に、手術後感染症に対して IVIG が有効であると判断することは困難であり、今後、IVIG の有効性を適正に評価するには、IVIG 非使用群を対照とした前方的な無作為比較臨床試験が必要と考えられる。

E．結論

全身麻酔下手術症例の内、手術後 IVIG を使用した患者は 1.4%と低頻度に留まった。男女比は 1：0.14 と明らかに男性が多かった。対象疾患としては、敗血症 31.3%、腹膜炎 25%、創部感染症 25%が中心となっており、肺炎は 12.5%と低頻度であった。対象病原体についてはウイルスや真菌は認められず、殆どが細菌であり、手術部位からの細菌感染症に対して IVIG が使用されたものと考えられた。さらに IVIG 投与開始の判断基準となる項目、基準値などについて検討した結果、症例全体として平均値、中央値などからみる限りは基準に準じているものの、個々の症例について、手術後から IVIG 投与までの日数や発熱、発熱期間、抗生物質の投与日数など詳細に検討すると、一部の症例は重症例と判断しがたく、予防的あるいは慣習的に IVIG を使用されたものと考えられた。今後、手術後感染症に対する IVIG の適正使用を推進するためにも、手術後感染症に適合した投与基準を作成する必要があると考えられる。

F．健康危険情報

特になし

G．関連する研究発表

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分担研究報告書

術中大量出血時におけるクリオプレシピテートおよびフィブリノーゲン製剤投与の有効性に関する研究

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研究要旨
過去3年間の大量出血症例について、術式、頻度、出血量、輸血量、術中止血能検査結果について検討した。あわせて、術中血小板アフェレーシスの効果について検討した。

A．研究目的

術中大量出血時の血小板および血液凝固因子の質的・量的評価を行ない、出血量と止血能低下の相関関係を明らかにし、希釈性凝固障害の本体の解明と、それに対する有効な輸血・止血（凝固因子補充）療法の確立を目的とする。

B．研究方法

平成１６年１月１日から１８年１２月３１日の３年間に名古屋大学医学部附属病院で行われた全手術症例のうち、循環血液量を超える出血か、もしくは４Ｌ以上の出血をきたした症例、ＭＡＰ２０単位以上の輸血を行った症例をピックアップし、その発生頻度ならびにその状況と予後を調査した。その中で、止血能検査が術中繰り返し行われた症例について、出血量の推移と血液凝固能との相関について検討した。あわせて、術中血小板アフェレーシスを行った症例について、出血量と血小板数との関係、輸血量について検討した。

（倫理面への配慮）

名古屋大学医学部倫理委員会で研究計画の審査を受け、承認されて研究を実施した。

C．研究結果

大量出血を来たした症例は、８３例で内訳は以下であった。胸腹部大動脈瘤手術２４例、肝切除術１８例、肝移植術１３例、心臓手術８例、骨盤内蔵全摘術３例、腎癌手術２例、腹部大動脈瘤手術２例、膵頭十二指腸切除術２例、食道手術、胃切除術、大腸穿孔手術、大網充填術、仙骨部腫瘍切除術、脊椎悪性腫瘍手術、神経芽種手術、前置胎盤帝王切開術、卵巣腫瘍手術、右肺全摘術、脳動静脈奇形手術、各１例。また、大量出血を起こしやすい手術の３年間症例数は、胸腹部大動脈瘤手術９０例、肝切除術４８１例、肝移植術６０例、腎癌手術１０７例、腹部大動脈瘤手術９４例、全症例数は１３,４０９例であった。従って、発生率でみると、胸腹部大動脈瘤手術２６．７％、肝移植術２１．７％、肝切除術３．７％、腹部大動脈瘤手術２．１％、腎癌手術１．９％、全症例数に対する全大量出血症例は０．６％であった。

４７,２００ｍｌ以上出血し、ＭＡＰ１８４単位、ＦＦＰ２２５単位、血小板９５単位、フィブリノーゲン２３ｇ輸血したが、完全に止血し得た肝切除術の一例において、大量出血初期においてフィブリノーゲン値が５０

