ID: 468
TITLE: CREATIVE MUSIC THERAPY WITH PRETERM INFANTS TO IMPROVE BRAIN STRUCTURE, FUNCTION AND DEVELOPMENT: A RANDOMIZED CONTROLLED PILOT TRIAL
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CONTENT:

Preterm infants (PI) demonstrate a high incidence of white and grey matter abnormalities and as a consequence cognitive, motor, language, and behavioral problems. There is emerging evidence that stressful auditory intensive care environment may negatively impact brain maturation. As brain development is linked to early social contact and auditory experience, music has the potential to promote neurobiological processes and neuronal learning. Creative Music Therapy (CMT) provides meaningful interaction and auditory enrichment by infant-directed singing. We aimed to test feasibility in preparation for a multi-center trial and to explore whether CMT improves brain development in PI.

In this prospective, randomized controlled pilot trial 81 PI born before the 32nd week of gestation were randomized to CMT or standard care. CMT was performed 2-3 times a week for at least ten sessions during admission. MRI was performed at term equivalent age on a 3T scanner under natural sleep. Diffusion tensor imaging (DTI) was acquired using a spin echo EPI sequence with 30 diffusion-weighting directions. Group differences in the microstructure of the major white matter tracts were analyzed using tract-based spatial statistics. In a subset of 31-35 PI the effect of CMT on structural brain connectivity and resting-state functional MRI signals was analyzed. All statistical models are false discovery corrected and adjusted for gestational age at birth and time since birth until the MRI.

Clinical feasibility was successful. Recruiting and consenting the parents revealed moderate parental rejection. Structural brain connectivity and white matter microstructure, as reflected by DTI, appears to be only moderately affected by CMT when correcting the model for possible age-related confounders. On the other hand, functional brain activity and connectivity show a possible early beneficial effect of CMT which manifests in (1) lower thalamocortical processing delay, (2) stronger functional networks and (3) higher functional integration in predominantly left prefrontal, supplementary motor and inferior temporal brain regions. The analysis demonstrates a positive dose-dependent effect of CMT. The Structural connectomic analysis revealed one brain region to be affected by CMT, the left posterior cingulate gyrus.

Results suggest potential beneficial effects of CMT on thalamocortical processing, functional networks, and integration in prefrontal, supplementary motor and temporal brain regions. The latter is associated with acoustic processing, language, cognition, empathy, and fine motor coordination, and may demonstrate a promising influence on neurodevelopment that will be examined at two and five years of age and by a multi-center international RCT.

COI: None declared
ID: 594

**TITLE:** TREATMENT WITH INSULIN-LIKE GROWTH FACTOR (IGF) -1/IGF BINDING PROTEIN (BP) -3 IN PRETERM RABBIT PUPS - REGIONAL BRAIN DISTRIBUTION OF IGF-1 AND EFFECTS ON CHOROID PLEXUS ANGIOGENESIS AND EXTRACELLULAR MATRIX STRUCTURE

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**CONTENT:**

The preterm rabbit pup model incorporates essential physiological aspects of human preterm birth. Brain maturity at E 29 in the rabbit corresponds to gestational week 25 in the human. Following establishment of the pharmacokinetic (PK) profile of exogenous recombinant human (rh) IGF-1/IGFBP-3 we investigated the regional distribution of IGF-1 in the brain and its effects on genes involved in angiogenesis and extracellular matrix (ECM) structure in the choroid plexus (CP).

Rabbit pups were delivered by cesarean section at E29, housed in a controlled environment and fed twice daily via gastric tube with bovine colostrum. The PK of 1, 2, 4 and 8 mg/kg sc administered rhIGF-1/IGFBP-3 was evaluated. In a second study, pups were randomized to sc administered rhIGF-1/IGFBP-3, 8 mg/kg or saline, twice daily from birth up to 72 h. CP tissue was sampled and snap frozen at 4, 12, 24, 48 and 72h after first admin. of rhIGF-1/IGFBP-3 or vehicle. Expression of genes involved in angiogenesis and ECM structure was evaluated using RT PCR arrays in CP tissue. Paraffin sections from in vivo perfusion fixed brains were processed for immunohistochemistry (IHC), using monoclonal antibodies against IGF-1 and IGFBP3.

After sc admin. of rhIGF-1/IGFBP-3 complex at doses of 1, 2, 4 and 8 mg/kg, serum levels peaked between 1 and 4 h and returned to baseline levels at 48 h. A mean (SD) serum IGF-1 level of 264 (17) ng/ml was observed at 4h after 8 mg/kg (rhIGF-1/IGFBP-3). Brain IHC demonstrated an increased IGF-1 immunoreactivity at 4h after administration of rhIGF-1/IGFBP-3 (8mg/kg), with a wide distribution in different brain regions including the choroid plexus in comparison to the more restricted endogenous IGF1 expressing cell populations. In pups receiving IGF-1/IGFBP-3 (8 mg/kg), expression of genes in the CP involved in vessel maturation (eg Angiopoetin-1, Thrombospondin-1) and ECM structure (eg Fibronectin, Versican, Collagens) were upregulated (3-10 fold versus time-matched controls) at 24h after first dose of the rhIGF-1/IGFBP-3 complex.

Systemic admin. of rhIGF-1/IGFBP-3 at a dose counteracting the endogenous decrease was associated with an increase presence of IGF-1 protein in several brain regions. Exogenous IGF-1/IGFBP-3 caused an up-regulation of genes related to structural and functional vessel maturation. These findings indicate a possible therapeutic effect of rhIGF-1/IGFBP-3 on vascular fragility and thereby a role in prevention of preterm intraventricular hemorrhage.
Figure. The relatively higher insulin-like growth factor-1 immunoreactivity in the brain at 4 h after administration of rhIGF-1/IGFBP-3 (8 mg/kg, sc) is illustrated in the brainstem (top images) and in the cerebellum, in a treated and vehicle injected preterm rabbit pup.

