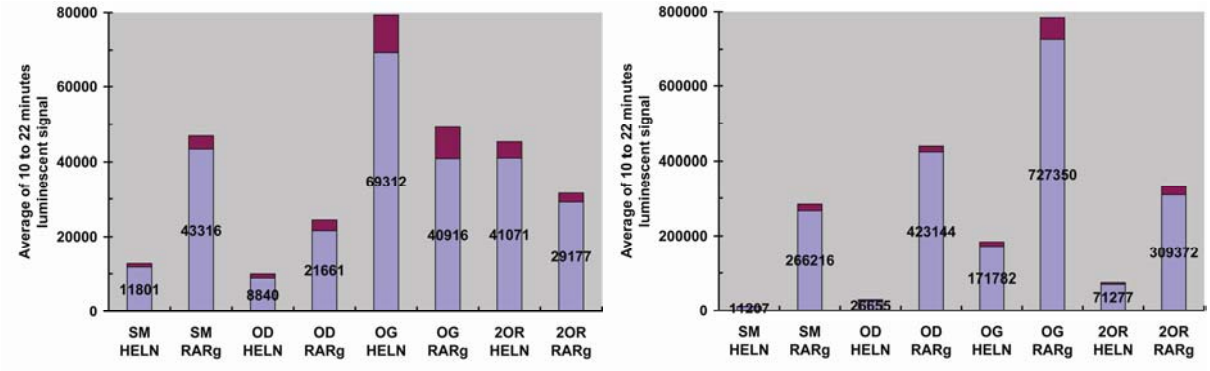


Figure 6: Implantation of inducible and constitutive cells as an internal standard. Four mice subcutaneously double grafted with cells expressing a constitutive luminescent signal (HELN cells) on their left flank (red arrows) and with cell expressing a retinoid inducible luminescent signal (RARγ) on their right flank (green arrows).

Mice were imaged using the sequential mode before and after 16 hours induction by a ligand of the inducible cells (retinoid agonist) administered orally. Averages of luminescent signal between 10 and 22 minutes were calculated for each tumor and reported in figure 7 and table below.



	Cells	Basal luminescent values (photon/pix)	Inducted luminescent values (photon/pix)	Induction simple	Induction corrected
Mouse 1 (SM)	Constitutive (HELN)	11801	11207	0.95	**6.47**
	Inducible (RARγ)	43316	266216	**6.15**	
Mouse 2 (OD)	Constitutive (HELN)	8840	26655	3.02	**6.48**
	Inducible (RARγ)	21661	423144	**19.53**	
Mouse 3 (OG)	Constitutive (HELN)	69312	171782	2.48	**7.17**
	Inducible (RARγ)	40916	727350	**17.78**	
Mouse 4 (2OR)	Constitutive (HELN)	41071	71277	1.74	**6.11**
	Inducible (RARγ)	29177	309372	**10.60**	

Figure 7: Averages of luminescent signal. Four mice were imaged using the sequential mode before and after 16 hours induction by a ligand of the inducible cells (retinoid agonist) administered orally. Averages of luminescent signal between 10 and 22 minutes were calculated. HELN were constitutive luciferase expressing cells and RARγ were retinoid luciferase inducible cells. Induction factor were calculated with or without constitutive cells correction.

Without any correction, fold induction of retinoid inducible cells were 6.15, 19.53, 17.78 and 10.60 for the four mice. Those results were very heterogeneous and could be due to variability of luciferin injection and its bioavailability as well as cell proliferation. Using the internal standard correction, fold induction were 6.47, 6.48, 7.17 and 6.11, respectively. This homogeneity allows us to determine the real fold induction of this experiment: 6.56 ± 0.44.

Literature

Burgos, J.S., M. Rosol, R.A. Moats, V. Khankaldyyan, D.B. Kohn, M.D. Jr. Nelson, and W.E. Laug. 2003. Time course of bioluminescent signal in orthotopic and heterotopic brain tumors in nude mice. Biotechniques 34:1184-8.