０ｍｇ／ｄｌ以上、血小板数５万／μｌ以上に維持され止血が得られた症例を確認した。

胸腹部大動脈瘤手術において平成１８年４月１日から平成１９年３月３１日までの１年間に血小板アフェレーシスを行った２７例とその直前１年間にアフェレーシスなしで行った３６例を比較検討し、アフェレーシス症例で出血量、輸血したＭＡＰ、血小板、ＦＦＰいずれにおいても有意に減少した。

D．考察

大量出血となる頻度の高い術式として、胸腹部大動脈瘤手術、肝移植術、肝切除術があり、それらだけで大量出血症例の２／３を占める。血小板フェレーシスは同種血小板輸血量を減らすのみならず、同種赤血球輸血、同種ＦＦＰの輸血量を減らし、総出血量も減らすことがわかった。

E．結論

過去の大量出血症例において、術中に止血能の検査が充分に行われていない症例もあった。超大量出血となっても輸血により完全な止血が得られる場合もあることがわかった。大量出血とならなかった症例も含めて前向き検討が重要と考えられ、大量出血となる可能性の高い、胸腹部大動脈瘤手術、肝移植術、肝切除術などにおいて、止血能を検査しながら、輸血療法を前向きに検討することが有用と考えられた。

F．健康危険情報

特になし

G．研究発表

１．論文発表

なし

２．学会発表

・「胸部大血管手術における血小板アフェレーシスの有用性について」梶田博史、西脇公俊　Cardiovascular Anesthesia vol.10S P131, 2006

・“The efficacy of bicarbonated Ringer's solution duling living-donor liver transplantations” A. Umeda, K. Nishiwaki, Y Shimada. ASA Annual Meeting P.246, 2006

H．知的財産権の出願・登録状況

なし

III. 研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yamamoto K, Honda T, MatsushitaT, Kojima T, Takamatsu J	Anti-HCV agent, ribavirin, elevates the activity of clotting factor VII in hemophilia patients: a possible mechanism of decreased events of bleeding in hemophiliacs by ribavirin	J Thromb H aemost	4	469-470	2006
Yamakage N, Ikejiri M, Okumura K, Takagi A, Murate T, Matsushita T, Naoe T, Yamamoto K, Takamatsu J, Yamazaki T, Hamaguchi M, Kojima T	A case of coagulation factor V deficiency caused by compound heterozygous mutations in the factor V gene	Haemophilia	12	172-178	2006
Yamamoto K, Takamatsu J, Saito H	Intravenous immunoglobulin therapy for acquired coagulation inhibitors: a review	Int J Hematol, in press			
Miyamoto K, Nishigami K, Nagaya N, Akutsu K, Chiku M, Kamei M, Soma T, Miyata S, Higashi M, Tanaka R, Nakatani T, Nonogi H, Takeshita S	Unblinded pilot study of autologous transplantation of bone marrow mononuclear cells in patients with thromboangiitis obliterans	Circulation	114	2679-2684	2006
Banno F, Kokame K, Okuda T, Honda S, Miyata S, Kato H, Tomiyama Y, Miyata T	Complete deficiency in ADAMTS13 is prothrombotic, but it alone is not sufficient to cause thrombotic thrombocytopenic purpura	Blood	107	3161-3166	2006
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宮田茂樹	外科周術期輸血トリガー値に関する考察	別冊・医学のあゆみ 輸血医療・医学の新展開	218 (6)	585-592	2006
中谷武嗣、宮田茂樹	人工弁・補助循環における抗血小板療法	抗血小板療法の新しい使い方		153-158	2006
高本滋、加藤栄史、池田、比留間潔、浅井隆善、星順隆、倉田義之	静注用免疫グロブリンの使用状況調査	厚生労働省科学研究費補助金「医療機関における血液製剤の適正使用の推進に関する研究」平成17年度報告書		7-20	2006

