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Concise report

Bone-marrow derived mesenchymal stromal cells infusion in therapy refractory juvenile idiopathic arthritis patients

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Abstract

Objectives. To compare the total number of adverse events (AEs) before and after mesenchymal stromal cell (MSC) infusion in refractory JIA and to evaluate its effectiveness.

Methods. Single-centre Proof of Mechanism Phase Ib, open label intervention study in JIA patients previously failing all biologicals registered for their diagnosis. Six patients received 2 million/kg intravenous infusions of allogeneic bone-marrow derived MSC. In case of ACR-Ped30-response but subsequent loss of response one and maximal two repeated infusions are allowed.

Results. Six JIA patients with 9.2 years median disease duration, still active arthritis and damage were included. All had failed methotrexate, corticosteroids and median five different biologicals. MSC were administered twice in three patients. No acute infusion reactions were observed and a lower post-treatment than pre-treatment incidence in AEs was found. The one systemic onset JIA (sJIA) patient had again an evolving macrophage activation syndrome, 9 weeks after tocilizumab discontinuation and 7 weeks post-MSC infusion. Statistically significant decreases were found 8 weeks after one MSC infusion in VAS well-being (75–56), the JADAS-71 (24.5–11.0) and the cJADAS10 (18.0–10.6).

Conclusion. MSC infusions in six refractory JIA patients were safe, although in sJIA stopping the 'failing' biologic treatment carries a risk of a MAS flare, as the drug might still suppress the systemic features.

Trial registration. Trial register.nl, http://https://www.trialregister.nl, NTR4146.

Key words: Juvenile arthritis, therapeutics, mesenchymal stromal cells, stem cell transplantation

CLINICAL SCIENCE

Rheumatology key messages

- Mesenchymal stromal cell infusions in 6 refractory JIA patients were safe.
- For systemic JIA patients, there are risks from withdrawing current therapy prior to mesenchymal stromal cell.
- Refractory JIA is worth investigating further as an indication for mesenchymal stromal cell treatment.

Introduction

JIA is not a single disorder, but consists of a heterogeneous group of inflammatory childhood diseases with a prevalence of 16–150 per 100 000 children [1]. Certain subtypes can only be found in children, while systemic onset JIA (sJIA) has the immunological signature of an auto-inflammatory rather than a classical auto-immune disease [2]. Although the introduction of biological agents has greatly improved the outcome, only 45–62% of JIA children on a biological reach an ACR-Ped 70% improvement at 12 months [3]. The patients remaining resistant to biological therapies might still suffer from a very severe, debilitating and potentially fatal disease. For such children, autologous haematopoietic stem cell transplantation has been performed since 1997 with a drug-free remission rate of 50–55%, but with considerable morbidity and even mortality [4]. Late relapses led to lower percentages for drug-free long-term outcome [5].

Cellular therapies are evolving and now include mesenchymal stromal cells (MSC). MSC are non-embryonic stromal cells present in bone marrow, fat, umbilical cord and many other tissues. MSC are widely studied for therapeutic purposes, because they are relatively easily

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harvested and expandable. MSC have strong immunosuppressive qualities *in vitro* inhibiting Th1-cells, B-cells, dendritic cells, NK-cells, and activating regulatory T cells [6]. Myelo-ablation may be omitted because MSC do not express MHC-class-II and only little MHC-class-I. Allogeneic MSC are thus valuable off-the-shelf third-party donor cells with only a small chance of *in-vitro* alloimmune reactions and low rates of treatment-related serious adverse events similar to autologous MSC [7]. In 2004, a patient with severe graft-*vs*-host disease received MSC from his mother with a striking clinical response [8]. Since then, over 1000 patients have been described treated with i.v. MSC for various diseases [9]. As far as we know, JIA patients were never before treated with allogeneic bone-marrow derived MSC. We hypothesized refractory JIA patients who failed registered biologics may profit from i.v. MSC and therefore conducted this small Proof of Mechanism phase Ib clinical trial.

Methods

Study population

The study is conducted according to the Declaration of Helsinki, registered at EUDRACT (2012–002067-10) and approved by the Dutch Ministry of Health and The Central Committee on Research Involving Human Subjects (NL40454.000.13). The study is registered in the Dutch National Trial Register (NTR4146). All parents and patients consented to the study.

