Clinical Immunology 179 (2017) 47–53

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Phase I trial of low-dose interleukin 2 therapy in patients with Wiskott-Aldrich syndrome

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ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 21 December 2016
accepted with revision 10 February 2017
Available online 14 February 2017

Keywords:
Low dose IL-2
Wiskott-Aldrich syndrome
X-linked thrombocytopenia

ABSTRACT

Background: Low dose IL-2 can restore the function of T and NK cells from Wiskott-Aldrich (WAS) patients. However, the safety of in vivo IL-2 in WAS is unknown.
Objectives: A phase-I study to assess safety of low dose IL-2 in WAS.
Methods: Patients received 5 daily subcutaneous IL-2 injections, every 2 months, for three courses. A "3 + 3" dose escalation method was used.
Results: 6 patients received the 0.5 million units/m^2/day dose without serious adverse events. However, 2 of 3 patients receiving the 1 million units/m^2/day dose developed thrombocytopenia requiring platelet transfusions. A statistically significant platelet increase occurred in patients receiving the 0.5 million units/m^2/day dose. A trend toward higher T, B and NK cell numbers and higher T regulatory cell percentages was observed.
Conclusion: We have identified a safe IL-2 dose for WAS patients. Additional trials are indicated to study the efficacy of this immunostimulant as a therapy for WAS.

1. Introduction

Wiskott-Aldrich syndrome (WAS) is a severe X-linked immunodeficiency characterized by susceptibility to recurrent bacterial and herpes virus infections, eczema, thrombocytopenia, autoimmunity, and malignancies [1]. It is caused by mutations in the gene encoding the WAS protein (WASp), which facilitates filamentous actin (F-actin) branching and is required for NK cell immunological synapse formation and NK cell cytotoxicity. Some patients have a milder phenotype of X-linked thrombocytopenia (XLT) and mainly suffer from bleeding complications. However, even patients with XLT are at risk for increased severe infections, autoimmunity and malignancies with an event-free survival period of nine years [2]. Hematopoietic stem cell transplantation (HSCT) offers a potential cure for patients. However, the use of HSCT for patients with a milder XLT phenotype is controversial, especially if there is no HLA matched donor (only 25% of WAS patients will have a matched sibling donor), given the high risk of morbidity and mortality from the procedure itself [3]. The use of intravenous immunoglobulin therapy and antimicrobial prophylaxis can reduce the risk of bacterial infections, but its effectiveness is only perceived as partial [4]. Furthermore, there are currently no therapies to reduce the risk of herpes virus infections and malignancies that are often attributed to reduced NK cell function. As a result, severe herpes infection and hematological malignancies remain causes of significant morbidity and mortality in WAS patients [1, 5–7].

Interleukin 2 (IL-2) is a T cell stimulatory cytokine that is also able to potently activate NK cells. In 1993 Azuma et al. first reported the use of extremely low dose (10 units/kg) recombinant interleukin 2 (IL-2) therapy in a patient with WAS suffering from severe eczema and herpes virus infection refractory to acyclovir therapy. Following IL-2 administration, the patient had both improvement of his eczema and herpesvirus infection [8]. This patient was shown to have improved T cell numbers and normalization of low T cell proliferation in response to

Abbreviations: WAS, Wiskott-Aldrich syndrome; XLT, X-linked thrombocytopenia; IL-2, interleukin 2; Treg, T regulatory cell.

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http://dx.doi.org/10.1016/j.clim.2017.02.001
1521-6616/

Table 1
Criteria for inclusion and exclusion.

Inclusion criteria
1. Age: subjects age > 24 months
2. Weight: subjects > 12.5 kg
3. Disease status: WAS classified as grades 1–4
4. Informed consent: written informed consent of the subject (if an adult) or parental permission, and assent of the child subject provided justification is made for the inclusion of children in the study
Exclusion criteria
1. Prior or planned hematopoietic transplant
2. WAS classified as currently grade 5 (autoimmune disease or malignancy)
3. Known previous reaction to IL-2
4. Subjects taking nephrotoxic, cytotoxic, cardiotoxic, or hepatotoxic medications (including medications for hypertension)
5. Subjects currently taking systemic corticosteroids (not included here: topical and inhaled corticosteroids)
6. Subjects taking Interferon alpha
7. Use of any other investigational agent in the last 30 days
8. Subjects with abnormal cardiac, hepatic and CNS function

Table 2
Stopping criteria based on serious adverse events.

