Analysis of thromboembolic risk related to high-dose intravenous immunoglobulin treatment: a preliminary clinical study of 10 patients with autoimmune mucocutaneous blistering diseases

M. D. Mignogna, G. Fortuna, S. Leuci, E. Ruoppo, D. Adamo and S. Fedele*

*Section of Oral Medicine, Department of Odontostomatological and Maxillo-facial Science of the School of Medicine and Surgery, University of Naples Federico II; and *Oral Medicine Unit, Division of Maxillofacial Diagnostic, Medical and Surgical Sciences, Eastman Dental Institute, University College London, London, UK*

doi:10.1111/j.1365-2230.2008.02809.x

Summary

Background. Intravenous immunoglobulin (IVIg) treatment is a well-known treatment that has been used successfully in a broad spectrum of autoimmune diseases. Currently no data are available in the literature about the role of IVIg in the pathogenesis of thromboembolic events in patients with autoimmune blistering diseases refractory to conventional immunosuppressive treatment.

Aim. To determine the relationship between IVIg and thromboembolism in patients with autoimmune blistering diseases and to establish a protocol to deal with the thromboembolic risk.

Methods. In our preliminary clinical study, 10 patients with autoimmune blistering diseases underwent IVIg cycles to a total of 133 cycles in all (total number of infusions in the patient group: 399), at a standard dose of 2 g/kg/infusion accompanied by an accurate and a complete clinical and laboratory screening for thromboembolism. Preventive measures, such as hydration before and after IVIg, and administration of 100 mg of acetyl salicylic acid (aspirin) or 1000 IU of subcutaneous heparin calcium per day for 3 weeks, were introduced to reduce the thromboembolic risk.

Results. Throughout the 2 years of IVIg treatment, no patient developed a superficial and/or deep venous or arterial thrombosis, even though some of the patients had underlying thromboembolic risk factors and had tested positive for some congenital and acquired thrombophilia markers.

Conclusions. Our results indicate that thromboembolic events are uncommon, despite the presence of risk factors. However, as these disorders are very rare and the percentage of nonresponder patients is very low, further investigations are needed to better understand whether IVIg alone is able to trigger these fatal events in blistering disorders.

Introduction

Over the past 5 years, several investigations have shown that intravenous immunoglobulin (IVIg) treatment is one of the most successful treatments for autoimmune mucocutaneous blistering diseases (AMBDs) refractory to conventional immunosuppressive treatment (CIST).[1–3]

IVIg has been approved by the US Food and Drug Administration (FDA) as a safe and effective treatment for a variety of autoimmune and inflammatory diseases, such as idiopathic thrombocytopenic purpura (ITP), paediatric human immunodeficiency infections, graft-versus-host disease and Kawasaki's syndrome.[4] Unfortunately, it is associated with a wide range of adverse events, which have an incidence ranging from 1% to 42%[5] and a somewhat variable frequency. These

Correspondence: Professor. M. D. Mignogna, Head, Division of Oral Medicine, Department of Odontostomatological and Maxillofacial Sciences, University of Naples Federico II, Via Pansini 5, 80131 Naples, Italy.
E-mail: mignogna@unina.it

Conflict of interest: none declared.

Accepted for publication 15 December 2007

adverse events are categorized as: (i) minor self-limiting side-effects, such as headache, chills, myalgia and fever; (ii) moderate, such as skin reactions; (iii) severe, such as anaphylactic shock or fatal renal complications.[6] One of the most serious side-effects appears to be the onset of venous and arterial thromboembolic complications, such as stroke or myocardial infarction, usually in patients with underlying risk factors.[7]

To our knowledge, only three reports have previously described a thromboembolic event in patients with pemphigus vulgaris after treatment with high-dose IVIg.[8–10] In these reports, it seemed that the presence of predisposing risk factors, such as lipid profile, diabetes, obesity and the role of corticosteroids in the pathogenesis of thromboembolism was not taken into consideration during treatment. Thus, it is likely that the thromboembolic complications might be due to a lack of appropriate screening of patients before and insufficient monitoring during IVIg treatment. Assuming IVIg to be the cause of thromboembolism is not entirely appropriate and certainly not supported by the data.