Subjects eligible for this study needed to meet all of the following: patients (4–18 years of age) diagnosed with JIA according to ILAR-criteria with active arthritis resistant to intra-articular steroids and systemic use of methotrexate previously failing all biologicals registered for their JIA subtype. There were no biologicals yet registered for (extended) oligoarthritis JIA patients; however, the failure of two classes of biologicals was required for these patients. Patients are followed for moderate or worse adverse events via the Pharmachild pharmacovigilance database [10]. Exclusion criteria for participation in this study were refusal to withdraw from biologicals, concurrent infection, febrile illness, malignancy or pregnancy.

Use of co-medication

NSAIDs, paracetamol and tramadol could be used as escape medication during acute pain attacks and stable doses of systemic steroids and synthetic DMARDs were allowed in order to qualify for a next MSC infusion. Biologicals and additional IA steroid injections were reasons to disqualify for a next MSC infusion.

Sample size calculation

Using an 80% one-sided confidence interval, it was estimated by Cocks and Torgerson that a pilot trial should have at least 9% of the sample size of the main planned trial [11]. The number of biological-allocated patients in the randomized phase of the JIA-registration trial was 25 for etanercept, 68 for adalimumab and 60 for abatacept. If MSC were to be studied with an equal randomized controlled trial with six patients in this tolerability pilot study, we fulfilled the above-mentioned requirements.

Investigational product

The MSC used were isolated from bone marrow mononuclear cells obtained from healthy (consenting) donors by plastic adherence and expanded under GMP-conditions in our Cell Therapy Facility using human platelet lysate derived from five pooled platelets donations as source of growth factors as described before [12]. Density separated bone-marrow cells were seeded in two-layer CellStacks in αMEM with 5% platelet lysate and 2 IU/ml Heparin. After 7 days, non-adherent cells were depleted and when 80–100% confluency was reached, the cells were harvested using trypsin. The cells were put into new CellStacks for further expansion till passage 3, harvested and cryopreserved before infusion.

The MSC differentiated towards osteoblasts, adipocytes and chondrocytes. The release criteria (all needed) were >70% expresses the MSC phenotype (CD73+, CD90+ and CD105+); <10% haematopoietic cells (CD45+); <1% T cells (CD45+, CD3+ cells); Sterility testing (no bacteria, fungi or yeast); Mycoplasma tests <10 CFU/mL, and Endotoxins <1 IU/ml

The MSC were thawed, counted, tested for viability and 2 million living cells/kg bodyweight with a maximum of three doses were injected i.v. Repeated i.v. administrations with this dose (up to 2 million/kg) was already used in children for the HOVON-MSC-112 graft-*vs*-host disease study in our hospital. In case of repeated infusion, the MSC from the same donor was used.

Study design

Single centre Proof of Mechanism Phase Ib, open label, non-randomized study during 64 weeks per patient with continuous follow-up for adverse events (AEs).

The study consisted of nine visits (V) at week -12(V0), 0(V1), 4(V2), 8(V3), 12(V4), 16(V5), 26(V6), 39(V7), 52(V8). At V0, patients and physicians recorded the AEs on the current therapeutic regimen in the 12 weeks before the MSC-therapy. At V1, questionnaires, physical examination, venepuncture and a MRI of a clinically active large joint were performed with subsequently the first MSC infusion. At V2–8, questionnaires, physical examination and venepuncture were performed. The MSC infusion could be repeated at V3 and V5 if there is at least ACR Ped 30% improvement at V2 or V4 but weaning of the effect at the following visit. The patients were their own historic controls regarding both safety and efficacy.

Collection of the study data

The visits encompassed complete medical history, medication use, Childhood Health Assessment Questionnaire, the Juvenile Arthritis Multidimensional Assessment Report, a complete physical examination including a physician global assessment. The ACR Ped-30 was only used to decide if another MSC-infusion was needed. A

weighted joint score, a scoring system for JIA in which joints are weighted to reflect their relative importance to children's function [13] and Juvenile Arthritis Disease Activity Scores (JADAS) were calculated.

Endpoints

The primary outcome was the number of AEs per 3 months after MSC infusion compared with 3 months prior to the MSC. Secondary objectives were the 8-week (before 2nd MSC) and end-of-study parameters: ESR (mm/h), CRP (mg/L), active joint count, tender joint count, limited joint count, weighted joint count, VAS well-being, VAS pain, physician global assessment, JADAS-71, cJADAS-10, Childhood Health Assessment Questionnaire, Quality of Life and Juvenile Arthritis Functionality Scale (derived from the Juvenile Arthritis Multidimensional Assessment Report).

