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Topical tacrolimus—a novel treatment alternative for cutaneous chronic graft-versus-host disease

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Abstract Graft-versus-host disease (GVHD) may be resistant to accepted treatments. Therefore, the aim of the present preliminary study was to evaluate the efficacy of topical treatment with tacrolimus, an immunosuppressive agent, for cutaneous GVHD. Ten patients with chronic steroid-dependent cutaneous GVHD were treated with 0.03–0.1% tacrolimus ointment, twice to three times a day. The dermal manifestations of GVHD were monitored, and a score was given to the cutaneous response by both the physician and patient. Seven patients demonstrated a response to the tacrolimus ointment. Three out of the ten patients were scored as showing a "good" or "complete" response in the objective examiner's view or subjective patient's view. Another four patients showed "moderate" or "mild" response; only three patients showed "no response". Topical tacrolimus is suggested as an alternative treatment for cutaneous chronic steroid-dependent GVHD. This conclusion concurs with a previous study on this medication.

Keywords GVHD · Skin · Tacrolimus · Topical

Introduction

Graft-versus-host disease (GVHD) is a well-known complication of hematopoietic stem cell transplantation (HSCT) and small bowel transplantation. GVHD occurs when transplanted donor T lymphocytes react to foreign host cells and cause damage to a wide variety of host tissues. In addition to extensive tissue injuries, inflammatory cytokines, including interleukin-2, tumor necrosis factor-α, granulocyte-macrophage colony-stimulating factor, and interferon-γ, are released. The pre-transplant conditioning regimen enhances the presentation of recipient major histocompatibility complex antigens. Recognition of the foreign host antigens by donor T cells, and activation, stimulation and proliferation of T cells, is crucial in the post-transplant phase GVHD [1]. Extensive, progressive-onset type GVHD is a prognostic factor for poor outcome [2]; therefore, the importance of treatment is clear. When the damage of GVHD is multi-organic, the treatment is mainly systemic with cyclosporine, steroids, and other immunosuppressive agents [1]. Second-line treatments include thalidomide, azathioprine, tacrolimus, and mycophenolate mofetil [3]. Topical treatment is adjunctive and consists of steroid creams [4]. However, improvement of the cutaneous GVHD is not always achieved with the above-mentioned systemic and/or topical treatments. When the skin is the sole organ involved in GVHD, topical treatment can rapidly improve the condition, allowing time for the systemic therapy to work. When limited skin involvement is the only GVHD manifestation, topical treatment alone may be appropriate and, thus, the severe side effects of various systemic treatment options can be avoided. Another treatment alternative is phototherapy [5, 6], which can be regarded as a topical treatment when ultra-violet B is used [7].

Tacrolimus ointment offers a new treatment alternative for cutaneous GVHD. A major advantage over topical corticosteroid therapy is that skin atrophy has not been observed [8]. The topical application of tacrolimus

ointment avoids systemic effects, with no apparent increased risk of cutaneous or systemic infections [9, 10, 11]. Clinical studies of atopic dermatitis, clinically similar to cutaneous GVHD, have shown that tacrolimus ointment was effective and safe [10, 11, 12, 13], with no reduction in cell-mediated immunity [10]. In light of these facts, our aim was to evaluate the efficacy of tacrolimus as a topical treatment for chronic cutaneous GVHD.

Patients and methods

Study population

The group in our pilot study consisted of ten patients following HSCT with steroid-dependent cutaneous chronic GVHD. All patients signed an approved consent form. Patients were eligible for inclusion if they had developed GVHD that was unresponsive to conventional therapy. The duration of steroid and/or cyclosporine administration was usually 3 months before the second line of treatment was introduced. GVHD diagnosis was based on clinical criteria, and, in some patients, was supported by biopsy. Other etiologies of skin eruptions were clinically excluded. All patients received GVHD prophylaxis consisting of single-drug, low-dose, short-term cyclosporine at 3 mg/kg i.v. daily in two divided doses, starting on day –4. Once the patients were mobile, cyclosporine was administered orally. Cyclosporine dosage was tapered during the second or third month post-transplantation, according to chimeric status and evidence of GVHD. Steroid-dependent cutaneous GVHD has been defined as the lack of complete response to conventional first-line (systemic prednisone and cyclosporine, with or without topical hydrocortisone aquasum 2%) treatments. Patients not responding to other accepted treatments for cutaneous GVHD, namely methotrexate, azathioprine, thalidomide, cyclosporine, fludarabine, and phototherapy with ultra-violet type B, were also included in the study. In all these patients the cumulative effects of first-line and second-line treatments was limited and, therefore, they were appropriate candidates for topical treatment with tacrolimus ointment. Systemic immunosuppression was unchanged during topical tacrolimus treatment. No new immunomodulating agents were introduced during the follow-up period.