COI: DL and AH hold stock/stock options in Premalux AB, and have received consulting fees from Shire. NB and GC are employees of Shire and own stock/stock options in Shire.
ID: 742

TITLE: INTRANASAL INSULINE-LIKE GROWTH FACTOR 1 (IGF1) TREATMENT TO COMBAT PRETERM WHITE MATTER INJURY

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CONTENT:

White matter injury (WMI) is a common cause of neurological morbidity in the preterm neonate. Previous studies have established that impaired maturation of oligodendrocytes (OLs), followed by myelination failure, is the main underlying pathophysiological mechanism in preterm WMI. IGF1, an endogenous growth factor vital for normal white matter development, is shown to be downregulated following extreme preterm birth. Here we evaluated endogenous IGF1 changes in a mouse model of preterm WMI. Moreover, we explored the potential of intranasal IGF1 treatment to restore myelination in vivo. Furthermore, we assessed in vitro whether IGF1 could support OLs to overcome their maturational arrest.

WMI was induced in C57Bl/6j mouse pups at postnatal day (P) 5 by combining a hypoxic-ischemic insult with systemic inflammation (1mg/kg LPS i.p.). Endogenous IGF1 levels were measured in blood and brain between P5-8. Mice were treated with intranasal IGF1 at different dosages during 6 consecutive days following WMI induction. At 3 weeks post-WMI, we assessed motor outcome using the cylinder rearing test. Brain sections were analyzed for myelination (MBP), cerebral inflammation (Iba-1, GFAP) and axonal injury (NF200) by immunohistochemistry. To explore the potential of IGF1 to support OLs in overcoming their maturational arrest, WMI was modelled by subjecting primary cultured immature OLs to inflammatory stimuli.

Induction of preterm WMI led to a transient decrease in endogenous IGF1 levels in both plasma and brain compared to sham-operated control pups. Intranasal treatment with 25ug IGF1 for 6 days post-WMI restored myelination at 3 weeks post-WMI to levels in undamaged sham control mouse pups (p<0.001). Motor function of WMI mice was restored by ~80% after IGF1 treatment compared to vehicle treatment (p<0.001). We did not observe any axonal or neuronal damage in our in vivo model of preterm WMI. Intranasal IGF1 treatment dampened astrocyte activity compared to vehicle-treated WMI animals while microglia activation remained unaffected. ELISA for hIGF1 confirmed the cerebral distribution of IGF1 at 30 min after treatment. In our in vitro model we showed that addition of IGF1 to OLs arrested in maturation directly boosted OL differentiation and subsequently increased myelin production.

Induction of WMI in newborn mice is associated with a transient decrease in endogenous IGF1 levels between P5 and P8, comparable to the human preterm neonate. Restoring IGF1 levels using intranasal administration of IGF1 is a potent new strategy to restore myelination in a mouse model of preterm WMI. IGF1 aids in white matter regeneration after preterm WMI by boosting OL differentiation following OL maturation arrest.

COI: None declared
ID: 764

TITLE: NEURODEVELOPMENTAL OUTCOME AT PRESCHOOL AGE AFTER EARLY HIGH-DOSE RECOMBINANT HUMAN ERYTHROPOIETIN IN VERY PRETERM BORN CHILDREN: RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND TRIAL.

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CONTENT:

While erythropoietin (EPO) was shown to be neuroprotective in animal experimental and human clinical studies. The findings of the primary outcome analysis of this randomized controlled trial showed that prophylactic early (within the first 2 days of life) high-dose recombinant human (rh) EPO does not improve neurodevelopment of very preterm infants at 2 years. Since rhEPO reduced brain injury assessed by MRI at term equivalent age in a subgroup of study infants, we aimed to determine whether prophylactic early high-dose rhEPO in preterm infants improves neurodevelopmental outcome at preschool age (secondary outcome).

448 infants born between 26.0 and 31.9 gestational weeks were enrolled in this randomized, double-blind, placebo-controlled, multi-center trial in Switzerland in 2005-2012. Participants were randomly assigned to receive either rhEPO (3000 IU/kg) or placebo (NaCl 0.9%) intravenously within 3 hours, at 12-18 hours and 36-42 hours after birth. Outcomes at age 5 years (secondary outcomes) were the intellectual development assessed by the Mental Processing Composite [MPC, norm (SD) 100 (15)] of the Kaufman Assessment Battery for Children and survival without severe neurodevelopmental impairment (NDI, MPC<70, severe cerebral palsy, severe auditory or visual impairment).

Among 448 randomized infants [mean (SD) gestational age 29.0 (1.7) weeks and birth weight 1210 (345) gram; 185 (59%) female], 228 were allocated to rhEPO and 220 to placebo. Outcome data were available for 345 (77%) children at a mean (SD) age of 5.8 (0.4) years. We observed no difference in the mean (SD) MPC between the rhEPO group [96.0 (12.6)] and the placebo group [97.3 (13.0); mean difference (95%-CI) -1.4 (-4.1;1.4), p = 0.338] and in the rate of survival without severe NDI [170/179 in the rhEPO and 157/166 in the placebo group (both 95%), odds ratio (95%-CI) 1.1 (0.4;3.1), p = 1]. Results were similar after adjustment for sex, study center, and socioeconomic status.

Prophylactic early high-dose rhEPO administered immediately after birth and subsequently over the first two days of life is not associated with neurodevelopment in this cohort of very preterm infants at 5 years. An ongoing follow-up study will test whether the previously observed reduced rate of white matter injuries after rhEPO exposition will be reflected in better executive function abilities at age 7-12 years.

COI: None declared.