Statistical analysis

For the comparative pre- and post-MSC incidences of adverse events, the two-sided χ^2 test (Fisher's exact) for related samples was used. For the comparison of start- and end-of-study results the Wilcoxon signed Ranks test (2-tailed) for related samples was used. The significance level was set at $P < 0.05$. We use SPSS version 21.0.0.0.

Results

Patients

Six therapy-refractory JIA patients (four males) were included (see Table 1). All patients had articular joint damage and/or extra-articular damage at baseline. All had failed methotrexate, corticosteroids (intra-articular and/or systemic) and median 5 (2–7) different biologicals (see Table 1). All patients had stable persistent disease activity at study start and synovitis on a MRI-scan. Three patients referred from other centres also had follow-up visits elsewhere, unfortunately resulting in some missing data. For all patients, complete safety data was obtained.

Treatment during the study

All patients had discontinued their biological therapy at median 9 weeks before MSC administration. MSC were administered at week 0 in all and again in week 8 (or ultimately delayed till week 11) in the three patients qualifying for a repeated infusion (see Table 2). None of these three patients qualified for the third MSC-infusion. Other anti-rheumatic therapy changes than the MSC were made in patients 2, 4, 5 and 6 at week 28, 22, 13 and 9, respectively (see Table 2).

Safety

No acute infusion reactions were observed during any of the nine MSC administrations. Overall we found a non-significant (P =0.60) lower monthly incidence of serious adverse events and a non-significant (P =0.36) lower monthly incidence of moderate-severe AE post-MSC compared with pre-MSC (see Table 2).

In the 3 months pre-MSC, two serious adverse events were recorded in patient 1 with hospitalizations for (drug-induced) haematemesis and for faecal impaction. She needed to be readmitted for the latter condition twice in the year post-MSC.

Patient 2 was admitted 50 weeks post-MSC for bilateral pneumonia and 20 weeks after her second rituximab infusion while still using 10 mg/day prednisolone.

Patient 6, the only systemic JIA patient with a medical history of a macrophage activation syndrome (MAS) presented to the emergency room with significant headache and afebrile lethargy at week 7. Compared with the routine visit 2 days before, he now had a marked polyarthritic flare and a sharp drop in his white blood cell count (from 3.2 to 1.7×10^9/l), platelet count (from 170 to 89×10^9/l), rising ESR (from 40 to 82 mm/h), normal stable CRP (from 0.4 to 2.8 mg/l), normal ferritin level 41.2 μg/l (41.2 ng/ml), normal stable fibrinogen (from 3.8 to 3.4 g/l) and elevated rising triglycerides (from 1.2 to 2.8 mmol/l). Both clinical and laboratory features suggested an evolving MAS, although being afebrile with a normal ferritin he did not (yet) fulfil the criteria [14]. He was admitted and treated with 3 days i.v. methylprednisolone 1 g/day with a dramatic clinical improvement within 24 h. There was no intercurrent infection found and blood cultures stayed negative. He restarted tocilizumab on the second day of admission and was discharged a day later with normalization of all the aforementioned laboratory values.

Efficacy

For efficacy we analysed the results at 8 weeks after the first MSC all patients received. Statistically significant decreases were found between baseline and the 8-week results in VAS well-being (75–56), the JADAS-71 (24.5–11.0) and the cJADAS10 (18.0–10.6) (see for more details Supplementary Table S1, available at *Rheumatology* online).

At the end of the study, three of six patients had clinically inactive disease with a fourth almost reaching this; however, two of these four also received additional treatments half way (see Supplementary Table S2 and Supplementary Fig. S1A-N, available at *Rheumatology* online, for the analysis of all end-of-study results and the individual graphs).

Discussion

In this study we did not see any acute infusional reactions after allogeneic MSC administration, which is in agreement with the meta-analysis of 13 studies [9]. We found a lower incidence for AEs post-treatment than pre-treatment, even though we ascribed all found AEs to the MSC infusions. Some of the AEs that we encountered post-treatment were, however, due to a chronic pre-existent problem (faecal impaction). The bilateral pneumonia in patient 2 was more likely due to the combination of corticosteroids and repeated rituximab infusions than resulting from the single MSC infusion 50 weeks earlier.