Except for these three cases, thromboembolic risk in AMBDs treated by IVIg has not been assessed previously, as these diseases are rare and it is therefore difficult to perform a sufficiently powerful statistical analysis. To our knowledge, our investigation is the first preliminary clinical study to assess thromboembolic risk in such patients. We report our experience in evaluating the relationship between high-dose IVIg and thromboembolic events in patients with AMBDs, proposing our clinical approach to this risk and our hypothesis of the physiopathological mechanism that underpins these dangerous and unpredictable phenomena.

Methods

The preliminary study began in November 2004 and concluded in December 2006. From a group of 82 patients with AMBDs registered with the Division of Oral Medicine at the Department of Odontostomatological and Maxillo-facial Science, a subgroup of 10 patients (3 men, 7 women; mean age 52.9 years, range 23–77) was selected. All 10 patients were classified as non-responders to CIST, composed of high-dose corticosteroids and immunosuppressants; duration of treatment had varied between 4 and 72 months. Of the 10 patients, 3 had mucous membrane pemphigoid and 7 had pemphigus vulgaris. In addition, all patients had a variety of thromboembolic risk factors (Table 1). The other 72 patients were classified as responders to CIST, so were not included in the study, but later follow-up showed that none of these had experienced any thromboembolic events.

To reduce thromboembolic risk in the study group, we set up a four-step protocol: (i) diet control; (ii) evaluation of β-cryoglobulin and IgA concentration; (iii) evaluation of underlying risk factors by thorough history-taking and physical and laboratory examinations, including screening for acquired and congenital thrombophilia; and (iv) low infusion rate. Consequently, all patients underwent a thorough clinical examination before starting IVIg, and routine blood tests were carried out. In addition, to identify any underlying hypercoagulable state, a complete coagulation profile was performed, and patients were tested for congenital and acquired thrombophilia markers. Thus, in agreement with guidelines in the international literature,[11,12] we measured international normalised ratio, prothrombin time, thrombin time, activated partial thromboplastin time (aPTT), and levels of β-fibrinogen (β-FG), antiphospholipid antibody, IgG and IgM-anticardiolipin, lupus anticoagulant, D-dimers and plasminogen activator inhibitor 1 (PAI-1) to test for the acquired form. To test for the inherited form, patients were tested for the gene mutations related to Factor V Leiden (G1691A), prothrombin (G20210A), methylenetetrahydrofolate reductase (*MTHFR*; C677T and A1298C), β-FG, PAI-1, congenital deficiency of protein C, protein S and antithrombin III (ATIII), and for activated protein C (APC) resistance. Finally, all patients underwent tests for β-cryoglobulin and IgA; a nutritionist devised a targeted regimen to be meticulously followed by the patients at home; and a thorough cardiological examination, including electrocardiography and colour Doppler ultrasonography of the supraaortic branches, was carried out.

The 10 patients received IVIg cycles to a total of 133 cycles in all. The treatment was not used as monotherapy; during the IVIg maintenance phase, all 10 patients took low-dose corticosteroids and immunosuppressive agents (12–60 mg of deflazacort per week and 50–250 mg of azathioprine per week).

The optimum dose, frequency and duration of IVIg treatment was based on the previously reported consensus statement.[3] All patients received pre-treatment medication with acetaminophen 500 mg, chlorpheniramine 20 mg and methylprednisolone 40 mg, 30 min before each infusion. Human immunoglobulin 5% solution [Flebogamma (Grifols, Barcelona, Spain), Ig vena NIV (Kedrion, Lucca, Italy), Endobulin (Baxter, Pisa, Italy)] was infused intravenously using an electronic pumping device (Optima MS; Fresenius Vial, Brezins, France) at a total dose of 2 g/kg/cycle, given

over three consecutive days (Table 1). The infusion was administered slowly at not more than 50 mg/kg/h. These infusions were given initially at an interval of 3 or 4 weeks between each cycle until complete clinical remission and the absence of new lesions was achieved. Thereafter, the intervals between infusions were slowly increased to 6, 8, 10, 12, 14 and 16 weeks (defined as maintenance treatment period). The IVIg end-point of treatment was defined as the time by which patients had been disease-free for 16 weeks.