Tacrolimus ointment protocol

Patients were instructed to spread the 0.03% tacrolimus ointment (Protopic, Fujisawa Healthcare) on the affected skin site, twice to three times a day. After an initial response to the 0.03% ointment was noted with the absence of side effects, the dose was increased to 0.1%.

A clinical and laboratory follow-up was conducted. As part of the clinical monitoring, dermal status was scored by the same examiner and the patients using a 5-step scale: 0, no response; 1, mild response; 2, moderate response; 3, good response; 4, complete response. The examiner's score was based on the criteria for clinical evaluation of cutaneous GVHD (Table 1). The patient provided a score on a 5-step scale where the level of inconvenience/discomfort was described. The laboratory follow-up consisted of differential blood count and biochemical analysis. Follow-up visits were done every 2–3 weeks.

Results

The study group consisted of ten patients with an average age of 36.7 years, diagnosed with leukemia, lymphoma or myelodysplastic syndrome. Patient characteristics are presented in Table 2, including gender, age, indications for HSCT, type of HSCT, type of GVHD, grade/class of GVHD [1], presence of histological diagnosis, site of GVHD involvement, day and type of GVHD onset, and type of cutaneous GVHD. In most of the patients, GVHD involving additional organs was found, following the diagnosis of oral GVHD. The most frequent target organs, besides the skin, were the oral mucosa and conjunctiva (ten of ten patients each), the liver (seven of ten patients), and gastrointestinal tract (two of ten patients). The maximum prednisone dose that was administered prior to the tacrolimus treatment was 1 mg/kg per day (Table 3). Although this medication was combined with cyclosporine, azathioprine, thalidomide, methotrexate, fludarabine, and phototherapy, only a partial response was achieved.

A subjective improvement was reported by most of the patients (Table 4). One of the patients scored a "complete" response, two patients scored a "good" response, four patients scored a "moderate" response, and three patients scored "no" response. An objective response to the tacrolimus was observed in seven of the ten patients. The levels of response reported by the physician were the same or lower than the score given by the patients. Six patients were scored as a "good" or "moderate" response, one was scored as a "mild" response, and three were scored as a "no" response.

Table 1 Criteria for clinical evaluation of cutaneous GVHD (*IA* involved area)

Grade	Score	Definition
0	No response	No change in the oral GVHD signs
1	Mild response	A limited reduction of the involved surface (25% IA) or early healing of ulcerated lesions or mild decrease in lesion's thickness
2	Moderate response	A reduction of the involved surface (50% IA) or moderate decrease in lesion's thickness or a reduction of the erythema severity
3	Good response	A marked reduction of the involved surface (75% IA) and marked reduction in lesion's thickness and a marked reduction of the erythema severity
4	Complete response	A complete resolution of the oral GVHD

Table 2 Patient characteristics (*NHL* non-Hodgkin's lymphoma, *AML* acute myelogenous leukemia, *CML* chronic myelogenous leukemia, *ALL* acute lymphocytic leukemia, *MDS* myelodysplastic syndrome, *Ac.* acute, *Chr.* chronic, *Exten.* extensive, *Prog.* progressive, *GIT* gastrointestinal tract, *NA* not applicable, *Lich.* lichenoid, *Scler.* scleroderma tous)

