How can we help patients with refractory chronic graft versus host disease- single centre experience

P. RZEPECKI*, J. BARZAL, T. SAROSIEK, S. OBORSKA, C. SZCZYLIK

BMT Unit, Department of Oncology, Military Institute of Health Services, 128 Szaserow street, 00-909 Warsaw, Poland, e-mail: piotr_rzepecki@poczta.onet.pl

Received January 29, 2007

Chronic graft-versus-host disease (cGVHD) is a major cause of morbidity and mortality in long-term survivors of allogeneic haematopoietic stem cell transplantation (alloHSCT). Ocular involvement as well as dermal sclerosis, joint contractures and pathological changes in oral cavity are often refractory to treatment. This kind of patients require complex aggressive immunosuppressive therapy. We are still waiting for drugs against cGVHD, characterized by decreased infectious complications, encouraging efficacy and rare and reversible side effects. We describe eight patients who developed extensive chronic graft versus host disease with eye involvement after alloHSCT. All had ocular manifestations, which were refractory to the first and second line of systemic immunosuppressive therapy. All patients responded to the topical cyclosporine therapy, but clinical improvement was seen only since the fifth month of starting treatment. Topical cyclosporine was well tolerated. Other four patients with sclerodermoid type of skin changes, refractory to second line systemic immunosuppressive therapy, were treated with clofazimine. Clofazimine is a drug used to treat leprosy. Because of its anti-inflammatory effects, clofazimine is used also as a second or third line therapy for various skin disorders including: pyoderma gangrenosum, lupus erythematosus, palmoplantar pustulosis and chronic graft versus host disease. All patients,who received clofazimine due to dermal sclerosis, joint contractures and oral manifestations, achieved partial or complete responses and were able to reduce other immunosuppressive drugs. Clofazimine was generally well tolerated.

Key words: Clofazimine- chronic graft-versus-host disease- dermal sclerosis- ocular involvement- topical cyclosporine

Despite improvements in the practice of allogeneic hematopoietic stem cell transplantation (alloHSCT) over the last three decades, chronic graft versus host disease (cGVHD) remains a substantial problem with little change in the incidence, morbidity, and even mortality from this complication. It affects 40% of HLA identical sibling allogeneic recipients, 50% in one antigen HLA mismatched sibling allografts and 70% in matched unrelated transplants [1, 2]. Approximately half of affected people have 3 or more involved organs and treatment requires immunosuppressive drugs. Because of the higher treatment-related (non-relapse) mortality, cGVHD remains the major cause of late death despite its association with a lower relapse rate [3, 4]. Primary therapy for extensive cGVHD typically includes corticosteroids and cyclosporine. However, this treatment is often unsuccessful in patients with multiorgan involvement and is associated with significant therapy-related complications [5, 6]. Alternative approaches are needed for patients who cannot tolerate or do not respond to the first-line therapy. Clinical reports exist showing response to type of other agents, such as tacrolimus, mycophenolate mofetil, thalidomide, rituximab and psolaren and ultraviolet A (PUVA) therapy or extracorporeal photochemotherapy [7-12]. We are still waiting for drugs against cGVHD, characterized by decreased infectious complications, encouraging efficacy and rare and reversible side effects.

Ocular manifestations are found in a majority of patients and may be the predominating symptom. They occurs in 45% to 60% of patients with cGVHD [13-16]. Ocular findings include keratoconjunctivitis sicca, pseudomembranous conjunctivitis, cicatricial conjunctivitis, corneal epithelial sloughing, corneal ulceration and microvascular retinopathy. Cicatricial conjunctivitis can lead to entropion, trichiasis, corneal ulceration and vision loss. Dry eyes developed in 76% of acute GVHD patients and between 62.5% and 81.8% of cGVHD [17-21]. Systemic immunosuppressive therapy and

* Corresponding author

Table 1. Treatment with clofazimine- Patients' characteristics

Nr	Age	Sex	Diagnosis	Donor sex	The source of HSC	Type of cGVHD	Platelet count at diagnosis of cGVHD	Involved organs	Treatment	Response to clofazimine
1	33	M	AML	F	PB	Quiescent, extensive	< 100 G/l	Liver, skin, oral cavity, joints, ocular manifestations	CsA, prednisolone, FK506, MMF, clofazimine	partial
2	44	M	AML	F	PB	De novo, extensive	> 100 G/l	Liver, skin, oral cavity, joints, ocular manifestations	CsA, prednisolone, FK506, MMF, clofazimine	Skin and joint mobility – complete oral cavity – partial
3	48	M	AML	F	PB	Quiescent, extensive	< 100 G/l	Liver, skin, oral cavity, joints, ocular manifestations	CsA, prednisolone, FK506, MMF, clofazimine	partial
4	47	F	MS	F	PB	Quiescent, extensive	> 100 G/l	Liver, skin, oral cavity, joints, ocular manifestations	CsA, prednisolone, FK506, MMF, clofazimine	Skin and joint mobility – partial oral cavity – complete

