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BENEFICIAL EFFECTS OF SUBCUTANEOUSLY INJECTED HUMAN UMBILICAL CORD STEM CELLS ON CEREBRAL PALSY AND TRAUMATIC BRAIN INJURY IN CHILDREN AND A POSITED MECHANISM

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ABSTRACT. CLINICAL CASE HISTORY DATA provided by research-oriented physicians doing human umbilical cord stem cell therapy (hUCSC) in Mexico indicates that children with cerebral palsy treated by a subcutaneous injection of ~1.5 million CD34+/CD133 human umbilical cord stem cells near the umbilicus experienced clinically significant improvements in various cognitive and motor skill functions. Conventional wisdom would argue that human umbilical cord stem cells (hUCSC) would tend not to confer substantive benefit in the brain of human patients, much less so hUCSC injected subcutaneous in a body site quite distant from main blood vessels and the brain itself. In this paper tentative evidence is advanced indicating that hUCSC injected subcutaneously near the umbilicus does bring about notable clinical improvement in children with cerebral palsy and traumatic brain injury (TBI). A hypothetical mechanism is posited to explain how hUCSC injected in abdominal fat tissue would facilitate neurologic repair.

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1. BACKGROUND

Stem-cell laden human cord blood has proven of clear benefit in a diverse range of diseases including but not limited to leukemia [1], breast cancer [2], aplastic anemia [3], Fanconi's anemia [4], and various immune disorders. CD34+ progenitor cells immunomagnetically separated from cord blood and introduced into animal models of various neurological conditions such as ALS, Alzheimer's, Huntington's, and stroke has demonstrated efficacy in terms of effecting notable improvement [5-9]. Laboratory research has shown that cord blood progenitor cells can be differentiated into osteoblasts, chondroblasts, adipocytes, and hematopoietic and neural cells including astrocytes and neurons [10]. hUCSC implanted into intact adult rat brains revealed that "human Tau-positive cells persisted for up to 3 months and showed migratory activity and a typical neuron-like morphology" [11]. "In vivo differentiation of hUCSC along mesodermal and endodermal pathways was demonstrated in animal models" [11]. In addition, human umbilical cord stem cells injected into the tail vein of rodents were subsequently found in the brains of the animals, thus providing proof these cells reach and penetrate the blood brain barrier [12].

In Mexico, research physician Fernando Ramirez carried out a pilot study in which he treated 8 children with cerebral palsy with 1.5-2 million CD34+/CD133 human umbilical cord progenitor (stem) cells as part of a six month pilot study (2004). No bone marrow ablation or use of immunosuppressant drugs was involved. All the children showed clinically significant gains in mobility and cognitive function. Six children (75%) were reported to have improved in multiple areas of motor function by the end of the study [13]. One cortically blind, aphasic child began tracking objects visually and speaking using brief phrases.

In addition to these children, Ramirez and his associates have treated pediatric and adult patients suffering from a variety of physical challenges including macular degeneration, diabetic neuropathy, primary and secondary progressive multiple sclerosis, traumatic brain injury, and emphysema. The Steenblock Research Institute (with which the author is affiliated) provided technical support to this research-oriented clinical application of hUCSC in the form of data collection and analysis, patient interviews and other forms of follow-up, as well as development of protocols to enhance stem cell delivery and post-treatment activity (USPTO Patent Pending). The results to-date has in most instances been very encouraging.

What is especially intriguing is that several cortically blind, aphasic, severely locomotor impaired children who received hUCSC in Mexico experienced amelioration of these during the first 120 days following treatment. Recently (May 2005) a female child with advanced genetic metachromatic leukodystrophy also experienced a resolution of cortical blindness and motor function gains in the first 60 days following a hUCSC injection. In addition, at least 5 children with mild to moderate seizure activity prior to hUCSCT experienced a significant reduction in this and were able to reduce reliance on anti-seizure medications and even stop them in a few instances.

By far the best responder to the hUCSC being administered by Ramirez et al. has been cerebral palsy and TBI in children. The study cited above involved only eight children with CP, but a total of 13 with CP have been treated to date (June 2005). As indicated, all eight of those in the pilot study have shown clinically significant improvements in at least 7 areas of function (Caregiver assessment using a specially designed questionnaire one month prior to hUCSCT, then 1 month, 3 months and 6 months following the treatment. Questions were taken from McKeena's report on side effects from cord blood and included changes in heart rate and blood pressure, nausea, back pain, rashes, chills, excessive thirst, rapid breathing, headaches, and others. There was also a section on symptoms and symptom improvements [14]. Eighty-five percent (85%) of the total complement of 13 children treated since March 2003 has experienced moderate or better improvement in their condition (2005 Caregiver survey). Four children and young people with TBIs were treated during 2004 with 75% reported to have experienced clinically significant

improvements in overall cognitive and motor skill function.

This data is far from conclusive, though we at SRI feel it is compelling enough to justify additional, more rigorous study (SRI has, in fact, begun the pre-IND process with the F.D.A. requisite to ultimately gaining approval to conduct a double blind, placebo controlled crossover trial involving use of hUCSC in children with cerebral palsy). These admitted limitations aside, if it is granted that some favorable neurologic influence is being exerted by these treatments, one is left with questions concerning how these cells are bringing this about. If these cells were being given by IV, then a probable mechanism would be obvious: Some of the hUCSC are getting into the brain. The body of studies indicating these cells can be differentiated into neurons in the Petri dish [15-17] and penetrate the blood brain barrier [18-19] readily suggest that this would be the operative mechanism in cases in which hUCSC are infused by IV drip (something done by Ramirez et al for adult patients with neurologic diseases and insults). However, Dr. Ramirez injected ~1.5 million hUCSCs subcutaneously near the umbilicus in all his pediatric patients. What mechanism would most likely account for the neurologic improvements seen following this? Especially those that take place literally within the first day or so following treatment?

