Final progress report

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Reversal of heroin neurobehavioral teratogenicity with neural progenitor.

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In this project, we have established a model for heroin neurobehavioral teratogenicity and its reversal with stem cell transplantation in mice and then made the first steps toward expanding it to the more efficient avian model.

1. Prenatal exposure to heroin in mice abolished cholinergic receptor-induced activation/translocation of PKCγ in the mouse hippocampus; transplantation of neural stem cells totally reversed the heroin-induced deficits (fig. 1).

2. Prenatal exposure to heroin in mice induced deficits in the hippocampus-related Morris maze behavior in the mouse hippocampus; transplantation of neural stem cells totally reversed the heroin-induced behavioral deficits (fig. 2).

3. In search of the mechanism by which the stem cells exert their therapeutic action, transplantation of NSC to the prenatally heroin-exposed offspring, enhanced neurogenesis in their brain, concomitant to the reversal of the neurobehavioral deficits (fig. 3a,b).

The chick model is fast and efficient (it’s actually near complementary) and particularly suitable for work with small grants. In the latter part of our research we have attempted to develop a novel chick model for heroin neurobehavioral teratogenicity of heroin.
4. We developed the methodology for deriving neural stem cells from a chick embryo at the age equivalent to the birthdate of the mouse. In addition, we developed the methodology and the appropriate day for administrating the stem cells to the chick embryo via the peripheral blood vessels. Stem cells injected into the embryonic blood vessels, reached the brain and migrated to various locations (Fig. 4).

5. Expending the model to include mesenchymal stem cells: mesenchymal stem cell (MSC) transplantation enables autologous transplantation, thus avoiding obstacles related to immune rejection and moral issues. We transplanted MSC into the embryonic blood vessels. The cells reached the brain and migrated to various locations (Fig. 5).

6. Once the avian model was established we started our research on consequences of prenatal (pre-hatch in the case of the chick) exposure to the teratogen. Prenatal exposure to the teratogen in the chick embryo markedly decreases neurogenesis as is attested to by the 43% decrease of doublecortin expression in the lateral striatum at hatching (fig.8).

In our continuing studies (present grant) we will study the reversal of the deficits by the novel technique of transplantation of mesenchymal stem cells and will ascertain the mechanism of both the deleterious action of heroin and the mechanism of the reversal of the deficits by transplantation of mesenchymal stem cells. It should be noted that mesenchymal stem cells enable autologous transplantation i.e. from the patient to himself/herself thus avoiding immune-rejection. It is hoped that all this will provide the preliminary date and prepare the groundwork for the clinical work on humans.

Preliminary results:
1. Mice exposed prenatally to heroin showed deficits in Morris maze behavior. Transplantation of neonatal cortical-derived neural stem cells (NSC) reversed both the molecular alterations and behavioral deficits. Mice were exposed to heroin prenatally on gestation days 9-18, as described in the original proposal, and the neural behavioral deficits were assessed at adulthood (Figs. 1 and 2).

Fig. 1.
PKCγ: Ach-induced translocation/activation

Fig. 2

Effects of prenatal heroin exposure and subsequent grafting of neural progenitors on carbachol-induced PKCγ in the hippocampal cell membrane. As is shown in the figure, incubation with carbachol induced a significant increase in membrane PKCγ level in the control (C-C). Mice exposed prenatally to heroin (H-C) displayed total abolishment of translocation/activation of PKCγ (p<0.05 for the difference from control levels). By itself, grafting of neural progenitors had no effect on PKCγ level (C-NP), but neural progenitors (H-NP) reversed the deficits evoked by prenatal heroin exposure (p<0.05 from H-C).
2. Our hypothesis was that beyond their effect on replacing lost cells and repairing damaged circuitries, one major mechanism by which NSC exert their therapeutic action, is by induction of proliferation of endogenous neural stem cells in the host brain. Indeed, transplantation of NSC to the prenatally heroin-exposed offspring, enhanced neurogenesis in their brain, concomitant to the reversal of the neurobehavioral deficits (Fig. 3a,b).

Fig. 3a (Ben-Shaanan et al 2008)
Endogenous cells in the DG of the heroin-exposed offspring

A. Sham transplanted
B. Transplanted with NSC

Fig. 3b
3. The chick model:
a) Developing minimally invasive routes of stem cell transplantation: we developed the methodology for deriving neural stem cells from a chick embryo at the age equivalent to the birthdate of the mouse. This day was found to be the incubation day (ID) 10. In addition, we developed the methodology and the appropriate day for administrating the NSC to the chick embryo via the peripheral blood vessels. The most appropriate day was established as ID 13. NSC injected into the embryonic blood vessels, reached the brain and migrated to various locations (Fig. 4).
Chick’s neural stem cells, transplanted via the vascular system, surviving in the host brain (Dil cell marker)

b) Enabling autologous transplantation: mesenchymal stem cell (MSC) transplantation enables autologous transplantation, thus avoiding obstacles related to immune rejection and moral issues. We transplanted MSC into the embryonic blood vessels. The cells reached the brain and migrated to various locations (Fig. 5).
c) Xenographic transplantation: Mouse subventricular zone-derived adult NSC transplanted into the embryonic blood vessels, reached the brain, survived there and migrated to various locations (Fig. 6). The development of this protocol of xenographic transplantation will enable our subsequent studies, where we will be able to assess the efficacy of human stem cells in the therapy of neurobehavioral teratogenicity.

Fig. 6
4. **Medium throughput model for heroin effect on development using chick NSC culture:** chick NSC were cultured and after the removal of the growth factors, differentiated into the major lineages. Addition of neuroteratogens to the media specifically impaired differentiation to neurons, but did not affect differentiation to astrocytes and other glial types *(Fig. 7).*
Effect of 5 and 10 mg chlorpyrifos on the survival of chick NSC neurospheres and the differentiating nerve cells
Fig. 8 Prenatal exposure to heroin in the chick embryo markedly decreases neurogenesis as is attested to by the 43% decrease of doublecortin expression in the lateral striatum at hatching. Immunocytochemistry study, bar indicates a reduction from control (injected with saline) among the heroin exposed offspring.
N= 12 p<0.0001

References Cited


**Other publications partially supported by the present grant**