F – female
M – male

AML – acute myeloid leukemia
BM – bone marrow

MS – myeloid sarcoma (granulocytic sarcoma)
PB – peripheral blood

CsA – cyclosporine A
FK506 – tacrolimus

MMF – mycophenolate mofetil
cGVHD – chronic graft versus host disease

local treatment may be used to control an ocular disease. Conventional initial local therapy such as lubrication and topical steroids is directed to treat decreased tear production and ocular surface abnormalities. However, some patients with ocular manifestations of cGVHD get worse despite systemic treatment [15, 22-25].

Clofazimine, previously used in the treatment of leprosy, is now used for treatment of Mycobacterium avium complex infection in patients with acquired immune deficiency syndrome, it's also used to treat dermatologic disorders, including: pyoderma gangrenosum, lupus erythematosus and palmoplantar pustulosis and chronic graft versus host disease [26, 27]. The mechanism of clofazimine action in these conditions is unknown. The effects of clofazimine on neutrophil activities such as random motility, migration to the leukoattractants, endotoxin-activated serum and N-formyl-L-methionyl-L-leucyl-L-phenylalanine phagocytosis of Candida albicans, postphagocytic hexose-monophosphate shunt activity, and myeloperoxidase-mediated iodination and the effects of clofazimine on lymphocyte transformation to mitogens were assessed in vitro and after ingestion of the drug by normal individuals and patients with lepromatous leprosy. For in vitro studies, the concentration range of the drug investigated was 10(-6) M to 10(-2) M. For in vivo studies, subjects ingested 200 mg of clofazimine daily for a period of 5 days. At concentration of 5 X 10(-6) M to 5 X 10(-3) M clofazimine caused a progressive dose-dependent inhibition of neutrophil motility without detectable effects on phagocytosis, postphagocytic hexose-monophosphate shunt activity, or myeloperoxidase-mediated iodination. Over the same concentration range, clofazimine inhibited lymphocyte transformation. The inhibitory effect on neutrophil motility was associated with a spontaneous stimulation of oxidative metabolism. After ingestion of clofazimine responsiveness of lymphocytes to mitogens was decreased in normal volunteers and leprosy patients; neutrophil motility in normal individuals was likewise inhibited [28]. Although the drug is relatively well tolerated, side effects, when they occur, involve the skin, eyes and gastrointestinal tract, that is all major sites of cGVHD involvement [26, 27]. Clofazimine is secreted in sweat, sebum, tears and saliva. Clofazimine crystals are found precipitated in Peyer's patches, lymph nodes, skin and other organs in patients receiving long-term therapy [26, 27]. Thus, clofazimine may be effective in cGVHD because of its tissue distribution and immunomudulatory activity.

The purpose of this study was to illustrate the efficacy of topical cyclosporine 0,05% as an adjunct in managing ocular surface abnormalities and clofazimine for refractory skin, joint and oral cavity changes caused by chronic graft versus host disease.

Patients and methods

Hospital Ethics Committee approved the study and patients gave consent to participate. Four patients with cGVHD were treated with clofazimine (Lamprene- Novartis). The detailed characteristics of patients are summarized in table 1. All patients had a sibling donor. All developed extensive chronic graft versus host disease, manifested by sclerodermoid and lichenoid involvement of the skin with joint contractures. In oral cavity following changes were observed: mucositis with ulcerations, lichen planus like lesions and erosions ranging