Table 1 Patient characteristics at baseline

	Patient number	1	2	3	4	5	6	Total (% or median)
Patient characteristics								
	Sex (female%)	F	F	M	M	M	M	**33%**
	Age at 1st MSC	12.1	15.9	09.4	14.0	16.8	16.2	**15.0 yrs**
	Disease duration at 1st MSC	4.4	9.2	6.7	13.3	9.3	12.7	**9.2 yrs**
	Extended oligo-articular	✓			✓			**33%**
	Poly-articular RF-		✓	✓		✓		**50%**
	Systemic						✓	**17%**
	Antinuclear antibodies +	-	-	-	+	+	-	**33%**
	Uveitis ever	-	-	-	-	-	-	**0%**
	JADI-Articular damage	1	7	18	1	12	0	**4**
	JADI-Extra-articular damage	1	0	1	1	3	7	**1**
Medication history (duration in months)								
Corticosteroids	IA steroids (ever)	✓			✓	✓	✓	
	MP pulse i.v.					3x	7x	
	Oral steroids		70	67	18	15	153	**43**
sDMARDs	MTX	43	16	59	152	81	153	**70**
	Sulfasalazine		6			41		
	Leflunomide					22		
	Ciclosporine						2	
	Thalidomide						34	
Alkylating agents	Cyclophosphamide						9	
Kinase inhibitor	Tofacitinib						3	
Biologicals	Abatacept		5		19	3	7	**4**
	Anakinra					1	5	
	Adalimumab	30	11	1	9	46		**10**
	Certolizumab		4					
	Canakinumab			2			3	
	Etanercept		18	8	100	15	3	**12**
	Golimumab			23				
	Infliximab						3	
	Rituximab						6	
	Tocilizumab	6	3	3	4	2	75	**4**
	aHSCT (CYC & ATG)					✓		
Last time biological prior to MSC (weeks)		5	31	12[a]	5	17	2	**9**
Concurrent medication use								
	MTX (mg/wk)	25			7.5		25	
	Prednisolone (mg/d)		10	13	5	14	2	**8**

The underlined biologicals are the ones last used by that specific patient. [a]This patient used his last tocilizumab as 2-weekly subcutaneous injections. aHSCT: autologous haematopoietic stem cell transplant; ATG: antithymocyte globulines; JADI: Juvenile Arthritis Damage Index; Mo: months; MP: methylprednisolone; MSC: mesenchymal stromal cells; sDMARDs: synthetic DMARDs.

The one sJIA patient in our study with a history of both MAS and JIA flare 4 weeks after discontinuation of tocilizumab, did now suffer from an evolving MAS 9 weeks after the discontinuation of tocilizumab. This patient had neither clinical nor laboratory effect of the MSC, and already suffered from a JIA flare 3 weeks before the evolving MAS, which is also more likely due to an again unsuccessful discontinuation of tocilizumab. We can, however, not discern the role of the single infusion MSC 7 weeks earlier.

In our study four of six patients showed a decrease of clinically active joints 8 weeks after the first MSC administration, with a decrease of CRP and ESR in three of the four patients with an elevated value at the start. In the six study patients, only JADAS-71, cJADAS10 and the VAS well-being decreased significantly in that short period. The median scores of the patient reported outcome measures of VAS pain, Childhood Health Assessment Questionnaire and Quality of Life all improved non-significantly during that same episode. The only other study describing twice 40 million umbilical cord derived intravenously in 10 steroid-using JIA patients also found MSC to be safe and observed an improvement in DAS28, as well as a decrease of ESR and CRP and better functionality and growth [15]. The efficacy results of both that and our studies should, however, be interpreted with caution, as the non-blinded fashion induces bias. Furthermore, in a meta-analysis of randomized JIA trials, the placebo rate response found was already 35% on physician global

Table 2 Primary outcome: adverse events and the concomitant treatments per patient