Ⅳ．研究成果の刊行物・別刷

Journal of Thrombosis and Haemostasis, 4: 469–487

LETTERS TO THE EDITOR

Anti-HCV agent, ribavirin, elevates the activity of clotting factor VII in patients with hemophilia: a possible mechanism of decreased events of bleeding in patients with hemophilia by ribavirin

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The combination therapy with ribavirin and interferon-α (IFN-α) has been reported to be more effective than IFN-α monotherapy for eradicating hepatitis C virus (HCV) [1,2], including patients with concomitant hemophilia [3]. We observed significant decreases in doses of clotting factors used for hemostatic therapy in hemophiliacs during ribavirin administration (e.g. 3780 units per month before ribavirin treatment and 1600 units per month during ribavirin on the average) [4]. In our hospital, 47 hemophilic patients who had been treated for chronic hepatitis C with IFN-α alone demonstrated no significant reduction in the use of clotting factor. This observation strongly suggests that the addition of ribavirin leads to the reduction of clotting factors used for bleeding in hemophiliacs. One suggestion comes from a case report that described an increase in warfarin dose requirement in a patient with heart valve prosthesis after starting this anti-HCV combination therapy [5].

These observations led us to investigate the ribavirin-induced change in vitamin K-dependent coagulation factors. To this purpose, we have measured the clotting activity of factor (F)VII, X, and prothrombin in hemophilic patients who were receiving the anti-HCV combination therapy. The protocol of therapy and analysis was approved by the Nagoya University institutional review board and written informed consent was obtained from each patient before treatment. Nine hemophilic patients, including seven hemophilia A and two hemophilia B (mean age ± SD: 42.5 ± 10.4 years old), whose characteristics were previously described [4], were entered in this study.

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The liver biopsy performed before starting the combination therapy did not show cirrhosis but chronic hepatitis in all patients analyzed. During this study, all patients were treated with the same 24-week regimen of IFN-α 2b (Intron A®, Schering Plough, K.K., Osaka, Japan) and oral ribavirin (600–800 mg day^{-1} of Rebetol; Schering-Plough, Kenilworth, NJ, USA). All statistical analyses were performed with STATA ver.7 software (STATA Corp., College Station, TX, USA) and the P-value < 0.05 was considered statistically significant.

The procoagulant activity of FVII in plasma has been elevated in all of nine ribavirin-treated hemophilic patients in comparison with that before ribavirin administration (Fig. 1A). The average and standard deviation for the elevation of FVII activity was 15.7% ± 8.8% ($P < 0.04$ in before vs. during ribavirin treatment; max. 28%; min. 5%). This elevation of FVII activity was independent of improvement of liver function (i.e. albumin, total billirubin, cholinesterase) in the patients (not shown). Only two patients, one has HIV infection and the other has hepatitis B virus concomitant with HCV, did not show a substantial elevation of FVII activity (i.e. 5% and 8%, respectively). We then measured activated FVII (FVIIa) levels in patients' plasma before and during ribavirin treatment using STACLOT® VIIa-rTF (Diagnostica Stago, Asnieres, France) [6] and observed substantial increases in FVIIa (e.g. 25.3 ± 14.8 mU mL^{-1}), which were almost compatible with elevation of FVII clotting activity. The plasma levels of FX and prothrombin were unchanged by ribavirin treatment in all of nine hemophilic patients (not shown). The elevation of FVII clotting activity by ribavirin is consistent with the previous observation of warfarin resistance in a ribavirin-treated patient [5].

