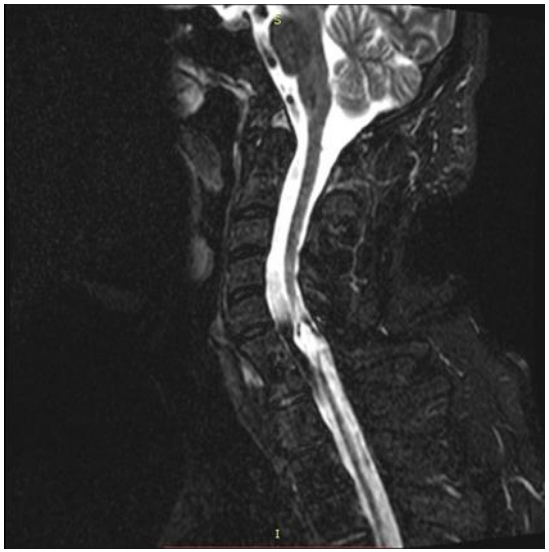
Treatment of Spinal cord injury with autologous bone marrow-derived mononuclear cells.



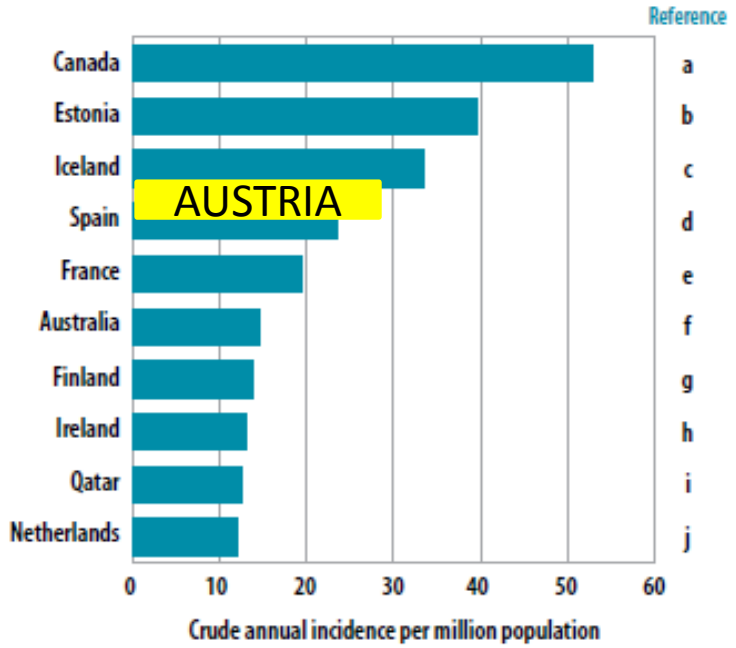
Situation

SPINAL CORD INJURY

Every year, between 250 000 and **500 000 people** become spinal cord injury victims. (WHO 2013). Austria has 150 to 200 cases per year (25 per million population) (http://www.ascis.at/)

"Spinal cord injury is **a medically complex and life-disrupting condition**," notes Dr. Etienne Krug, Director of the Department of Violence and Injury Prevention and Disability, WHO. "However, spinal cord injury is preventable, survivable, and needs not preclude good health and social inclusion.

Figure 2.2. Global variation in country-level estimates of annual incidence of TSCI



http://www.who.int/mediacentre/news/releases/2013/spinal-cord-injury-20131202/en/

Rationale for aBM-SC treatment

Adult SC in BM (HSCs and MSCs)

The mechanisms involved are: Paracrine and cellular.

Secrete all kind of factors, which influence the direct environment of injured cells

(Gnecchi et al. 2008, Caplan et al. 2006).

