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THE FATE OF LUCIFERASE-LABELLED MYOBLASTS INJECTED IN THE RAT

Hypothesis / aims of study

One of the causes of stress urinary incontinence is intrinsic urethral sphincter deficiency. This condition occurs mostly in women after menopause and in men in whom during a radical prostatectomy the sphincter was damaged. A minimally invasive technique to repair the sphincter involves the injection of cultured myoblasts. Do myoblasts survive in the muscle, do they migrate, do they survive in the bloodstream? In order to answer these questions it is necessary to identify and track the transplanted cells. Using the luciferase expressing Lewis transgenic rat (1) as a donor, we monitored labelled myoblasts injected into the quadriceps, the tail vein and the urethral sphincter of host rats.

Study design, materials and methods

Myoblasts were isolated from luciferase positive (LEW-Tg(Rosa-luc)11Jmsk) 'firefly' rat striated muscle biopsies and grown in culture. Virgin female Lewis rats (mean weight: 213 g $\pm$ 7 g) were anesthetized with isoflurane and injected with these myoblasts into the quadriceps (9 animals), the tail vein (5 animals) or the urethral sphincter via a midline abdominal incision (4 animals). A total of 500.000 or 1.500.000 cells in 33 or 50 μl were injected using a 0.3 ml insulin syringe with a 30G needle. Gentamycine (antibiotic, 20mg/kg im) and Fynadine (analgesic, 2.5mg/kg im) were given to the sphincter-injected animals and muscle and skin were sutured separately with vicryl 4.0 and silk 4.0. The quadriceps and vein injections were given transcutaneously. Immediately after the injection on day 0, the animals received an intraperitoneal injection of luciferine (30mg luciferin in 2 ml Dulbecco's phosphate buffered saline (DPBS)). They were then placed in the non-invasive bioimaging system IVIS200^{TM} (Xenogen, CA) under isoflurane inhalation anesthesia. Luminescence was measured for 30 min (3x10 min). The measurements were repeated on day 1, 2, 4, 7, 11, 14, 18 and 25. After 25 days, the animals were sacrificed and the target organ was dissected and frozen in liquid nitrogen for future histology. The images were analyzed with IVIS Living Image® 3.2 and the signal intensity was quantified as photons flux in the region of interest (ROI). A non-luminescent area was monitored to calculate background values (BKG) (Fig. 1). All measurement data were exported to Excel. Average background flux was subtracted from the average flux in the region of interest. Animals with the same injection site were pooled and the average flux was calculated on each of the measurement days.

Results

None of the animals showed signs of infection or change of behaviour. The weight of the animals decreased a little but then returned to normal levels (mean on day 25: 216 g $\pm$ 8 g).
The luminescence data are depicted in Fig. 2:

- In the quadriceps-injected rats, luminescence was seen only at the site of the injection on day 0, 1 and 2, thereafter no signal could be detected.
- Injection of the tail vein resulted in very small luminescent signals, first in the thoracic region and later on in the tail.
- Injection of the sphincter showed very diverse results, small signals were seen in the thoracic and abdominal region and in the tail throughout the period of 25 days.

Figure 1
IVIS image of a rat injected with labelled myoblasts in the left quadriceps. Flux in region of interest (ROI) and background (BKG) on day 0.

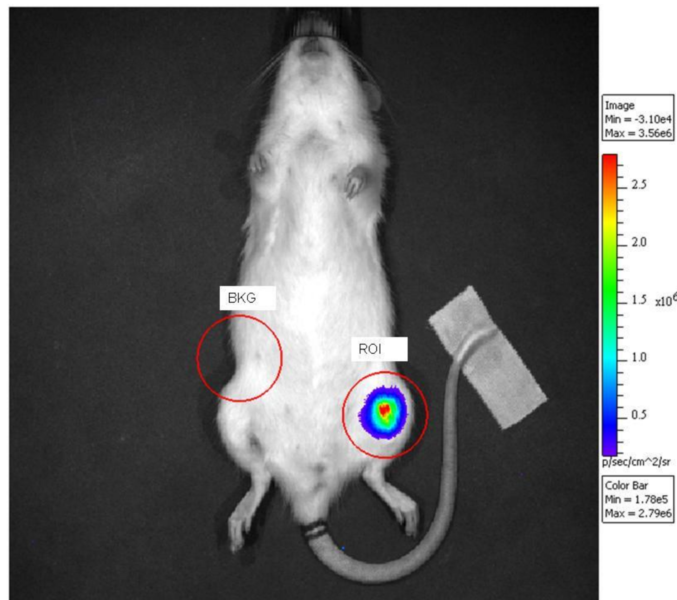
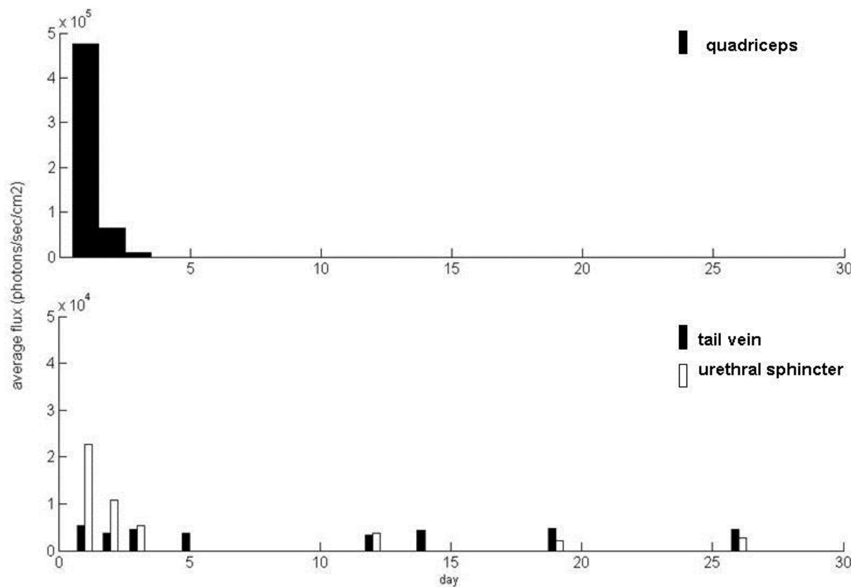


Figure 2
Average flux on day 0 – 25 for quadriceps, tail vein and urinary sphincter injected animals.



Interpretation of results
Luminescence is correlated with the presence of viable myoblasts. In the quadriceps, transplanted cells could be visualized up to the second day after injection, thereafter the signal disappeared. It is not clear whether the cells migrated from the muscle or failed to survive. After injection of the vein, from day 0 on, very little luminescence was seen, which suggests that the myoblasts did not survive and did not spread to other tissues via the bloodstream. Injection of the urinary sphincter proved to be very difficult and we fear that most of the cells were not confined to the muscle but ended up in the abdominal cavity or blood stream, from where they could not be traced thereafter.

Concluding message
In our set up, the myoblasts in the quadriceps were visible for only 2 days. Cells injected into the bloodstream did not show up in other tissues but disappeared from view. The technique of injecting the rat urinary sphincter as described here needs to be refined using smaller syringes and smaller volumes. Prior to its implementation in patients, any treatment involving the injection of myoblasts needs to be investigated in detail on an experimental research basis.

References

1. Transplantation 81(8): 1179-1184 (2006)

Specify source of funding or grant	**none**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**- Approval for the animal experiments was obtained from the local Dier Experiment Commissie (Animal Experiment Committee)**