To investigate the mechanism of ribavirin-induced elevation of FVII activity, we analyzed the gene expression of FVII in cultured normal human hepatocytes (Cambrex Bio Science Walkersville, Inc., Walkersville, MD, USA) or human hepatoma cell line, HepG2 cells (ATCC, Manassas, VA, USA),

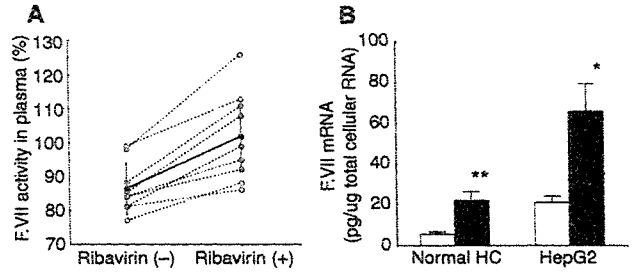

Fig. 1. Clotting activity of FVII in plasma of hemophilic patients and the mRNA expression of FVII in cultured human hepatocytes with or without ribavirin. (A) Each clotting activity of FVII in plasma of nine hemophilic patients before and at 4 weeks after starting ribavirin therapy was shown as open circle and dashed line, respectively. The average and SD of all patients was expressed as closed circle and error bar (without ribavirin: 86.3% ± 7.6%; with ribavirin: 102.0% ± 10.3%; $P < 0.04$). (B) Normal human hepatocytes or HepG2 cells had been cultured with (■) or without (□) ribavirin for 48 h. The mRNA expression of FVII was quantitated by real-time RT-PCR assay. Each value is expressed as the mean and SD from three sets of experiments. All of real-time RT-PCR assays were performed in duplicate. $*P < 0.02$; $**P < 0.01$.

which were cultured in medium with ribavirin at clinically therapeutic concentration (150 μg mL^{-1}) in the presence of IFN-α 2b (0.75 μg mL^{-1}; kindly provided by Schering Plough, K.K.). The expression level of mRNA for FVII, FX, and prothrombin, was determined by real-time quantitative RT-PCR with the ABI Prisms 7700 Sequence Detection (Perkin-Elmer Biosystems, Foster City, CA, USA) and SYBR Green PCR Kit (Perkin-Elmer Biosystems), according to the manufacturer's recommendations. The sequences of primer pairs used to quantify mRNA of the above genes were described in the NCBI Sequence Viewer. Variations in sample loading were assessed by measuring β-actin mRNA. Comparison of quantitative RT-PCR results between two groups was performed with the two-sample *t*-test. Welch's method was applied when variance between two groups was unequal (statistical significance: $P < 0.05$).

Significant induction of FVII mRNA was demonstrated in cultured normal hepatocytes (fourfold; $P < 0.01$) or HepG2 cells (threefold; $P < 0.02$) at 48 h after ribavirin treatment (Fig. 1B). No significant induction of mRNAs for FX and prothrombin was detected in ribavirin-treated cultured hepatocytes or HepG2 cells (not shown). In hepatocytes, ribavirin may stimulate to synthesize FVII by binding specifically to the promoter region of FVII gene (under current investigation).

It is possible that not only the induction of FVII but also changes in other coagulation factors during ribavirin therapy may be responsible for the decreased events of bleeding in hemophiliacs. However, the elevation of FVII activity in plasma could contribute most to the increased hemostatic potential in hemophilic patients because the cell-based tissue factor-activated FVII would play a central role in initiating coagulation and in activating platelets followed by large scale thrombin generation [7]. Clinically, recombinant activated FVII has been widely used as an antidote to control and prevent excessive hemorrhage in hemophilic patients with inhibitors [8]. Meanwhile, it was reported that even 10–20% of increase in plasma FVII/FVIIa would be an independent risk factor for coronary heart disease in healthy individuals [9,10], suggesting that a substantial elevation of endogenous FVII levels could result in an increased thrombotic potential. In general, the occurrence of spontaneous bleeding events in hemophiliacs is dependent on the critical hemostatic balance. In these conditions, 15–20% elevation of intrinsic FVII activity in plasma (Fig. 1A), because of the continuous induction of endogenous FVII by ribavirin (Fig. 1B), would contribute to the prevention from spontaneous bleeding in hemophiliacs. As a half-life of FVII in plasma is the shortest in all of coagulation factors, the continuous induction of FVII can maintain or increase the hemostatic value *in vivo*. If the prophylaxis to bleeding in hemophilic patients by ribavirin treatment were executable, it would result in much improvement of quality of their life and in large reduction of medical expenses in the country.