Induce **NEUROPROTECTION, inflammatory suppression and neural repair**, allowing reconstruction of totally damaged tissues or preventing partially damaged cells from evolving to cell demise. (Erceg and Stojkovic, 2009)

Gnecchi, M., Zhang, Z., Ni, A., Dzau, V.J., 2008. Paracrine mechanisms in adult stem cell signaling and therapy. Circ. Res. 103, 1204–1219. doi:10.1161/CIRCRESAHA.108.176826

Caplan, A.I., Dennis, J.E., 2006. Mesenchymal stem cells as trophic mediators. J. Cell. Biochem. 98, 1076–1084. doi:10.1002/jcb.20886

Erceg, S.M.R. and M. Stojkovic, 2009. Human embryonic stem cell differentiation toward regional specific neural precursors. Stem Cells, 1: 78-87. DOI: 10.1634/stemcells.2008-0543

Rationale for aBM-SC treatment

Adult SC in BM (HSCs and MSCs)

- Stimulate **neovascularization** and increase oxygenation **(Zhang et al. 2008).**
- **Transdifferentiate** into specific neuronal cells **(Zeng et al. 2011).**
- Promote **synaptic** connections **(Bareyre et al. 2008).**
- Promote **neuroplasticity (Kucia et al. 2005).**

NO Ethic or immune rejection concerns

aBM-SC are easy to harvest from the iliac crest and a gradient centrifugation can separate the buffy coat layer, which contains the highest concentration of HSCs and MSCs.

Zhang H, Zhang N, Li M, Feng H, Jin W, Zhao H, Chen X, Tian L (2008) Therapeutic angiogenesis of bone marrow mononuclear cells (MNCs) and peripheral blood MNCs: transplantation for ischemic hindlimb. Ann Vasc Surg 22:238–247

Zeng, R., Wang, L.-W., Hu, Z.-B., Guo, W.-T., Wei, J.-S., Lin, H., Sun, X., Chen, L.-X., Yang, L.-J., 2011. Differentiation of human bone marrow mesenchymal stem cells into neuron-like cells in vitro. Spine 36, 997–1005. doi:10.1097/BRS.0b013e3181eab76

Bareyre, F.M., 2008. Neuronal repair and replacement in spinal cord injury. J. Neurol. Sci. 265, 63–72. doi:10.1016/j.jns.2007.05.004

Kucia, M., Reca, R., Jala, V.R., Dawn, B., Ratajczak, J., Ratajczak, M.Z., 2005. Bone marrow as a home of heterogenous populations of nonhematopoietic stem cells. Leukemia 19, 1118–1127. doi:10.1038/sj.leu.2403796

Spine (Phila Pa 1976). 2011 Jun;36(13):997-1005. doi: 10.1097/BRS.0b013e3181eab764.

Differentiation of human bone marrow mesenchymal stem cells into neuron-like cells in vitro.

Zeng R[1], Wang LW, Hu ZB, Guo WT, Wei JS, Lin H, Sun X, Chen LX, Yang LJ.

Author information

Abstract

STUDY DESIGN:

Responses of human mesenchymal stem cells from bone marrow (hBMSCs) were analyzed under chemical conditions, and then characterization of ion channels was evaluated by whole-cell patch clamp.

OBJECTIVE:

To explore the possibility of differentiation of human bone marrow-derived mesenchymal stem cells into neuron-like cells in vitro under different conditions.

SUMMARY OF BACKGROUND DATA:

The generation of mesenchymal stem cells into neuron-like cells has been studied. However, few of these studies characterized functional properties of the differentiated hBMSCs.

METHODS:

hBMSCs (Passage 2) were expanded and cultured in vitro. After Passage 5 was subcultured, the cells were induced by cytokines and antioxidants. Morphologic observation, immunocytochemistry, Western blot analysis, and patch-clamp techniques were performed to evaluate properties of treated and control groups.

RESULTS:

The differentiated neuronal cells from hBMSCs not only expressed neuron phenotype and membrane channel protein including Nav1.6, Kv1.2, Kv1.3, and Cav1.2 but also exhibited functional ion currents. Both hBMSCs and differentiated cells expressed Nav1.6, Kv1.2, Kv1.3, and Cav1.2 and voltage-activated potassium currents, including delayed rectifier, noise-like and transient outward currents. However, expression of channel proteins, such as sodium channel Nav1.6 and potassium channels Kv1.2 and Kv1.3, were upregulated. Consistently, their potassium currents were also enhanced in the differentiated cells.