In addition, patients were hydrated with 500 mL of normal saline before and after infusions, and preventive treatments such as aspirin 100 mg (given to seven low-risk patients),[13] and 1000 IU of subcutaneous heparin calcium (given to three multimorbidity and multi-therapy high-risk patients) per day for 3 weeks were given, to prevent hyperviscosity syndrome and thus lessen thromboembolic risk. A high-risk patient for thromboembolism was defined as a patient with > 50% of the total risk factors present.

Results

Of the 10 patients, 7 presented underlying clinical and laboratory risk factors for thromboembolism, (Tables 1 and 2). Despite this, all 10 patients completed the full treatment, resulting in prolonged, sustained clinical remission. No patient developed any thromboembolic event during the 3 days of IVIg, 24–72 h later, or within 30 days of the last infusion. Usually a case of IVIg-associated thrombosis is defined as a patient who develops a thrombotic event within 30 days of receiving an IVIg infusion.[13]

The routine blood tests and evaluation of β-cryoglobulin and IgA concentration gave normal results. Electro-cardiography and eco-colour Doppler ultrasonography were negative for heart and/or carotid diseases, despite the presence of several underlying risk factors in the patients.

Some patients showed mild and/or moderate variations in the results of serum screening for acquired and congenital thrombophilia. Four patients (patients 1, 4, 5 and 7) showed an increase in β-FG (359.7, 664, 386 and 355 mg/dL, respectively; normal range 160–350) and in PAI-1 (4.5, 5.0, 8.3 and 4.4 IU/mL, respectively; normal range 0.3–3.5). Two patients showed an increase in D-dimers (200.1 and 201.5 μg/L for patients 3 and 5, respectively; normal range 0–200), two others an increase in homocysteine (15.3 and 17.3 μmol/L for patients 2 and 5; respectively; normal range 5–15) and, finally, two others a decrease in aPTT (24.2 and 23 s for patients 4 and 7, respectively; normal range 26–44) (Table 2).

Some patients also showed an increase in protein C (149.94%, 140.93% and 144.7% for patients 2, 4 and 7, respectively; normal range 70–120%), an increase in ATIII (122.6% for patient 7, normal range 70–120%), and a decrease in APC resistance (113.7, 77.8 and

Table 1 Patient characteristics.

Patient	Age (years)	Gender	AMBD	Risk factors	Weight (kg)	Total dose (g/cycle)	No. of cycles
1	29	F	PV	Central venous catheter, bed-ridden, solid tumour, radiotherapy, chemotherapy, corticosteroids, smoking, post-surgical state, previous thromboembolic events, HRT	60	120	21
2	37	M	PV	Corticosteroids	67.5	135	22
3	77	F	PV	Advanced age, hypertension, coronary artery disease, bed-ridden, diabetes, CVC, corticosteroids, post-surgical state, previous thromboembolic events, smoking	60	120	19
4	65	F	MMP	Advanced age, hypertension, hyperlipidaemia, obesity, diabetes, smoking, corticosteroids, HRT, coronary artery disease, CVC	82.5	165	15
5	57	M	PV	Hypertension, hyperlipidaemia, corticosteroids	75	150	9
6	42	F	PV	Corticosteroids	60	120	9
7	76	F	MMP	Advanced age, hypertension, obesity, corticosteroids	67.5	135	10
8	57	M	MMP	Coronary hear disease, hypertension, corticosteroids	75	150	10
9	66	F	PV	Coronary artery disease, hypertension, previous thrombo-embolic events, diabetes, HRT	60	120	9
10	23	F	PV	Corticosteroids	60	120	9

AMBD, autoimmune mucocutaneous blistering diseases; CVC, central venous catheter; HRT, hormone-replacement therapy; MMP, mucous membrane pemphigoid; PV, pemphigus vulgaris.

Table 2 Alteration in acquired and congenital thrombophilia markers and of genotyping polymorphisms.