Table 2: Treatment with topical cyclosporine- Patients' characteristics

Nr	Age	Sex	Diagnosis	Donor sex	The source of HSC	Type of cGVHD	Involved organs (other than the eyes)	Treatment
1	50	F	CML II CP	F	BM	Progressive	Liver, oral cavity, skin, vagina, BOS	CsA, prednisolone, FK506, MMF
2	44	M	CML II CP	M	BM	Progressive	Liver, oral cavity, skin, BOS	CsA, prednisolone, FK506, MMF
3	28	F	AML	F	PB	Progressive	Liver, skin, oral cavity	CsA, prednisolone, FK506, MMF
4	26	F	AML	F	PB	Progressive	Liver, skin, oral cavity	CsA, prednisolone, FK506, MMF
5	30	M	AML	F	PB	Progressive	Liver, skin, oral cavity	CsA, prednisolone, FK506, MMF
6	42	M	AML	F	PB	Progressive	Liver, skin, oral cavity	CsA, prednisolone, FK506, MMF
7	50	M	AML	F	PB	Progressive	Liver, skin, oral cavity	CsA, prednisolone, FK506, MMF
8	22	F	MS	M	PB	Progressive	Liver, skin, oral cavity	CsA, prednisolone, FK506, MMF

CML – chronic myeloid leukemia
II CP – the second chronic phase
BM – bone marrow
PB – peripheral blood
AML – acute myeloid leukemia
MS – myeloid sarcoma (granulocytic sarcoma)
F – female
M – male
MMF – mycophenolate mofetil
CsA – cyclosporine A
FK506 – tacrolimus
BOS – bronchiolitis obliterans

from fine white lines to broad plaques, dryness and xerostomia. In three cases and one de novo the cGVHD was without manifested symptoms. All patients had also liver disease and ocular manifestations. Liver disease was manifested as cholestatic abnormalities. Ocular manifestations of cGVHD included keratoconjunctivitis sicca and sterile conjunctivitis. Two patients had platelet count < 100 G/l when diagnosis of cGVHD was established. All patients were treated with cyclosporine and prednisolone. No improvement was observed. Mycophenolate mofetil with tacrolimus were used as the second line therapy. After three months of this kind of therapy liver and ocular manifestations improved, but sclerodermoid changes of the skin, pathological changes in oral cavity and joint contractures were refractory to treatment. Clofazimine, as an addition to the second line immunosuppressive therapy was started. All patients received clofazimine 300mg in a single oral dose with food for 90 days. Then the dose of clofazimine was reduced to 100 mg daily. Patients received pneumocystis and pneumococcal prophylaxis and immunoglobulins when hypogammaglobulinemia occured. Response rates were determined by review of medical records. Complete organ responses were defined as a resolution of all pathological changes. Partial responses were defined as a greater than 50% response in all involved organs.

Other eight patients with ocular involvement during cGVHD were treated with topical cyclosporine A (ophthalmic emulsion 0.05%; Restasis produced by Allergan). The detailed characteristics of patients are summarized in table 2. All patients developed extensive chronic graft versus host disease with eye involvement after the allogeneic stem cell transplantation. In all cases it was progressive cGVHD. All patients had also liver disease, skin changes and oral mucosa involvement. Liver disease was manifested as cholestatic abnormalities. Skin abnormalities included extensive lichenoid changes, thin hair and nails with vertical ridging and fragility. In oral cavity mostly mucositis with ulcerations were observed. Two patients had bronchiolitis obliterans (BOS). Ocular manifestations of cGVHD included keratoconjunctivitis sicca and sterile conjunctivitis. All patients were treated with cyclosporine and prednisolone. Patients with BOS additionally received intravenous immunoglobulins. No improvement was observed. Mycophenolate mofetil with tacrolimus were used as a second line therapy. Topical therapy consisted of preservative-free tears during the day, preservative-free ointment at night and topical corticosteroids. After three to four months of the second line therapy most pathological symptoms in involved organs were resolved (including bronchiolitis obliterans) but patients still suffered from ocular manifestations of chronic GVHD: burning, pain, photophobia and keratoconjunctivitis sicca. Treatment with cyclosporine ophthalmic emulsion 0.05% was started. Two drops of Restasis were instilled twice a day in each eye with approximately 12 hours intervals. Systemic immunosuppressive therapy was continued.

Results

Four patients received clofazimine from 9 to 12 months. All were treated for skin, joint and mouth involvement which was refractory to mycophenolate mofetil with tacrolimus and achieved partial or complete responses in those organs. Three of them treated for sclerodermatous skin changes achieved partial and one complete response, respectively. In oral cavity partial response was seen in three patients and complete in one. We observed improvement in joint mobility in all patients, one of them attained complete response with release of contractures. However no complete responses were observed. None of patients with platelet count below 100 G/l (at diagnosis of cGVHD) achieved complete response in any of involved organs, only partial improvements were observed. Response to treatment with clofazimine for different organs are also shown in table 1. Treatment with clofazimine allowed to reduce doses of other immunosuppressive drugs by at least 50 %. They were still needed to control liver and ocular manifestations of cGVHD. The therapy with clofazimine was generally well tolerated. Side effects were mild and transient.