Acknowledgements

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CASE REPORT

A case of coagulation factor V deficiency caused by compound heterozygous mutations in the factor V gene

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Summary. We investigated the molecular basis of a severe factor V (FV) deficiency in a Japanese female, and identified two distinct mutations in the FV gene, a novel cytosine insertion (1943insC) and a previously reported point mutation (A5279G). We expected the patient to be a compound heterozygote for those mutations, as a 1943insC, but not an A5279G, was found in the mother and a sibling. The 1943insC will cause a frame-shift after ^{590}Gln, resulting in amino acid substitutions with two abnormal residues followed by a stop codon in the FV A2 domain (FS592X). The A5279G will cause an amino acid alteration in the FV A3 domain (Y1702C), which has been observed in several ethnic groups. We found that both mutant mRNAs were detected by reverse transcriptase polymerase chain reaction (RT-PCR) in the patient's platelets, whereas no FV antigen and activity were detected in plasma. On the one hand, the RT-PCR signal from the FS592X-FV mutant mRNA was markedly reduced, suggesting that the RNA surveillance system would eliminate most of the abnormal FS592X-FV transcripts with a premature termination. On the other hand, expression analyses revealed that only small amounts of Y1702C-FV with a low specific activity were secreted, and that the FS592X-FV was not detected in cultured media. These data indicated that both mutant FV molecules would be impaired, at least in part, during the post-transcriptional process of protein synthesis and/or in secretion. Taken together, it seems to suggest that each gene mutation could be separately responsible for severe FV deficiency, while this phenotype is due to the in-trans combination of the two defects.

Keywords: compound heterozygote, expression study, factor V deficiency, gene mutation, parahaemophilia, reverse transcriptase polymerase chain reaction

Introduction

Human coagulation factor V (FV) is a large (molecular weight of 330 kDa) single-chain glycoprotein that circulates in blood as an inactive procoagulant cofactor and plays an important role in the blood coagulation cascade [1,2]. The cDNA clones encoding human FV have been isolated [3], and the human FV gene has been mapped to chromosome 1q23 and spans approximately 80 kb of DNA [4]. The human FV gene consists of 25 exons and 24 introns, and the mRNA encodes 2224-amino acid protein containing a leader peptide of 28 amino acids [5]. It is comprised of three homologous A-type domains, two homologous C-type domains, and a heavily glycosylated B domain and shows a linear domain structure (A1-A2-B-A3-C1-C2) homologous to factor VIII (FVIII) with 35–40% homology existing in both the A-type and C-type domains [1,2]. Thrombin activates FV by the proteolytic release of the B domain, resulting in the formation of a non-covalently bound heterodimeric molecule of the heavy chain (residues 1–709, A1-A2 domains) and

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light chain (residues 1546–2196, A3-C1-C2 domains), activated FV (FVa) [6].

Activated FV functions as an essential molecule of the prothrombinase complex that catalyses the conversion of prothrombin to thrombin by factor Xa in the presence of calcium and a phospholipid membrane. The procoagulant function of FVa is down-regulated by the anticoagulant serine protease, an activated protein C (APC) [7] that cleaves to FVa at Arg306, Arg506 and Arg679, resulting in a loss of FVa activity. On the other hand, FV cleaved by APC before thrombin activation, FVac, shows an anticoagulant function as a cofactor in the APC-mediated inactivation of activated FVIII (FVIIIa). Thus, FV plays an important role in the procoagulant pathway as well as in the protein C anticoagulant pathway [8].

Around 75% of FV in blood is in the plasma, with the residual FV in the α-granules of blood platelets. In plasma, FV exists in two isoforms (FV1 and FV2) that have different molecular weights because of partial N-linked glycosylation in the C2 domain [9]. FV1 and FV2 have different characteristics in terms of procoagulant activity, inactivation by APC, and their anticoagulant function in the protein C pathway [10]. Consequently, FV1 has the overall potential to generate more thrombin than FV2.

