Cell Transplantation Therapy of Spinal Cord Injury

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SCI Cell Transplant Therapies

Embryonic stem cell (ESC)
- Mouse ESCs myelinate rat spinal cord (McDonald)
- Mouse and human ESCs replace motoneurons in rat (Kerr)
- Human ESC oligodendroglial precursors in rat SCI (Keirstead)

Fetal neural stem cell (NSC)
- Fetal NSCs improve recovery in rats (Blakemore, Okano).
- Radial glial cells reduce acute SCI damage (Grumet).

Umbilical cord blood (UCB)
- Human cord blood CD34+ cells improve recovery in rats (Saporta; Li; Zhao; Kuh; Nishio)

Olfactory ensheathing glia (OEG)
- Rat adult olfactory bulb (Ramon-Cuetos, Bunge).
- Rat nasal mucosa cells (Lu)
- Neonatal rat olfactory bulb (Liu, Koscis)

Bone marrow cells (BMC)
- Rat bone marrow cells remyelinate spinal cord (Koscis, Honmou, Dezawa)

Schwann cells (SC)
- Rat Schwann cells regenerate spinal cord (Xu, Bunge, et al.)
- Degenerated peripheral nerve segment in rat SCI (Feng)
SCI Cell Transplant Trials

- **Fetal spinal cord**
  - Fetal spinal cord transplants into chronic SCI (n>100, Gainesville, Karolinska, & Novosibirsk)

- **Porcine fetal neural stem cells**
  - Diacrin porcine neural stem cells (n=10, Wash U. & Albany)

- **Activated adult macrophage**
  - Proneuron phase 1 & 2 (n>40, Israel, Brussels, USA)

- **Bone marrow cell autografts**
  - Subacute SCI (n=10, Seoul)
  - Subacute SCI (n>220, Zhang in Zhengzhou, Henan, China)
  - Chronic SCI (n>100: Germany, Russia, Turkey, India, & others)

- **Olfactory ensheathing glia**
  - OEG from fetal olfactory bulb
    - n>700 chronic SCI (Huang)
    - n>30 chronic SCI (Sun)
    - n>57 chronic SCI (Shen)
    - n>20 chronic SCI (He)
    - n>10 subacute SCI (Zhu)
  - Nasal mucosa (n>160, Lima)
  - Nasal mucosa OEG (n=2, McKay-Sims in Brisbane)

- **Schwann cells (SC)**
  - Fetal SC (n>150, Zhu)
  - Adult SC (n=10, Feng)

- **Umbilical cord blood (UCB)**
  - IV/IT non-HLA (n>1000, Beike)
  - HLA-matched into chronic SCI (n=20, ChinaSCINet)
Combination Therapies

- **Bridging the gap.** Injury produces an area of dead cord, surrounded by glia and chondroitin-6-sulfate proteoglycan (CSPG). Cells can bridge this gap.

- **Sustained growth stimulation.** Regeneration may a long time, requiring years of sustained growth factor support. Lithium stimulates neurotrophic factors.

- **Blocking growth inhibitors.** Nogo and CSPG inhibit axon growth. Drugs can block these growth inhibitors: Nogo antibody, cethrin, chondrotinase.
Stem Cell Sources

- Embryonic stem cells
  - In vitro fertilized eggs
  - Cloned eggs (SCNT)
  - Parthenogenic eggs
  - Eggs from stem cells
  - Teratomas (stem cell tumors)
  - Induced pluripotent stem cells

- Fetal stem cells
  - Fetal neural stem cells
  - Germ cell line stem cells
  - Other fetal stem cells
    - Skin cells
    - Schwann cells
    - Vascular cells
    - Muscle cells
    - Bone marrow cells
    - Germ cells

- Neonatal stem cells
  - Umbilical cord/placenta
  - Placenta, umbilical cord
  - Baby teeth stem cells

- Adult stem cells
  - Neural stem cells
    - Hippocampus
    - Olfactory bulb
    - Spinal cord
  - Mesenchymal stem cells
  - Bone marrow stem cells
  - Nasal mucosa stem cells
  - Peripheral blood CD34+ cells
  - Skin and fat stem cells
  - Enteric glia
  - Testicular germ cells
Intravenous infusion of human UCB mononuclear cells (UCBMC) improves function in rat SCI (Saporta, 2004).

Intraspinal transplants of human CD34+ UCBC improve recovery in hemisected rats (Li, 2004; Zhao, 2004).

Intraspinal transplants of human CD34+ UCB cells BDNF improves recovery in rat SCI (Kuh, 2005).

Human UCB CD34+ cells survived >3 weeks in contused rat spinal cords and reduced tissue damage (Nishio, 2006).
Human UCB CD34+ cells reduce inflammation and increase myelination in contused rat spinal cords (Dasari, et al., 2008, 2009).