Patient no.	Gender	Age (years)	Indication for HSCT	Type of HSCT	GVHD type	GVHD grade/class	Biopsy proven	GVHD involvement	GVHD onset type	GVHD onset time	Skin GVHD
1	M	34	AML	Matched-sibling	Chr.	Exten.	–	Skin, oral, conjunctiva, liver	De novo	4 Months	Lich.
2	M	39	AML	Matched-sibling	Chr.	Exten.	+ Liver	Skin, oral, conjunctiva, liver	De novo	4 Months	Scler.
3	F	24	CML	Matched-sibling	Chr.	Exten.	+ Skin	Skin, oral, GIT, conjunctiva, liver	Prog.	3 Months	Lich.
4	F	54	NHL	Matched-sibling	Chr.	Exten.	–	Skin, oral, conjunctiva, liver	De novo	7 Months	Scler.
5	M	23	AML	Matched-sibling	Chr.	Exten.	+ GIT	Skin, oral, GIT, conjunctiva, liver	Prog.	3 Months	Scler.
6	F	38	AML	Matched-sibling	Chr.	Exten.	–	Skin, oral, conjunctiva	De novo	6 Months	Lich.
7	F	18	ALL	Matched-sibling	Chr.	Limited	+ Skin	Skin, oral, GIT, conjunctiva	Prog.	3 Months	Lich.
8	M	40	ALL	Matched-sibling	Chr.	Exten.	–	Skin, oral, conjunctiva, liver	Prog.	3 Months	Lich.
9	F	44	CML	Matched-sibling	Chr.	Exten.	–	Skin, oral, conjunctiva, liver	Prog.	3 Months	Scler.
10	F	53	MDS	Matched-sibling	Chr	Exten.	–	Skin, oral, conjunctiva, liver	Prog.	4 Months	Lich.

The initial response was observed as early as 2 weeks after the commencement of treatment. A durable response required at least 3 weeks of treatment. The median follow-up period was 14 months (Table 5). Three patients died. All these patients suffered from deterioration from chronic GVHD.

Discussion

Tacrolimus (formerly FK506) is a macrolide immunosuppressant produced by the solid fungus *Streptomyces tsukubaensis*. The immunosuppressive effect of tacrolimus is due to inhibition of T-helper lymphocyte activation. Tacrolimus binds to the FK506-binding protein, and this complex inhibits calcineurin and, thus, the dephosphorylation needed for the transcription of cytokine genes is blocked. These cytokines include interleukin (IL)-2, IL-3, IL-4, IL-5, GM-CSF, IFN-γ, and TNF-α [14, 15]. Tacrolimus has inhibitory effects on other cell types, which may also contribute to its activity in skin disorders [16, 17]. Furthermore, it seems that the mechanism of entry of tacrolimus into cells is independent of the glucocorticoid receptor [18, 19], and this could account for the positive therapeutic response observed with tacrolimus ointment in patients with atopic dermatitis who were clinically insensitive to topical corticosteroid therapy [20]. A similar advantage is assumed in patients suffering from cutaneous chronic resistant GVHD.

The initial administration of systemic tacrolimus was in patients following solid-organ transplants. Since its implementation in kidney [21], liver [22], and heart transplantation [23, 24], it has become widely used in HSCT and even in inflammatory bowel diseases [25] and various dermatological diseases [17]. The common adverse reactions with oral and intravenous tacrolimus include insomnia, tremors, headaches, paresthesias, myalgias, pruritus, fatigue, photophobia, and gastrointestinal symptoms [26, 27]. Major adverse events include infection, hypertension, hyperglycemia, hyperkalemia, nephrotoxicity, neurotoxicity, and increased risk of neoplasia [28, 29]. Available data suggest that the bioavailability of topical tacrolimus ointment is below 0.5% for intravenously administered tacrolimus and below 5% for orally administered tacrolimus in patients with atopic dermatitis [9]. The most common adverse event of the topical formulation is a burning sensation of the skin, with no systemic adverse events reported [9, 11]. Though chronic immunosuppression may induce cutaneous malignancies [30], it is known that chronic inflammation may precede cutaneous malignancies [31]. Therefore, reduction of local inflammation with tacrolimus ointment may reduce this risk. Longer follow-up of these patients should be performed so that the risk of this complication can be evaluated.