CONCLUSION:

hBMSCs possess of great potential to differentiate into functional neurons, indicating that hBMSCs may be an ideal cell source in managing a variety of clinical diseases such as spinal cord injury.

The secretome of apoptotic human peripheral blood mononuclear cells attenuates secondary damage following spinal cord injury in rats

Thomas Haider [a,b], Romana Höftberger [c], Beate Rüger [d], Michael Mildner [e], Roland Blumer [f], Andreas Mitterbauer [b,g], Tanja Buchacher [b,g], Camillo Sherif [h], Patrick Altmann [b,g], Heinz Redl [i], Christian Gabriel [i,j], Mariann Gyöngyösi [k], Michael B. Fischer [d,l], Gert Lubec [m], Hendrik Jan Ankersmit [b,g,*]

ABSTRACT

After spinal cord injury (SCI), secondary damage caused by oxidative stress, inflammation, and ischemia leads to neurological deterioration. In recent years, therapeutic approaches to trauma have focused on modulating this secondary cascade. There is increasing evidence that the success of cell-based SCI therapy is due mainly to secreted factors rather than to cell implantation per se. This study investigated peripheral blood mononuclear cells as a source of factors for secretome- (MNC-secretome-) based therapy. Specifically, we investigated whether MNC-secretome had therapeutic effects in a rat SCI contusion model and its possible underlying mechanisms. Rats treated with MNC-secretome showed substantially improved functional recovery, attenuated cavity formation, and reduced acute axonal injury compared to control animals. Histological evaluation revealed higher vascular density in the spinal cords of treated animals. Immunohistochemistry showed that MNC-secretome treatment increased the recruitment of $CD68^+$ cells with concomitant reduction of oxidative stress as reflected by lower expression of inducible nitric oxide synthase. Notably, MNC-secretome showed angiogenic properties ex vivo in aortic rings and spinal cord tissue, and experiments showed that the angiogenic potential of MNC-secretome may be regulated by CXCL-1 upregulation in vivo. Moreover, systemic application of MNC-secretome activated the ERK1/2 pathway in the spinal cord. Taken together, these results indicate that factors in MNC-secretome can mitigate the pathophysiological processes of secondary damage after SCI and improve functional outcomes in rats.

Mechanisms of action

1. Cellular mechanism (Plasticity)

2. Paracrine effect (Plasma is important adjuvant): VEGF, HGF, IGF, NOs

3. Secretomes (released after cell death)

Spinal Cord Injury

(Park et al. 2005)

BMMN MSC–HSC autolog + GM-CSF	Open label study in acute SCI ASIA A; follow-up 6–18 months	Acute SCI ($N = 6$) IP: 1.9×10^8 BMMN cells SC: GM-CSF for 5 days for 5 months	6/6 Improved >5 points in motor and sensory ASIA scores 4/6 Improved from ASIA A to C 1/6 Improved from ASIA A to B No serious adverse events

(Sykova et al. 2006)

BMMN MSC–HSC autolog	Open label study in acute (10–30 days post injury) SCI; follow-up 1 year	IA: 4 patients received $104.0 \pm 55.3 \times 10^8$ BMMN cells ($CD34^+$: $89.7 \pm 70.7 \times 10^6$)	4/4 Improved in motor and sensory scores; 1/4 Changed from ASIA B to D 1/4 From ASIA A to B

Park DH, Eve DJ, Chung YG, Sanberg PR (2010) Regenerative medicine for neurological disorders. ScientificWorldJ 10:470–489

Sykova E, Homola A, Mazanec R et al (2006) Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. Cell Transplant 15:675–687

Spinal Cord Injury

(Yoon et al. 2007)

