Comparison of Embryonic Cerebellar Graft Survival in Adult Lurcher Mutant Mice of Strains C3H and C57Bl/7

Cendelín J., Korelusová I., Vožeh F.
Department of Pathophysiology of the Faculty of Medicine in Pilsen,
Charles University in Prague, Czech Republic

Received January 17, 2006, Accepted February 10, 2006

Key words: Cerebellar transplantation – Lurcher mutant mice – Olivocerebellar degeneration – Strain differences

This work was supported by the grant GA UK 75/2005/C/LFP and by the Research Program Project MSM ČR 021620816.

Mailing address: Jan Cendelín, MD., Department of Pathophysiology,
Faculty of Medicine in Pilsen, Lidická 1, 301 66 Plzeň, Czech Republic,
Phone: +420 377 593 366, Fax: +420 377 593 369, e-mail: jan.cendelin@lfp.cuni.cz

 Prague Medical Report / Vol. 107 (2006) No. 1, p. 89–94

Abstract: Lurcher mutant mice suffer from complete loss of cerebellar Purkinje cells. The aim of the work was to compare the solid embryonic cerebellar graft survival in adult Lurcher mutant mice derived from strains C3H and C57Bl/7 and to assess the morphology of the grafts. Embryonic cerebellar tissue was obtained from 12–13 days mice embryos expressing green fluorescent protein (GFP). Embryonic cerebellum was injected with a glass microcapillary into the cerebellum of adult Lurcher mutant mouse. Host mice were sacrificed 2–12 weeks after the transplantation. Brainstems and cerebella were examined histologically. The graft and graft derived GFP-positive cells were detected according to their green fluorescence. To visualise the structure of the graft Nissl staining was used. Graft survival percentage was evaluated in groups of mice sacrificed during the first, second or third month after the transplantation. The graft was found in all C57Bl/7 mice and in 90.9% of C3H mice examined within one month after the transplantation. In the second month the graft was present in 83.3% of C57Bl/7 and 50.0% of C3H mice. Till the third month the graft survived in 68.2% of C57Bl/7 mice and 22.2% of C3H mice. In C57Bl/7 mice a cerebellar structure was developed in the graft and migration of graft derived – cells to the host tissue was observed more often than in C3H mice. C567Bl/7 mice seem to be more suitable for experiments testing functional consequences of transplantation into the cerebellum requiring good long-term graft survival.

Introduction

Lurcher mutant mice represent a natural model of genetically determined olivocerebellar degeneration [1]. It is caused by a mutation of the δ2 glutamate receptor gene, which is in the cerebellum specifically expressed by Purkinje cells [2]. Heterozygous individuals (+/Lc), the Lurcher mutants, suffer from complete postnatal loss of Purkinje cells and substantial decrease of cerebellar granule cells and inferior olive neurons number. Purkinje cells became extinct with excitotoxic apoptosis induced by glutamate stimulation of the abnormal receptor [3]. Granule cell and inferior olive neurons death is secondary to the loss of Purkinje cells. The degeneration is completed at postnatal day 90, when there are no Purkinje cells in the cerebellum of Lurcher mutants and 10% of granule cells and 30% of inferior olive neurons remain [4]. Lurcher mutant mice are affected with cerebellar ataxia, deterioration of cognitive functions and some of their neurones are more sensitive to neurotoxic agents [5]. Wild type (+/+) littermates of Lurchers are completely healthy. Affected homozygots (Lc/Lc) are not viable due to a massive loss of neurons in the brainstem during the prenatal development [6, 7].

In our laboratory we have two strains of Lurcher mutant mice – C57Bl/7 and C3H. In both of them there is the same mutation and the same neurodegenerative affection. Despite of it, animals of the two strains differ in spatial learning ability, CNS excitability, and sensitivity to pain [8].

Lurcher mutant mice are used for investigation of functional and morphological consequences of the olivocerebellar degeneration and of therapeutical methods influencing the degenerative process or of subsequent functional deterioration.

The aim of the work was to compare the solid embryonic cerebellar graft survival in adult Lurcher mutant mice derived from two strains – C3H and C57Bl/7 – and to assess the morphology and development of the grafts in these animals.

Methods

Embryonic cerebellar tissue was obtained from 12–13 days mice embryos expressing the gene for green fluorescent protein (GFP) and without the Lurcher mutation. The embryos were obtained with cross-breeding of GFP-expressing mouse and a wild type C3H or C57Bl/7 mouse. Pregnant donor females were euthanized by overdosing with Thiopental at gestation day 12 or 13. The embryos were removed from the uterus and pooled in the cold aqueous solution of 0.9% natrium chloride and 0.6% glucose. GFP presence was checked with irradiating by UV-lamp. Only fluorescing embryos were used. Their brainstems were isolated and cerebella were dissected and pooled in the solution (0.9% natrium chloride and 0.6% glucose).