Neural progenitor cells grown from human UCB modestly repair and improve recovery in contused rat spinal cords (Cho, et al., 2008).

Human cord blood CD34+ cells increase vascular endothelia and neurotrophic factors in injured rodent spinal cords (Kao, et al. 2008).
Human mesenchymal stem cells from cord survive 15 week, stimulate corticospinal tract regeneration improve rat locomotor recovery (Yang, et al. 2008)

Review article by Cao & Feng (2009), including Chinese literature, revealed 51 reports of UCB cell treatments of spinal cord (1983-2007).

Human UCB increase matrix metalloproteinase-2 and reduce tissue plasminogen activator in contused rat cords (Veeravalli, et al. 2009).
Advantages of Cord Blood

- Umbilical cord blood (UCB) is the only banked stem cell that is readily available in sufficient diversity and amounts for clinical use.

- UCB requires only 4:6 HLA match for engraftment into bone marrow and therefore HLA-matched cells will be available for use in spinal cord injury.

- Over 20 years of experience with UCB transplants for hematopoietic conditions indicate the cells are very safe, non-tumorigenic, and non-infectious.
Lithium and Regeneration


N01.1 cells were cultured in 3 mM lithium chloride for 7 days. Lithium treated cultures had 359% more cells than control cultures grown without lithium.
Lithium Effects on N01.1

Lithium

Saline

Spinal cords at 2 weeks after injury and GFP NO1.1 cell transplants.
Lithium Effect on GFP in Vivo

GFP mRNA measured by real-time RT/PCT in un-injured rat spinal cord at 2 weeks after NO1.1 cell transplantation. GFP mRNA was detectable but very low in saline-treated rats. Lithium-treated rats had 1000x more GFP mRNA than in saline-treated rats.
Real-time PCR showed that LIF, GDNF, NT3, NGFa, and NGFb were 3-5 times higher in spinal cords of N01.1-transplanted and lithium-treated rats than in N01.1-transplanted and saline-treated rat.
China SCI Network

- **Beijing**: Beijing Army General Hospital, China Rehabilitation Research Centre, Peking University People’s Hospital, Beijing Xishan Hospital, People’s Liberation Army (PLA) 301 Hospital
- **Chongqing**: Third Military Medical University
- **Fuzhou**: Fuzhou University Hospital
- **Guangzhou**: Sun Yatsen Hospital, Nanfong Hospital (1st Military)
- **Kunming (Yunnan)**: Kunming Army (PLA) General Hospital
- **Hong Kong**: Hong Kong University (Queen Mary Hospital), Chinese University of Hong Kong (Prince-of-Wales Hospital)
- **Shanghai**: Fudan University Hospital, Shanghai Hospital (Second Military), East Shanghai Medical Center, and Ningpo #2 Hospital
- **Shantao**: Li Ka Shing Hospital
- **Taiwan**: Tzuchi Buddhist Hospital (Hualien, Taipei, Taichong, Tainan), China Medical University (Taichong), Chang Gung Memorial Hospital
- **Tianjin**: Tianjin Medical University Hospital
- **Xi’an**: Fourth Military University Hospital, Jiaotong Hospital
- **Zhengzhou**: People’s Provincial Hospital of Henan
Spinal Cord Injury in China

- Spinal cord injury (SCI) incidence in China increased tenfold (from 6.5 to 65 cases/million) from 1995 to 2005, i.e. >85,000 new cases/year.

- Prevalence of chronic SCI >800,000 in 2008 and is likely to exceed a million by 2010 in China.

- ChinaSCI-Net has 25 leading SCI centers in mainland China, Hong Kong, and Taiwan.

- Ability to randomize 6000 people with acute SCI/year and virtually unlimited numbers of chronic SCI.
ChinaSCINet Trials

CN100. Observational Trial
500 subjects with chronic SCI, followed for one year.

CN101. Phase 1 Oral lithium trial (at HKU)
20 subjects with chronic SCI, 6-week oral lithium carbonate.

CN102A. Phase 2 Oral lithium trial (in China)
40 subjects randomized to lithium or placebo, 6 months.

CN102B. Phase 2 Mononuclear cell transplant
20 subjects cord blood mononuclear cell transplant trial.