BMMN MSC–HSC autolog + GM-CSF	Open label study in acute (<2 weeks), sub acute (2–8 weeks) and chronic (>8 weeks) ASIA A SCI with matched controls; follow-up <10.4 months	Acute SCI ($N = 17$)	5/17 Changed from ASIA A to B/C
		IP: 1.9×10^8 cells + SC: 5 days monthly GM-CSF for 5 months	1/17 Reported neuropathic pain (1)
		Sub acute SCI ($N = 6$)	2/6 Changed from ASIA A to B/C
		IP: 1.9×10^8 cells + SC: 5 days monthly GM-CSF for 5 months	2/6 Reported neuropathic pain
		Chronic SCI ($N = 12$)	0/12 Changed from ASIA A to B/C
		IP: 1.9×10^8 cells + SC: 5 days monthly GM-CSF for 5 months	4/12 Reported neuropathic pain (4)
		Controls ($N = 13$)	1/13 Changed from ASIA A to B/C 1/13 Reported neuropathic pain

(Geffner et al. 2008)

BMMN MSC–HSC autolog	Open label study in acute (5–210 days) ($N = 4$) and chronic (5–21 years) ($N = 4$) SCI; follow-up: 24 months.	Acute SCI ($N = 4$)	3/4 Changed from ASIA A to C
		IP + IT + IV: 1.2×10^6 $CD34^+$/kg body weight in 80 ml suspension	
		Chronic SCI ($N = 4$)	1/4 Changed from ASIA B to C
		IP + IT + IV: 1.2×10^6 $CD34^+$/kg body weight in 80 ml suspension	1/4 Changed from ASIA C to D 1/4 Changed from ASIA A to C

Yoon SH, Shim YS, Park YH et al (2007) Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: phase I/II clinical trial. Stem Cells 25:2066–2073.

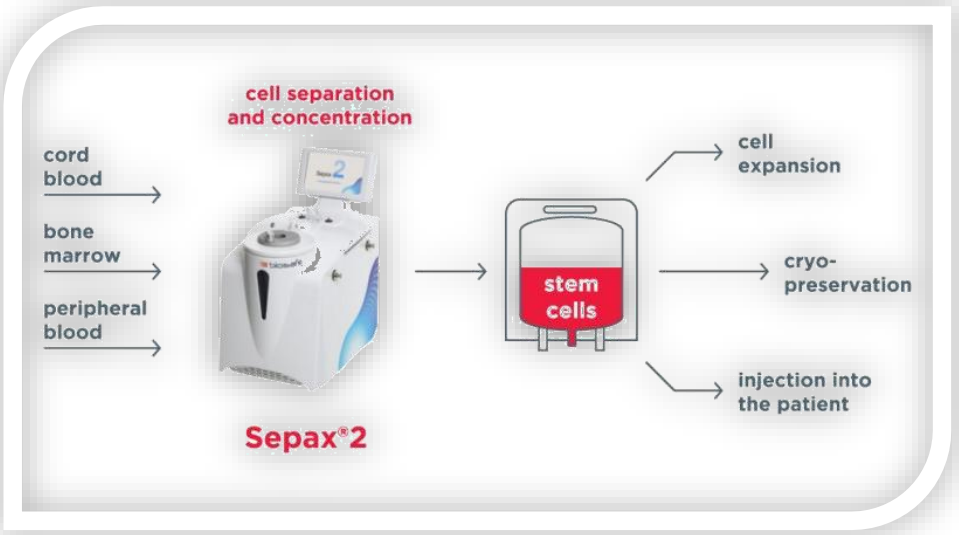
Geffner LF, Santacruz P, Izurieta M et al (2008) Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. Cell Transplant 17:1277–1293

Point-of-Care

Autologous bone marrow-derived stem cells transplant with on-site cell concentration for non-hematopoietic diseases.