As hosts adult Lurcher mutant mice of the C3H (n=30) or C57Bl/7 (n=44) strain were used. As adult the individuals older than 60 day were considered. Such animals have no Purkinje cells (if older than 90 days) or maximally 1% of their normal number [4]. The donor mouse was anaesthetized with intraperitoneal application of combination of Ketamine (100 mg/kg b.w.) and Xylazine (16 mg/kg b.w.). The mouse was fixed in a stereotaxic holder. Soft tissues of the occipital area of the head were cut in the midline and a hole (2 mm in diameter) was drilled in the middle of the occipital bone. Two solid pieces of the embryonic cerebellum (tissue obtained from one embryo) and 10 μl of vehiculum (aqueous solution of 0.9% natrium chloride and 0.6% glucose) were injected with a glass microcapillary into the host cerebellum. Finally, the wound was sutured in one layer.

The mice were sacrificed 2–12 weeks after the transplantation by overdosing with Thiopental and transcardially perfused with phosphate buffer solution

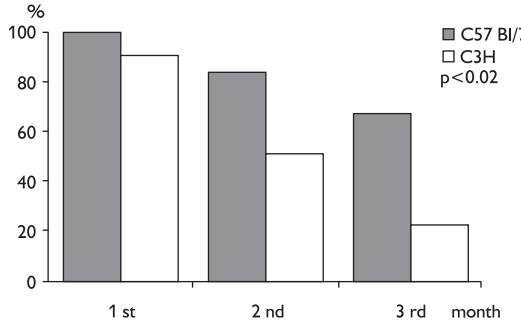

Figure 1 – Percentage of mice of the C57Bl/7 and C3H strains in which the graft survived and was found by histological examination in the 1st, 2nd and 3rd month after the transplantation. The difference between C3H and C57Bl/7 mice is statistically significant (p<0.02) in the 3rd month after the transplantation.

(pH 7.4) and 4% paraformaldehyde. Brainstems and cerebella were stored for several days in 4% paraformaldehyde for postfixation and sectioned with cryostat (50 μm frontal sections). The graft and graft derived GFP-positive cells were detected according to their natural green fluorescence in native sections. Size, shape and localisation were described also using a fluorescent microscope. Then the specimens were processed with Nissl staining to visualise histological structure of the graft.

Graft survival percentage was evaluated histologically within one month, during the second and third month after the transplantation. As individuals with surviving graft, mice in which at least small cluster of GFP-positive cells was found were considered. Graft survival percentage was compared in C3H and C57Bl/7 animals using χ^2 test. Presence of Purkinje cells and the development of typical cerebellar

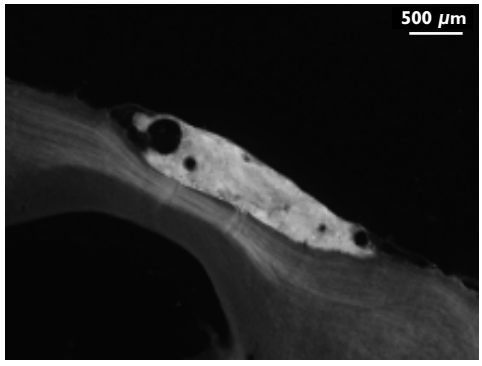

Figure 2 – Strictly delimited graft in a C3H mouse. Native specimen, GFP-fluorescence.

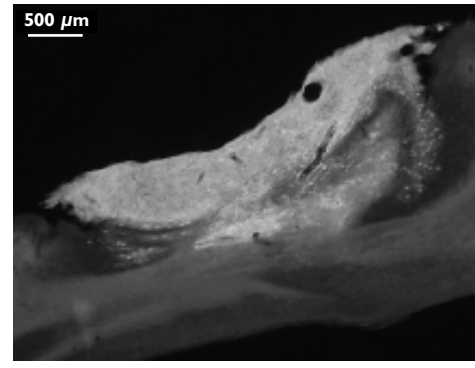

Figure 3 – Graft in a C57Bl/7 mouse, which is not strictly delimited against the host tissue. Native specimen, GFP-fluorescence.

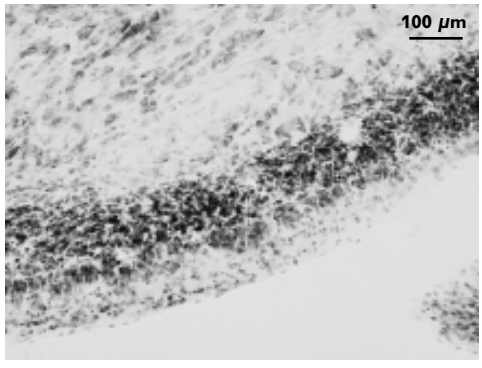

Figure 4 – Graft with developed cerebellar cortex structure in a C57Bl/7 mouse. Nissl staining.