Two patients had nausea and diarrhea and developed red-brown hyperpigmentation of the skin. None required discontinuation of clofazimine. No ophthalmologic complications were observed. All side effects were reversible upon stopping clofazimine treatment.

All patients treated with topical cyclosporine responded, but clinical improvement was seen not earlier than from the fifth month of starting treatment. The effectiveness of topical cyclosporine therapy was evaluated by the Schirmer's test in three patients. All of investigated ones with scores < 5mm at the initiation of the treatment had improvement in Schirmer's scores (≥ 5 mm) with therapy. Systemic therapy was successfully slowly tapered. We tried to reduce the dose of topical cyclosporine (after six months of treatment) .Unfortunately it was leading to exacerbation of eye manifestation of chronic graft versus disease in three patients. Nowadays these three patients still require systemic immunosuppressive therapy due to liver and skin involvement (with recuded doses of mycophenolate mofetil and/ or tacrolimus) and are also treated with topical cyclosporine to control eye disease. No secondary infections, such as bacterial or viral conjunctivitis occurred when using the topical cyclosporine. This ophthalmic emulsion 0,05% was generally well tolerated. Only mild side effects were observed including eye pain, foreign body sensation and transient visual disturbances (blurring). They occured in four patients, were temporary, did not require discontinuation of therapy and disappreared by the end of the second week of treatment.

Discussion

Clofazimine is an antymycobacterial agent that has been used in the treatment of leprosy and Mycobacterium avium complex. It has been reported to be useful in several immune-mediated skin disorders. Its mechanism of action is unknown, but its immunomodulatory effect is thought to reflect functional inhibition of pathogenic T lymphocytes. Clofazimine has been reported to have encouraging efficacy in the treatment of cGVHD in addition to a reduced infection risk, especially in patients with sclerodermatous skin, joint and oral involvement [4, 29]. The half-life of clofazimine in men is approximately 70 days due to its highly lipophilic nature. However, in vivo efficacy of clofazimine may be related to high local tissue levels of the drug. Clofazimine is secreted in sweat, sebum, tears and saliva. Clofazimine crystals are found precipitated in Peyer's patches, lymph nodes, skin and other organs in patients receiving long-term therapy [26, 27]. Thus, clofazimine may be effective in cGVHD because of its tissue distribution and immunomudulatory activity. Commonly reported side effects include skin changes such as pigmentation from pink to brownish-black in 75%-100% of the patients within a few weeks of treatment; ichthyosis and dryness (8%-28%); rash and pruritus (1%-5%); gastrointestinal abnormalities (abdominal and epigastric pain, diarrhea, nausea, vomiting, gastrointestinal intolerance (40%-50%); ocular disturbances (conjunctival and corneal pigmentation). Most of them are caused due to clofazimine crystal deposits. Other side effects are as follows: discoloration of urine, feces, sputum, sweat; elevated blood sugar; elevated ESR. Adverse reactions in less than 1% of patients include phototoxicity, erythroderma, acneiform eruptions, monilial cheilosis and methemoglobinemia [4, 29]. We observed only mild and transient side effects without ocular complications. They did not lead to discontinuation of the therapy. All patients treated for mouth, skin and joint involvement achieved partial or complete responses. We did not observe complete responses in patients with platelet count < 100 G/l. Thrombocytopenia and extensive skin involvement are known as negative prognostic factors leading to the poorer survival in patients with extensive cGVHD after allogeneic haematopoietic stem cell transplantation [30]. Treatment with clofazimine did not lead to discontinuation of mycophenolate mofetil and tacrolimus, but it allowed to reduce doses of these immunosuppressive drugs by at least 50 % (they were still needed to control liver and ocular disease).