	Acquired thrombophilia									Congenital thrombophilia				Genotyping polymorphisms					
Patient no.	PT	aPTT	β-FG	PAI-1	D-dimer	HOM	LA	aCL	aPL	PC	PS	ATIII	APC-R	Factor V Leiden	PRT	*MTHFR* (C677T)	*MTHFR* (A1298C)	PAI-1	β-FG
1			↑	↑									↓	WT	WT	WT	HT	HM	HT
2						↑				↑				WT	WT	HT	WT	HM	WT
3					↑									WT	WT	HT	WT	HM	WT
4		↓	↑	↑	↑					↑				WT	WT	HM	WT	HT	HT
5			↑	↑		↑						↑	↓	WT	WT	HM	WT	HT	WT
6													↓	HT	WT	HT	HT	HT	HT
7		↓	↑	↑						↑				WT	WT	WT	HT	HT	HT
8														WT	WT	WT	WT	WT	WT
9			↑											WT	WT	WT	WT	WT	WT
10														WT	WT	WT	WT	WT	WT

↑, Increased value; ↓, decreased value; aCL, anti-cardiolipin antibody; APC-R, activated protein C resistance; aPL, anti-phospholipid antibody; aPTT, activated partial thromboplastin time, ATIII, antithrombin III; β-FG, β-fibrinogen, HM, homozygous; HOM, homocystinaemia; HT, heterozygous; LA, lupus anticoagulant; *MTHFR*, methylenetetrahydrofolate reductase gene; PAI-1, plasminogen activator inhibitor 1; PC, protein C; PRT, prothrombin; PS, protein S; PT, prothrombin time; WT, wild type. Frequency of Leiden factor V heterozygosity in Italy is about 2–3%, and that of homozygosity is about 1 : 5000. Heterozygotes have a risk 2–8 times higher and homozygotes a risk 40–80 times higher than the general population of developing venous thrombosis. Frequency of heterozygosity for the C677T *MTHFR* mutation is about 40% and is not related to thromboembolic risk; homozygosity frequency is about 15% and is related to high risk of venous thrombosity if associated with an increase in serum levels of homocysteine. Frequency of heterozygosity for the A1298C *MTHFR* mutation is also about 40%; homozygosity frequency is about 11% and is responsible for reduced enzymatic activity. It is considered a risk factor if related to an increase in homocysteine serum levels. Homozygosity for the PAI-1 4G allele is associated with high levels of PAI-1, which in turn increases risk of coronary disease and early spontaneous abortion. The presence either heterozygosity or homozygosity of the β-fibrinogen 455 allele is associated with a high level of fibrinogen in the serum, which predisposes to venous therombosis.

116.9 s, for patients 1, 5 and 6, respectively; normal range 120–300). Genotyping of polymorphisms showed heterozygosity for factor V Leiden in one patient, for *MTHFR* C677T in three, for *MTHFR* A1298C in three, for PAI-1 in four and for β-fibrinogen in four, and homozygosity for PAI-1 in three and for *MTHFR* C677T in two (Table 2).

By correlating 16 risk factors [advanced age (defined as > 60 years), smoking, obesity (defined as body mass index ≥ 30 kg/m^2), postsurgical state, use of corticosteroids or hormone-replacement treatment, previous thromboembolic events, and presence of hypertension, coronary artery disease, central venous catheter, solid tumour, chemotherapy, radiotherapy, confinement to bed, hyperlipidaemia, diabetes, at-risk polymorphisms and congenital thrombophilia markers], we found the risk for all patients of developing thromboembolic events to be 5–65% (Fig. 1). However, it should be remembered that this is based on a small number of patients.