200 cc of BM

"The bone marrow collection in the acute phase of the brain injury did not cause hemodynamic disturbances and was well tolerated" (Cox et al. 2011) (TBI in children in the first 8 hours)

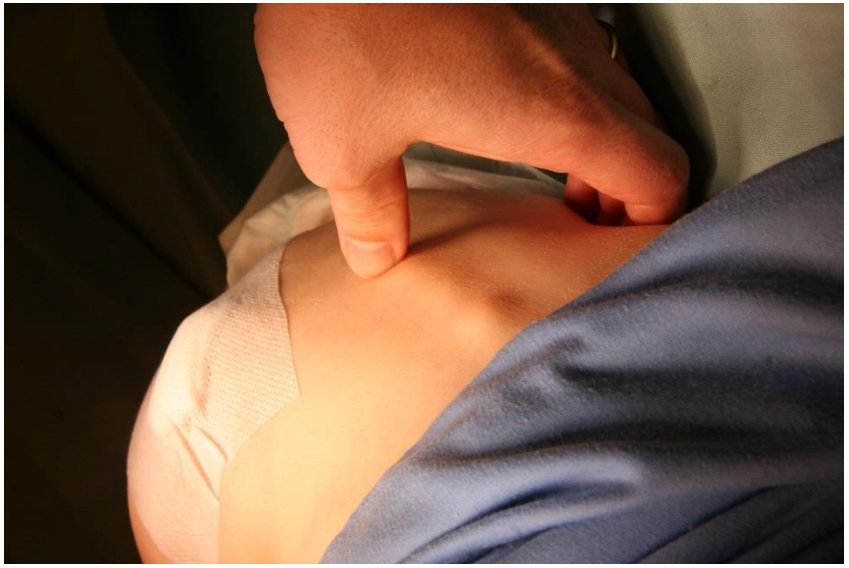


Minimal cell manipulation and effective separation (Aktas et. al. 2008)

Aktas, M., Radke, T.F., Strauer, B.E., Wernet, P., Kogler, G., 2008. Separation of adult bone marrow mononuclear cells using the automated closed separation system Sepax. Cytotherapy 10, 203–211. doi:10.1080/14653240701851324.

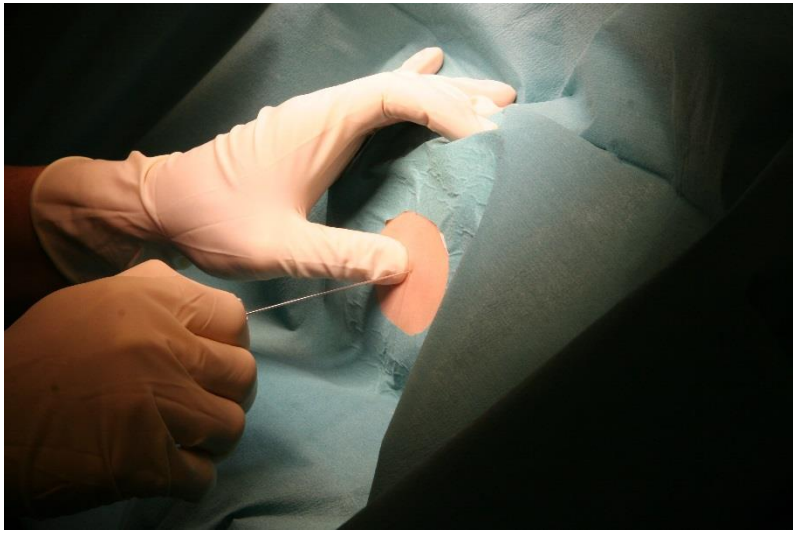
Cox CS, Baumgartner JE Jr, Harting MT et al (2011) Autologous bone marrow mononuclear cell therapy for severe traumatic brain injury in children. Neurosurgery 68:588–600

Lumbar Puncture?