The promise in controlling of ocular GVHD is shown by a variety of systemic and topical anti-inflammatory agents, including prednisolone acetate, cyclosporine, tacrolimus, autologous serum and retinoic acid. In spite of systemic therapy for cGVHD consisting of systemic immunosuppressants (ie cyclosporine and corticosteroids) it can sometimes be still insufficient to overcome the ocular manifestations of the disease [14, 31]. Cyclosporine ophthalmic emulsion 0,05% (Restasis- Allergan) is indicated to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca [32-36]. In our patients topical cyclosporine was added to the second line treatment regimen and the regression of the ocular disease was recorded. The addition of 0.05% ophthalmic emulsion of cyclosporine probably helped in controlling of the epithelial keratitis and melting process. The level of cyclosporine in the conjunctiva was significantly higher after topical application in comparison with systemic treatment and reduces or eliminates the drug's systemic side effects. Topical cyclosporine may help to promote the healing process and decrease the immunology activity from the donor lymphocytes which activate the immune cascade. It works only in the early period of ocular GVHD and it can minimize the immune destruction responsible for the development of the keratoconjunctivitis sicca. The local inhibitory effect of cyclosporine A on T lymphocytes and cytokine production may decrease the tissue destruction and allow wound healing [15, 37].

Conclusions

Treatment with clofazimine can be effective against refractory skin, joint and mouth pathological changes caused by chronic graft versus host disease. Its usage is characterized by lack of infectious complications, encouraging efficacy and

rare and reversible side effects. However, the number of patients treated with this drug in our centre was small and future studies are needed to estimate the place of clofazimine in treatment of chronic graft versus host disease after allogeneic stem cell transplantation.

Ophthalmic emulsion of cyclosporine A seems to be safe and may be beneficial to

patients with ocular GVHD. It may improve not only subjective signs but probably also objective clinical parameters of dry eye caused by chronic graft versus host disease. However, a further randomized clinical prospective studies are needed to verify these results.

References

[1] FLOWERS ME, PARKER PM, JOHNSTON LJ et al. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term folow-up of a randomized trial. Blood 2002; 100: 415–419.

[2] ZECCA M, PRETE A, RONDELLI R. Chronic graft-versus-host disease in children: incidence, risk factors and impact on outcome. Blood 2002; 100: 1192–1200.

[3] SOCIE G, STONE JV, WINGARD JR et al. Long-term survival and late deaths after allogeneic bone marrow transplantation: Late Effects Working Committee of the International Bone Marrow Transplantation Registry. N Engl J Med. 1999; 341: 14–21.

[4] LEE SJ, KLEIN JP, BARRETT AJ et al. Severity of chronic graft versus host disease: association with treatment-related mortalirty and relapse. Blood 2002; 100: 406–414.

[5] WINGARD JR, PIANTADOSI S, VOGELSANG GB et al. Predictors of death from chronic graft-versus-host disease after bone marrow transplantation. Blood 1989; 74: 1428.

[6] SULLIVAN KM, WITHERSPOON RP, STORB R et al. Alternating day cyclosporine and prednisone for treatment of high risk chronic graft-versus-host disease. Blood 1988; 72: 555.

[7] GREINIX HT, VOLC-PLATZER B, RABITSCH W et al. Successful use of extracorporeal photochemotherapy in the treatment of severe acute and chronic graft versus host disease. Blood 1998; 92: 3098–3104.

[8] OKAMOTO M, OKANO A, AKAMATSU S et al. Rituximab is effective for steroid-refractory sclerodermatous chronic graft –versus-host disease. Leukemia 2006; 20: 172–173.

[9] KAPOOR N, PELLIGRINI AE, COPELAN EA. Psolaren plus ultraviolet A (PUVA) in the treatment of chronic graft versus host disease: Preliminary experience in standard treatment resistant patients. Semin Hematol 1992; 29: 108.

[10] KULKARNI S, POWLES R, SIROHI B et al. Thalidomide after allogeneic haematopoietic stem cell transplantation: activity in chronic but not in acute graft-versus-host disease. Bone Marrow transplant 2003; 32: 165–170.

[11] VOGELSANG GB. How I treat chronic graft-versus-host disease. Blood 2001; 97: 1196–1201.

[12] LEE SJ. New approaches for preventing and treating chronic graft-versus-host disease. Blood 2005; 105: 4200–4206.

[13] EPSTEIN JB, REECE DE. Topical cyclosporine A for treatment of oral chronic graft-versus-host disease. Bone Marrow Transplant. 1994; 13: 81–86.

[14] ANDERSON NG, REGILLO C. Ocular manifestations of graft versus host disease. Curr Opin Ophthalmol. 2004; 15: 503–507.

[15] KIANG E, TESAVIBUL N, YEE R et al. The use of topical cyclosporine A in ocular graft-versus-host-disease. Bone Marrow Transplant. 1998; 22: 147–151.

[16] ROBINSON MR, LEE SS, RUBIN BI et al. Topical corticosteroid therapy for cicatrical conjunctivitis associated with chronic graft-versus-host-disease. Bone Marrow Transplant 2004; 33: 1031–1035.