Table 3 Treatments for GVHD (*MTX* methotrexate, *CSP* cyclosporine, *UVB* ultraviolet type B)

Patient no.	Duration of GVHD (months) (prior to the study)	Systemic (prior to the study)	
		Prednisone—maximum dose (mg/kg per day)	Other
1	16	0.3	MTX, azathioprine, thalidomide
2	45	0.2	Azathioprine
3	7	1	CSP, UVB
4	11	1	Azathioprine
5	7	0.25	Azathioprine, UVB
6	31	0.15	MTX, CSP, azathioprine, thalidomide, fludarabine
7	12	0.75	CSP
8	22	0.4	CSP
9	36	0.3	MTX, CSP, azathioprine, fludarabine
10	10	0.3	CSP, azathioprine

Table 4 Response to treatment with tacrolimus. Scale: 0, no response; 1, mild response; 2, moderate response; 3, good response; 4, complete response

Patient no.	Topical tacrolimus			Subjective response	Objective response
	Site of treatment	Duration of treatment (months)	Side effects		
1	Face	3	–	2	2
2	Face, arms	1	Burning sensation	3	3
3	Legs	1	–	0	0
4	Neck, thighs	1	–	0	0
5	Neck	5	–	2	1
6	Face, arms	2	–	2	2
7	Face	3	–	4	3
8	Face, arms	2	–	2	2
9	Neck, arms	2	–	0	0
10	Face, lips	2	–	3	Lips: 4, face: 3

Table 5 Overall clinical outcome

Patient no.	Overall GVHD outcome	Duration since HSCT (months)	Survival	Cause of death
1	Stable	34	Yes	–
2	Stable	62	Yes	–
3	Aggravated	14	No	Sepsis
4	Improved	60	Yes	–
5	Aggravated	11	No	Pulmonary hemorrhage
6	Improved	49	Yes	–
7	Improved	28	Yes	–
8	Stable	38	Yes	–
9	Aggravated	12	No	Multi-organ failure
10	Improved	35	Yes	–

GVHD develops when graft cells have immunologically different components from those of the host, such that host cells appear foreign to the graft. The host cells antigenically stimulate the graft, and the host is incapable of mounting an effective immunological reaction against the graft [9]. A multi-step process of antigen expression, cytokine production, T-cell activation and tissue injury occurs [32]. The histological manifestations of chronic GVHD range from lichenoid characteristics in the early phase to sclerodermatous characteristics in the late phase. In the early phase there is a lymphocytic infiltrate of the superficial dermis, and a thickened epidermis with acanthosis, hyperkeratosis and keratinocyte necrosis is noted. In the late phase there is epidermal atrophy, progressive destruction of appendageal structures, linearization of the dermo-epidermal junction, and

superficial collagen fibrosis. Keratinocytes are small, flattened and loaded with melanin. In the dermis there is a perivascular infiltrate [4]. Although some authors see the histological evaluation as an obligatory term for diagnosis of gastrointestinal GVHD [33], the diagnosis of cutaneous chronic GVHD can usually be based on the clinical examination of lichenoid or sclerodermatous lesions in a HSCT recipient [4]. In light of these cutaneous alterations, it is clear that tacrolimus, having both immunosuppressing and anti-inflammatory effects, should achieve at least partial control of the signs of GVHD.

Recently, tacrolimus was assessed for use as a topical agent for the treatment of chronic cutaneous GVHD [34]. Choi and Nghiem [34] provided the first evidence of the benefits of tacrolimus for topical treatment. In their case series, 13 of 18 patients benefited from this treatment and their responses occurred rapidly, within hours to days.

The results of the present study confirm the early evidence that tacrolimus is an important new adjunctive treatment for cutaneous GVHD. Ten patients suffering from cutaneous GVHD were included in the present preliminary study. All patients had received previous treatment for their GVHD, with limited improvement. Topical tacrolimus ointment, 0.03–0.1%, twice to three times a day, was prescribed for each of the patients. Concurring with Choi and Nghiem's data, the present results show that eight of the ten patients responded to the tacrolimus ointment to some degree. The clinical impression of the examiner was that the response was as soon as 1 day after initiation of the treatment. The mild side effect of a burning sensation at the site of application was reported in only one case. It seems that lichenoid GVHD responded better than sclerodermatous GVHD; however, for this observation to be evaluated scientifically, a larger sample should be assessed.

The results of this study emphasize the importance of topical tacrolimus for the treatment of steroid-dependent limited GVHD. This treatment approach spares high-dose systemic immunosuppressive agents. However, this pilot study points to the need for further larger randomized double-blind placebo-controlled studies to confirm these findings.

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