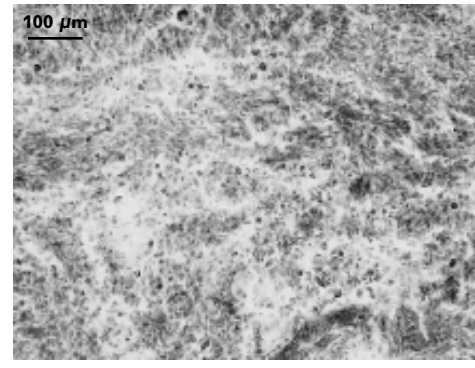

Figure 5 – Graft without organised histological structure in a C3H mouse. Nissl staining.

structure in the graft, as well as its delimitation against the surrounding host tissue were also assessed.

Results

The graft was found in the cerebella of all 10 C57Bl/7 mice examined histologically within one month after the transplantation (100% survival). In mice of the C3H strain the graft was present in 10 individuals of 11 examined at this time (90.9% survival). 12 C57Bl/7 mice were examined during the second month after the transplantation and the graft was present in 10 of them (83.3% survival), while in 10 C3H animals the graft was found in 5 cases (50.0% survival). In the third month the graft survived in 15 and disappeared in 7 C57Bl/7 mice (68.2% survival). In C3H mice the graft survived in 2 and was extinct in 7 C3H animals (22.2% survival) (Figure 1).

The size of the graft was variable in both strains. In some animals only small clusters of GFP-positive tissue were found. In other cases the graft grew up to several millimetres in diameter and covered almost all hemisphere of the host cerebellum. In almost all grafts the cells with size and shape corresponding to Purkinje cells were found.

In some cases the graft was strictly delimited against the host tissue (Figure 2). In several animals, more often of the C57Bl/7 strain, GFP-positive cells were dispersed in the host tissue close to the graft. Most of these cells showed Purkinje cell appearance and formed rich dendritic trees. Colonisation of distant parts the host cerebellum with graft-derived cells was not observed (Figure 3).

In approximately half of the C57Bl/7 mice with surviving graft a typical cerebellar structure with three cortical layers developed at least in a small part of the graft (in 40% of mice examined in the 1st month, 50% in the 2nd month and 76% in the 3rd month) (Figure 4). In the rest of C57Bl/7 mice and in almost all C3H animals no differentiated cerebellar structure was found in the graft and its structure was irregular (Figure 5).

Discussion

The embryonic cerebellar graft survived in high percentage of mice of both strains at least one month. Long-term graft survival (2–3 months) was higher in Lurcher mutant mice of the C57Bl/7 strain as compared with C3H mice. The development of the graft was also better in C57Bl/7 animals (typical cerebellar structure, cell migration to the host tissue). The strain differences in graft survival and its development could be explained by different affinity of C3H and C57Bl/7 host mice and donor GFP-expressing mice or by differences in the immune system of C3H and C57Bl/7 Lurcher mutant mice [9]. These findings indicate that mice of the C567Bl/7 strain are more suitable for experiments testing functional consequences of cerebellar tissue transplantation because of higher percentage of long-term graft survival than in mice of the C3H strain.

References

1. PHILLIPS R. J. S.: 'Lurcher'. A new gene in linkage group XI of the house mouse. *J. Genet.* 57: 35–42, 1960.
2. ARAKI K., MEGURO H., KUSHIYA E., TAKAYAMA C., INOUE Y., MISHINA M.: Selective expression of the glutamate receptor channel delta 2 subunit in cerebellar Purkinje cells. Biochem. *Biophys. Res. Commun.* 197: 1267–1276, 1993.
3. ZUO J., DE JAGER P. L., TAKAHASI K. J., JIANG W., LINDEN D. J., HEINTZ H.: Neurodegeneration in Lurcher mice caused by mutation of δ2 glutamate receptor gene. *Nature* 388: 769–773, 1997.
4. CADDY K. W. T., BISCOE T. J.: Structural and quantitative studies on the normal C3H and Lurcher mutant mouse. *Phil. Trans. Roy. So. Lond. B.* 287: 167–201, 1979.
5. CADDY K. W. T., VOŽEH F.: The effect of 3-acetylpyridine on the inferior olivary degeneration in the Lurcher mutant and wild type mice. *Europ. J. Pharmacol.* 330: 137–142, 1997.
6. CHENG S. S., HEINTZ N.: Massive loss of mid- and hindbrain neurons during embryonic development of homozygous Lurcher mice. *J. Neurosci.* 17: 2400–2407, 1997.
7. RESIBOIS A., CUVELIER L., GOFFINET A. M.: Abnormalities in the cerebellum and brainstem in homozygous Lurcher mice. *Neuroscience* 80: 175–190, 1997.
8. VOŽEH F., CENDELÍN J., YAMAMOTOVÁ A., ROKYTA R.: CNS excitability and pain perception in two strains of mice afflicted with the some type of cerebellar degeneration (Lurcher mutants). *Homeostasis* 41: 196–199, 2001/2002.
9. VOŽEH F., ŠÍMA P., VIRTOVÁ M.: Experimental study of neuro-immune relations in neurodefectiveLurcher mice derived from two strains. (Article in Czech and abstract in English) *Plzeň. lék. Sborn.* 74: 29–40, 2004.