CN103. Phase 3 UCBM cell transplants ± lithium
400 subjects with chronic SCI (>1 year, 6 months stable neurological function) will receive UCBMC transplant, then randomized to oral lithium or placebo; ASIA A, B, and C; followed for one year.
CN102a Phase 2 Clinical Trial

-4 weeks
ASIA Exam
Lab Tests
VAS/MAS

Screening

Inclusion Criteria
- Chronic SCI
- ASIA A, B, or C
- Age 18-60 years
- Informed Consent
- VAS capable

Screening

Day 0
Randomize

Day 14
Lithium
Treatment

Day 42

Day 84
Followup
Exams

Day 168

Screening

Lithium
Placebo

Titration 2wk

Treatment 4wk

Followup
6w, 12wk, 24wk

EXCLUSION
- General: Significant renal, cardiovascular, hepatic psychiatric disease, infection, and other medical conditions
- Specific: Brain injury, Addison's disease, debilitation or dehydration (sodium deficiency)
- Drugs: Diuretics (thiazides), tricyclic antidepressant, NSAID, tetracycline
- History: Lithium hypersensitivity
- Others: Alcohol or drug abuse, pregnancy or lactation, participation in another clinical trial

DISCONTINUATION
- Adverse events: Lithium toxicity
- Abnormal lab test: change from baseline
- Adverse neurological change: Increased pain (visual analog scale) or spasticity (modified Ashworth scale)
- Protocol violation: Subject withdraws consent or becomes pregnant
- Administration: Trial sponsor withdraws

OUTCOMES
ASIA motor and sensory scores, Functional Index of Motor (FIM) scores, Visual Analog Scale (VAS), Modified Ashford Scale (MAS)
CN102B Protocol

Cord Blood Mononuclear (CBM) Cells

→

HLA matched cord blood
>4/6 match
20 subjects
chronic SCI
CN103 Phase 3 Clinical Trial

SCREENING
- Chronic SCI (>1 year, from CN100)
- ASIA classification: A, B, or C
- Stable neurological exam ≥1 year
- Age: 18-60 years, male & female
- Informed Consent

HLA test

PROCEDURE (n=400)
Mini-laminectomies, inject (16 μl x 4) about 6.4 million HLA-matched (≥4/6) cord blood mononuclear cells into spinal cord above and below injury site.

Randomize
Placebo 6wk  Lithium 6wk

Outcomes (6-week, 6-month, 1-year):
- Motor/sensory scores
- Functional index motor (FIM) score
- Visual Analog Score (VAS)
- Modified Ashworth Scale (MAS)
- Adverse events

EXCLUSION
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- Drugs: Diuretics (thiazides), tricyclic antidepressant, NSAID, tetracycline
- History: Lithium hypersensitivity
- Others: Alcohol or drug abuse, pregnancy or lactation, participate in another clinical trial, HLA-match: cord blood not available

DISCONTINUATION
- Adverse events: surgical complications, lithium toxicity
- Abnormal lab tests: change from baseline
- Adverse neurological change: severe increase of pain (visual analog scale) or spasticity (modified Ashworth scale), or motor/sensory score deterioration
- Major protocol violation: Subject withdraws consent or becomes pregnant
- Administration: Trial sponsor withdraws
Hongyun Huang has transplanted OEG cells to >700 people with spinal cord injury by injecting 35-50 µliters of cell suspension into midline of spinal cord above and below the injury site.

We are concerned with both the volume and the midline approach, believing that it may be damaging to the spinal cord and particularly to the posterior columns.

We held a consensus conference in 2007 to develop a standard method of transplanting cells.
Diameter vs. Volume

\[ V = \frac{4\pi R^3}{3} \]
Injectate Volume

Rat

Human
Cells will be injected into the dorsal root entry zones of the spinal cord above and below the injury site, at a 45° angle and 1 mm deep. The injection volume will be 4, 8, or 16 µliters of 100,000 cells/µliter.
Central Injection Sites

Bevel Down

Bevel Up

45° angle, 1.5 mm depth, 0.5µl over 10 minutes

- Bevel direction is important. Down-bevel result in dye localizing to ventral gray while up-bevel has result in dye in dorsal gray matter.
- There is greater spread of dye with lumbar cord (right).
Floating Needle Injections

The spinal cord normally pulsates with respiration, moving 1-2 mm. Placement of any needle into the spinal cord will result repeated trauma to the cord.

We therefore propose the use of a 32-gauge “butterfly needle” (used for baby scalp veins), which would be hand inserted at 45° into DREZ.

The needle would be allowed to “float” and the injections would be made slowly with a Hamilton syringe able to inject 4, 8, and 16 µliters.
US102B. Dose-escalating Phase II trial.
- Compare 4, 8, 16 µliter UCBMC injections (x4)
- Assess Methylprednisolone (MP) effect on transplant
- Assess UCBMC, MP, & 6-week lithium

US103A. Phase III trial
- Placebo + Rehabilitation
- Lithium + Rehabilitation
- UCBMC + Rehabilitation
- UCBMC + lithium + Rehabilitation

US104 trials on older patients, high quads, & TM.
Conclusions

- Many therapies show promise in regenerating and remyelinating the spinal cord.
- Several therapies are ready to go to clinical trial. Combination therapies are likely to be needed.
- Much work needs to be done to prepare other therapies for clinical trial.
- Industry sponsorship of cell transplantation therapy clinical trials is beginning.
- The first successful therapy will transform the field.