[17] HIRST LW, JABS DA, TUTSCHKA PJ et al. The eye in bone marrow transplantation; I Clinical study. Arch Ophthalmol 1983; 101: 580–584.

[18] FRANKLIN RM, KENYON KR, TUTSCHKA PJ et al. Ocular manifestations of graft-versus-host-disease. Ophthalmology 1983; 90: 4–13.

[19] LAWLEY TJ, PECK GL, MOUTSOPOULOS HM. Scleroderma, Sjogren- like syndrome and chronic graft-versus-host-disease. Ann Intern Med 1977; 87: 707–709.

[20] JABS DA, HIRST LW, GREEN WR al. The eye in bone marrow transplantation; II Histopathology. Arch Ophthalmol 1983; 101: 585–590.

[21] JABS DA, WINGARD J, GREEN WR et al. The eye in bone marrow transplantation. Arch Ophtalmol 1989; 107: 1343–1348.

[22] LIVESEY SJ, HOLMES JA, WHITTAKER JA. Ocular complications of bone marrow transplantation. Eye 1989; 3: 271–276.

[23] VOGELSANG GB, LEE L, BENSEN-KENNEDY DM. Pathogenesis and treatment of graft versus host disease after bone marrow transplant. Ann Rev Med 2003; 54: 29–52.

[24] OGAWA Y, OKAMOTO S, KUWANA M et al. Successful treatment of dry eye in two patients with chronic graft versus host disease with systemic administration of FK-506 and corticosteroids. Cornea 2001; 20: 430–434.

[25] VOGELSANG GB. How I treat chronic graft-versus-host disease. Blood 2001; 97: 1196–1201.

[26] PARIZHSKAYA M, YOUSSEF NN, DI LORENZO C, GOYAL RK. Clofazimine enteropathy in a pediatric bone marrow transplant recipient. J Pediatr 2001; 138: 574–576.

[27] LEE SJ, WEGNER SA, McGARIGLE CJ et al. Treatment of chronic graft-versus-host disease with clofazimine. Blood 1997; 89: 2298–2302.

[28] vAN RENSBURG CE, GATNER EM, IMKAMP FM et al. Effects of clofazimine alone or combined with dapsone on neutrophil and lymphocyte functions in normal individuals and patients with lepromatous leprosy. Antimicrob Agents Chemother. 1982; 21: 693–697.

[29] MOREIRA DE VA, MEDEIROS DE BC, BONFIM C et al. Methemoglobinemia secondary to clofazimine treatment for chronic graft-versus-host disease. Blood 1998; 92: 4872-4881.

[30] Ratanatharathorn V, Ayash L, Lazarus HM et al. Chronic graft versus host disease: clinical manifestation and therapy. Bone Marrow Transplant 2001; 28: 121–129.

[31] OGAWA Y, OKAMOTO S, MORI T et al. Autologous serum eye drops for treatment of severe dry eye in patients with chronic graft versus host disease. Bone Marrow Transplant 2003; 31: 579–583.

[32] BARBER LD, PFLUGFELDER SC, TAUBER J, FOULKS GN. Phase III safety evaluation of cyclosporine 0,1% ophthalmic emulsion administered twice daily to dry eye disease patients for up to 3 years. Ophthalmology 2005; 112: 1790–1794.

[33] SAMARKOS M, MOUTSOPOULOS HM. Recent advances in the management of ocular complications of Sjogren's syndrome. Curr Allergy Asthma Rep. 2005; 5: 327–332.

[34] STONECIPHER K, PERRY HD, GROSS RH, KERNEY DL. The impact of topical cyclosporine A emulsion 0,05% on the outcomes of patients with keratoconjunctivitis sicca. Curr Med Res Opin. 2005; 21: 1057–1063.

[35] LELLI GJ, MUSCH DC, GUPTA A, FARJO QA et al. Ophthalmic cyclosporine use in ocular GVHD. Cornea 2006; 25: 635–638.

[36] RAO SN, RAO RD. Efficacy of topical cyclosporine 0,05% in the treatment of dry eye associated with graft versus host disease. Cornea 2006; 25: 674–678.

[37] PFAU B, KRUSE FE, ROHRSCHNEIDER K. Comparison between local and systemic administration of cyclosporine A on the effective level in conjunctiva, aqueous humor and serum. Ophthalmologe 1995; 92: 833–839.