The use of LP and interventricular routes allows more efficient delivery of cells to the injured cord compared with the intravenous route. **(Bakshi et al. 2004)**

We found that MSC delivered by LP reached the contused spinal cord tissues and exerted a significant beneficial effect by reducing cyst and injury size. **(Bakshi et al. 2006)**



Bakshi, A., Hunter, C., Swanger, S., Lepore, A., Fischer, I., 2004. Minimally invasive delivery of stem cells for spinal cord injury: advantages of the lumbar puncture technique. J. Neurosurg. Spine 1, 330–337. doi:10.3171/spi.2004.1.3.0330

Bakshi, A., Barshinger, A.L., Swanger, S.A., Madhavani, V., Shumsky, J.S., Neuhuber, B., Fischer, I., 2006. Lumbar puncture delivery of bone marrow stromal cells in spinal cord contusion: a novel method for minimally invasive cell transplantation. J. Neurotrauma 23, 55–65. doi:10.1089/neu.2006.23.55

Clinical study

A case series of 61 patients with SCI treated with intrathecal and intravenous autologous bone marrow-derived mononuclear cells and plasma as a point of care procedure.

Variable	Mean
Age	33,3 years SD 9,3
From diagnosis to treatment	72,5 months SD 50
Complete SCI (ASIA A)	16 cases
Incomplete SCI (ASIA B, C)	44 cases
CD34+ in whole BM	160, 841 cells per millilitre. SD 154
CD34+ in mononuclear concentrate	603, 349 cells per millilitre. SD 523
Vitallity in whole BM	82% SD 11
Vitallity in mononuclear concentrate	76% SD 12

PROGNOSIS OF CURRENT REGULAR TREATMENTS

ASIA A injuries remained mainly complete from admission to discharge and in no case reached functional levels.

Only a third of ASIA B patients showed improvement.

Improvement in ASIA C patients was 76.4%, these and all ASIA D patients were functional on discharge.

The condition a year after the injury remained unchanged in all cases, regardless of the extent of injury. Patients who showed improvement did so early on, mainly during hospitalization.

J Forensic Leg Med. 2008 Jan;15(1):20-3. Epub 2007 Sep 25.
Determining prognosis after spinal cord injury.
Vazquez XM[1], Rodriguez MS, Peñaranda JM, Concheiro L, Barus JI.

Clinical study

Preliminary results in the subgroup of 16 patients with Complete SCI.

According to a directed questionnaire of functionality:

62% improved muscular strength

50% improved spasticity

50% improved sensibility

37% improved the bladder control

93% of them referred a better general health status

NO PATIENT PRESENT MAYOR SIDE EFFECTS

WE PRESENT A COLLECTION OF CASES OF THIS GROUP

CD34+ correlations in whole bone marrow

Variable	Less than 100 CD34+ in whole BM	More than 100 CD34+ in whole BM
Improved muscular strength	25%	75%
Improved spasticity	20%	80%
Improved sensibility	0	100%
Improved bladder control	0	100%
Referred better general status	38%	62%

CD34+ correlations in mononuclear concentrate

Variable	Less than 500 CD34+	More than 500 CD34+
Improved muscular strength	83%	17%
Improved spasticity	66%	34%
Improved sensibility	100%	0
Referred better general status	90%	10%

Vitality correlations in whole BM

Variable	Less than 80%	More than 80%
Improved muscular strength	42%	57%
Improved spasticity	60%	40%
Improved sensibility	0	100%
Referred better general status	36%	64%

Vitality correlations in mononuclear concentrate

Variable	Less than 80%	More than 80%
Improved muscular strength	80%	20%
Improved spasticity	88%	11%
Improved sensibility	100%	0
Referred better general status	30%	70%

Cell analysis

The Number of CD34+ in complete BM sample may help as a predictor of success. As well as vitality.

Conclusions

Clinical safety ✓

Reproducibility ✓

Effectiveness ✓

Conclusions

SCT is the only treatment that shows improvement besides for spontaneous recovery.

MECHANISM

- Stem Cell
- Growth Factors

ACHIEVEMENTS

- 1 or 2 segments improvement specially in cervical injuries, where one segment means much more improvement than one segment in lumbar injuries.

- **Early treatments between 6 and 12 months after injury is possibly superior to treatment in the chronic state.**
- **Younger patients may have better results than older ones.**
- **More than one treatment should be performed in order to achieve higher number of cells**

NO RISK

SCT BRINGS CHANCES AND NO RISK