2. HYPOTHESIS

In reviewing the responses of children to the subcutaneously injected hUCSC during the first 72 hours or so following the treatment, I noticed that a few telltale side effects were cropping up: Many of the children experienced transient fluctuations in appetite in the first few weeks following the injection, as well as mildly elevated body temperature (for a few days to a week or so), uncharacteristic sleepiness, and in some instances a mild rash and muscular fasciculations in the region adjacent to the injection site. These symptoms and subsequent medical testing did not indicate a graft-versus-host or host-versus-graft reaction was taking place. While growth factors contained in the media in which the stem cells were suffused could account for some of the side effects — most of which were abolished following total wash out of these growth factors — some remained such as altered appetite, sleepiness and other signs that the injection was eliciting a response in the injection site (adipose tissue) that was influencing various neurologic centers in the brain.

This body of evidence and the sequence of events noted readily suggest a hypothetical mechanism: Namely, that hUCSC injected in adipose tissue stimulates adipocytes to synthesize and express TNF-alpha, which in turn disrupts the blood brain barrier, as well as upregulates production of nerve growth factor (NGF). NGF produced by the adipocytes then enters the CNS where it exerts an influence over cell growth, differentiation and function, and in addition affects cells of the immune system as well as inflammatory responses [20]. This adipocyte-generated NGF may also help quell seizures in children prone to them.

Normally, NGF crosses the blood brain barrier poorly if at all, but with permeability increased by TNF-alpha should gain access more readily and swiftly. It is the entry of NGF into the brain that makes explicable the many instances of neurologic improvement being reported as occurring during the first few hours following treatment. For while neurotrophins like NGF generally act slowly, it has been shown that this class of compounds also elicits rapid signaling that modulates and influences a diverse range of cellular functions such as synaptic transmission, membrane excitability and activity-dependent synaptic plasticity. These rapid action effects are mediated primarily through the interaction of Trk receptors with ionotropic receptors and ion channels in the plasma membrane [21].

A review of the literature produced numerous laboratory and animal studies that lend credence to this hypothetical mechanism:

Adipocytes exposed to proinflammatory stimuli such as lipopolysaccaride, or foreign antigens such as would be present in introduced hUCSC have been shown to begin synthesizing tumor necrosis

factor-alpha (TNF-alpha) [22]. TNF-alpha, in turn, has been shown to induce white adipocytes to synthesize NGF [23] which belongs to a family of neurotrophins that have been shown to induce the survival and proliferation of neurons. In addition, TNF-alpha has also demonstrated a blood brain barrier disrupting effect [24], and inhibited seizures in mice [25]. Interestingly, the latter effect parallels what we at SRI have seen take place in many children with a history of seizure activity, i.e., diminution and in some cases total cessation of seizures during the first few weeks following hUCSCT.

3. SUMMATION AND CONCLUDING REMARKS

Since March 2003 sixteen children with cerebral palsy and TBI have been treated in Mexico (by Fernando Ramirez et al] using a subcutaneous injection of 1.5 million CD34+/CD133 human umbilical cord stem cells in adipose tissue adjacent to the umbilicus. Eight-five percent of those with CP and 75% of those with TBI experienced clinically significant improvements in cognitive and motor skill function following this. What is intriguing is that many of these children began demonstrating benefit within the first day or so of receiving the injection. For example, many of the children began moving limbs that had previously were immobile, some grabbed objects with hands that previously were unable to uncurl and fasten around any object, and so forth. A few who demonstrated this kind and type of change were infants and toddlers.

Clearly too little time elapsed to attribute these positive changes to hUCSC migration to the brain, engraftment and proliferation.

However, these early onset clinically significant improvements become explicable when viewed as the end result of growth factor and neurotrophin activity. It is my contention (hypothesis) that the hUCSC deposited in adipose tissue causes adipocytes to synthesize blood brain barrier disruptive TNF-alpha and NGF. This would be consistent with published laboratory and animal studies, and with the rapid improvements seen in the treated children.

This hypothesis can be tested by doing quantitative assays of patients prior to hUCSCT and at regular intervals thereafter with respect to TNF-alpha and NGF. If the posited mechanism is at work, then a substantial increase in both TNF-alpha and NGF should be seen following injection of CD34+/CD133 human umbilical cord stem cells.

If this hypothesis is ultimately validated, the next logical step would be to mimic the effect of the human umbilical cord stem cells (hUCSC) by infusing patients with NGF and possibly the drug Mannitol to facilitate increasing the permeability of the blood brain barrier. Clinical responses obtained in these patients would then be compared and contrasted to those seen in age, sex, and disease and disability matched patients who receive hUCSC only. The outcome would help determine if infused NGF alone can bring about the type and kind of neurologic improvement seen in the patients treated by Ramirez et al., or conversely point to benefits induced or facilitated by hUCSC that NGF alone cannot match or rival. In either case, progress in the treatment and amelioration of neurologic challenges such as cerebral palsy in children would be at-hand.

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