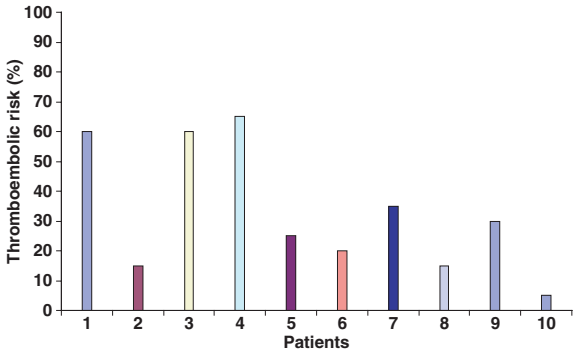

Figure 1 To draw the graphic, for each patient, we have considered three parameters: 1) risk factors (Table 1); 2) acquired and congenital thrombophilia markers (Table 2); 3) genotyping polymorphisms (Table 2). We assigned to the first parameter, the following score: +1 for every risk factor shown by patient and to the second and third parameter the following score: +1 if the patient shows <50% and +2 if a patient shows ≥50% than all genotyping polymorphisms and all acquired and congenital thrombophilia markers. In order to calculate the percentage of thromboembolic risk we divided the relative risk (score gained by every patient) for the absolute risk (maximum score: 20) and, then, multiplied per 100.

Discussion

Previous investigations have clearly shown that administration of immunoglobulins may provoke thromboembolic events[7,14–17] such as strokes,[18] acute myocardial infarction[19] and even central retinal vein occlusion.[20] Our study emphasises the necessity of a thorough medical evaluation, including physical

examination, laboratory and genetic tests, and complete medical history of the patient before commencing infusional treatment, in order to identify possible risk factors. Although there is no clinical evidence in the international literature, we believe that our protocol could be of use in reducing life-threatening events, as no thromboembolic event of any kind occurred in our group. Our protocol included hydration of patients with normal saline before and after infusions, and the use of preventive treatments such as aspirin[13] and subcutaneous calcium heparin, which we believe played a pivotal role in controlling any hyperviscosity syndrome, and thus, in lessening the thromboembolic risk.

It is known that adjunctive immunosuppressive and steroid treatment may affect the occurrence of thromboembolic events,[21] but the reasons behind this are not known. A case–control study should be performed, but there are ethical difficulties in such as study because, in this kind of disorders, patients cannot be treated without adjunctive steroid treatment.

Furthermore, to make our protocol more complete and accurate, we also performed tests for congenital thrombophilia. It is difficult to evaluate the necessity of such tests, which are very expensive, as few investigations have been carried out.[14,15] Nevertheless, our results have highlighted some intriguing alterations (Table 2), indicating that results of these analyses are very heterogeneous and, although not predictive for thromboembolism by themselves, may be predictive if correlated with alterations in clinical and laboratory risk factors.

Currently, several theories of pathogenesis, which may contribute to the genesis of thromboembolic events, have been suggested: (i) increase in plasma viscosity,[17] due to a transient state of hyperproteinaemia with subsequent pseudohyponatraemia[22] and/or to a synergistic action of albumin and FG; (ii) increase in platelet number and activation; (iii) arterial vasospasm;[14] (iv) presence of activated coagulation factor XI;[23] (v) administration of IVIg as a single high dose;[24] and (vi) high infusion rate.[13] All these theories should also consider the pivotal role played by corticosteroids in the development of these events, through platelet activation, decrease in prostacyclin/nitric oxide and inhibition of the fibrinolytic system.[14]

Nevertheless, although all these theories are valid and worthy of further evaluation, we must consider that IVIg has been used successfully as a valid and safe treatment in the prevention of thromboembolism in some disorders such as antiphospholipid antibody syndrome[25,26] or Kawasaki's syndrome,[27] demonstrating a paradoxical effect. However, in such disorders, the benefit of removing the pathological antibodies is higher than the risk of triggering thromboembolic events. It is understood that the underlying disorder seems to play a pivotal role in the pathogenesis of these fatal events through still unknown mechanisms. Therefore, we believe that further investigations into this mechanism should be carried out.

In conclusion, our results indicate that IVIg administration, in combination with a meticulous and accurate protocol of screening and prevention of thrombophilia, is a successful, safe and very helpful treatment for AMBDs. We fully agree with those authors who consider thromboembolism related to IVIg as a rare event that may occur only if it is associated with a wider cohort of clinical and laboratory risk factors. We also agree with the concept that IVIg may be restarted in patients with IVIg-associated thrombotic complications.[14] and believe that evaluation of these patients by a haematologist could be of marked benefit. None of the patients in our study developed a superficial and/or deep venous or arterial thrombotic event, despite all the risk factors present, thus we believe that IVIg may simply be the final element required for a thrombotic event in a patient with a predisposing condition.

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