Episodes (months)	−3–0	0–3	3–6	6–9	9–12	Monthly Incidence Pre-MSC	Monthly Incidence Post-MSC	Incidence /person/month Pre-MSC	Incidence /person/month Post-MSC	*P*-value
Serious Adverse Events										
Pt 1	*Hematemesis faecal impaction*	Fecal impaction	-	Fecal impaction	-	0.67	0.17	0.111	0.055	0.60
Pt 2		-	-	-	Bilateral pneumonia	0	0.08			
Pt 3	-	-	-	-	-	0	0			
Pt 4	-	-	-	-	-	0	0			
Pt 5	-	-	-	-	-	0	0			
Pt 6	-	-	Evolving MAS	-	-	0	0.08			
Total	*2*	*1*	1	1	1	**0.67**	**0.33**			
Adverse Events										
Pt 1	-	-	-	-	-	0	0	0.055	0.014	0.36
Pt 2	-	-	-	-	-	0	0			
Pt 3	*Flu-like illness*	-	URTI		-	0.33	0.08			
Pt 4	-	-	-		-	0	0			
Pt 5	-	-	-	-	-	0	0			
Pt 6	-	-	-	-	-	0	0			
Total	*1*	0	1	0	0	**0.33**	**0.08**			
Anti-rheumatic therapy										
Pt 1	(Wk -5 Toclilizumab) MTX 25mg/wk sc	Wk 0 & 11: MSC MTX 25mg/wk po	MTX 25mg/wk po	MTX 25mg/wk po	MTX 25mg/wk po					
Pt 2	*Pred. 10 mg/d*	Wk 0: MSC Pred. 10 mg/d	Pred. 10 mg/d	Wk 28 & 30 Rituximab Pred. 10 mg/d	Pred. 10 mg/d					
Pt 3	(Wk -10 Tocilizumab[a]) Pred. 12.5 mg/d	Wk 0: MSC Pred. 12.5 mg/d	Pred. 12.5 mg/d	Pred. 12.5 mg/d	Pred. 12.5 mg/d					
Pt 4	***(Wk -5 Abatacept)*** *Pred. 5mg/d* *MTX 7.5 mg/wk po*	Wk 0 & 10: MSC Pred. 5mg/d MTX 7.5 mg/wk	Wk 23: Sirolimus 2mg/d Pred. 7.5mg/d Wk 22: MMF 1500mg/d	Wk 38: IA steroids (4) Wk 38: Etanercept Pred. 10mg/d MMF 1500mg/d	Etanercept 50mg/10d Pred. **7.5**mg/d MMF 1500mg/d					
Pt 5	*Pred. 14 mg/d*	Wk 0 & 8: MSC Pred. 14 mg/d	Wk 13: IA steroids[b] Wk 16: Adalimumab Pred. 10 mg/d Wk 13: MTX 15mg/wk sc	Wk 32: MMF 1000mg/d Adalimumab 40mg/wk Pred. 5 mg/d MTX 20 mg/wk sc	MMF **1500**mg/d Adalimumab 40mg/wk Pred. **2.5** mg/d MTX 20 mg/wk sc					
Pt 6	***(Wk-2 Tocilizumab)*** *Pred 2 mg/d* *MTX 25 mg/wk po*	Wk 0: MSC Wk 9: MP 3x pulse Wk 9: Tocilizumab /e.o.w. Wk 9: Pred. 60->30 mg/d *MTX 25 mg/wk po*	Tocilizumab / e.o.w. Pred. **30->10** mg/d MTX 25mg/wk po	Tocilizumab / e.o.w. Pred. **10->5** mg/d MTX 25mg/wk po	Wk 39: MMF 1440mg/d Tocilizumab / e.o.w. Pred. 2 mg/d MTX 25mg/wk po					

Serious and moderate-severe adverse events are shown for 3-month periods before and 12 months after the first MSC-infusion. Also, the anti-rheumatic medication given during the study is shown per patient; therapy changes are in bold. The biologics used prior to week 0 are between brackets with their last administrations displayed. Incidences are displayed per patient/month. The two-sided χ^2 test (Fisher's exact) for related samples was used for statistical analysis of the incidences of events per person per month comparing the Pre-MSC with the Post-MSC episode. [a]subcutaneous. [b]5 joints injected. e.o.w: every other week; MP: methylprednisolone; MSC: mesenchymal stromal cells; po: per os; Pred: prednisolone; URTI: upper respiratory tract infection; Wk: week.

assessment improvement [16]. The additional therapy changes in our study beyond week 9 in two out of four responders make it impossible to interpret the exact reason for their improvement at 1 year. In conclusion, from the findings in our study we believe that MSC are safe in JIA patients, but one should be aware of (evolving) MAS in sJIA patients and therefore consider adding MSC to the failing biologic treatment, as it might still unknowingly suppress the systemic features.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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