Cardiovascular	Sustained ventricular tachycardia
	Cardiac rhythm disturbances not controlled or unresponsive to management
	Chest pain with ECG changes consistent with myocardial infarction (MI)
	Symptomatic atrial fibrillation
	Symptomatic supraventricular tachycardia
	Symptomatic bradycardia
	Hypotension with pressor requirement
	Cardiac tamponade
CNS	Coma or toxic psychosis
	Repetitive or difficult to control seizures
	Encephalopathy
GI	Bowel ischemia/perforation
	GI bleeding requiring surgery
	Hepatic failure
Respiratory	Compromise requiring intubation
Renal	Renal failure requiring dialysis
Dermatological	Bullous Dermatitis
General	Sepsis Syndrome

anti-CD3 stimulation [9]. Interestingly, T cells, which are the main source of IL-2 are defective in their ability to produce this cytokine in WAS, thus resulting in a potential and relative deficiency of IL-2 in this disease [10].

In light of the relative deficiency of IL-2 production and defects in NK cells, exogenous application of IL-2 to NK cells in vitro had been demonstrated to restore defective NK cell function [11]. In our earlier work, we elucidated that IL-2 stimulation activates the WASp homolog WAVE2 which in turn can facilitate F-actin organization and restore NK cell function [12]. This is especially relevant in NK cells which do not endogenously produce IL-2 like T cells, and utilize WASp as their main actin branching enzyme [12,13]. More recently, in vivo IL-2 administration to WASp-deficient mice was demonstrated to restore NK cell functions [14]. Thus IL-2 treatment could theoretically provide much needed adjunctive treatment for prevention of malignancies and severe herpes infections through recovery of NK cell function.

High dose IL-2 therapy (9 million U/m^2/day) has been used for the treatment of malignant melanoma, but this treatment can be associated with significant drug related toxicities (sometimes resulting in death) including leukopenia, granulocytopenia, thrombocytopenia, anemia, nausea, vomiting, severe infection, renal failure and myocardial infarction [15]. In contrast, low dose IL-2 studied in HIV, type 1 diabetes, alopecia areata, and hepatitis C virus induced vasculitis (0.33–3 million IU/day) was associated with only minor side effects which included injection site reactions and transient influenza-like symptoms (fever, headache, chills, nausea) [16–19].

The purpose of this phase I clinical trial was to study the safety and tolerability of low dose IL-2 in WAS patients. Secondary endpoints including, T, B, NK, and T regulatory cell (Treg) enumeration were also obtained and are reported here as well.

2. Methods

2.1. Study design

This was a single center open label study of WAS patients who received 5 once daily IL-2 subcutaneous injections every 2 months for a total of three cycles during a 6 month period. A standard 3 + 3 dose escalation method was used for this trial design [20]. The first 3 subjects were to receive 0.5 million U/m^2/day, the next 3 subjects were to

Table 3
Patient characteristics.

Patient	Age	WAS grade	WAS gene mutation	Baseline platelet count	Infection history	Baseline medications	Other
Patient 1	13	1	c.134 C>T (p.T45M)	21,000	Otitis media Otitis externa Warts	None	No eczema
Patient 2	4	3	c.390 G>A (p.G119E)	28,000	Pneumonia Otitis media	Trimethoprim – sulfamethoxazole prophylaxis	Eczema
Patient 3	3	2	c.390 G>A (p.G119E)	30,000	Otitis media	Trimethoprim – sulfamethoxazole prophylaxis	Eczema
Patient 4	14	3	c.713 T>C (p.I238T)	152,000	Otitis media Pneumonia Molluscum Warts	None	Alopecia Eczema
Patient 5	7	3	c.116T>C (p.L39P)	24,000	Otitis media Sinus infections Pneumonia	Subcutaneous Ig Trimethoprim - sulfamethoxazole	Eczema
Patient 6	4	3	c.116T>C(p.L39P)	78,000	Sinus infections Salmonella sepsis	Subcutaneous Ig	Eczema
Patient 7	2	4	c.379G>A (a)	82, 000	Otitis media Sinus infection Mastoiditis Lymphadenitis	IVIG	Eczema
Patient 8	11	2	c.167 C>T (p.A56V)	96,000	Perianal warts	None	Eczema
Patient 9	14	2	c.167 C>T (p.A56V)	53,000	Otitis media	None	No eczema Nosebleeds

[a] Data not available.