Factor V deficiency, also known as parahaemophilia, was first described in 1947 by Owren [11]. It is a rare bleeding disorder inherited in an autosomal recessive manner with an incidence of about one in 1 million [1]. Bleeding symptoms in FV-deficient patients are varied; heterozygotes are usually asymptomatic, whereas homozygotes may show a mild, moderate or severe bleeding tendency.

To date, more than 40 identified cases of mutations in the FV gene were described in FV-deficient patients in the homozygous or compound heterozygous state [12]. In this study, we investigated the molecular basis of severe FV deficiency in a Japanese patient, and demonstrated that she was another compound heterozygote for FV gene mutations resulting in the post-transcriptional impairment of FV synthesis and/or secretion.

Materials and methods

Preparation of plasma, genomic DNA and total RNA of platelets

Ethical approval for the study was obtained from the Ethics Committee of the Nagoya University School of Medicine. Following informed consent, blood samples from the patient, family members and volunteers were collected in a 1:10 volume of 3.13% sodium citrate. Plasma was separated by centrifugation at 2000 *g* for 20 min, and aliquots were stored at −70°C until use. The patient had not received substitution therapy for 3 months prior to blood sampling for FV antigen and activity measurements. Genomic DNA was isolated from peripheral blood leucocytes as described previously [13]. Citrated blood samples from the patient and her sibling were centrifuged at 250 *g* for 5 min at 4°C to collect platelet-rich plasma. Subsequently, the total RNA was extracted from platelets by RNA STAT-60 (Tel-Test Inc., Friendswood, TX, USA), and subjected to a reverse transcription (RT) reaction as described below.

FV antigen and activity assays

Factor V procoagulant activity and FV antigen in plasma as well as in culture media containing recombinant FV proteins were measured as described below. FV procoagulant activity was measured by one-stage clotting assay, of which the sensitivity limit and the normal range are 3% and 70–135%, respectively, using human FV-deficient plasma (George King Bio-Medical, Overland, KS, USA) and Simplastin (Biomerieux, Inc., Durham, NC, USA). FV antigen was measured by enzyme-linked immunosorbent assay (ELISA), of which the sensitivity limit and the normal range are 1% and 70–135%, respectively, using an affinity-purified sheep anti-human FV IgG as a coating antibody with a peroxidase-conjugated sheep anti-FV antibody as a second antibody, according to the manufacturer's protocol (Cedarlane Lab. Ltd, Hornby, ON, Canada). In both assays, FV levels were expressed as a percentage of control plasma pooled from 25 healthy individuals.

PCR and DNA sequencing

The polymerase chain reaction (PCR) primers were synthesized to amplify all exons and splicing junctions of the FV gene, based on the reported genomic DNA sequence of human FV (GenBank Z99572). Information of the primer sequences is available from the authors. PCR amplification of the FV gene was performed with rTaq polymerase or exTaq polymerase (Takara Bio Inc., Kusatsu, Japan) in 30–35 cycles under the following conditions: 30 s denaturing at 94°C, 30 s annealing at 47–58°C and 30 s extension at 72°C.

Polymerase chain reaction products were separated by agarose gel electrophoresis, and authentic fragments were collected and purified with a QUAEX II kit (Qiagen K.K., Tokyo, Japan). The samples were then directly sequenced by a Big Dye Terminator

Cycle Sequencing FS Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) using forward or reverse PCR primers, according to the manufacturer's protocol. The sequencing products were then precipitated with 0.15 M NaOAc (pH 8.0) and cold ethanol, washed once with 70% ethanol, dried, resuspended in 25 μL of Template Suspension Reagent (Applied Biosystems), and analysed by an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

Analysis of FV mRNA

To investigate the presence of FV transcripts from the mutant allele in platelets, we analysed platelet RNAs from the proband by mRNA-based PCR-restriction fragment length polymorphisms (RFLPs). In brief, total RNA extracted from the platelets was reverse-transcribed using the respective gene-specific primers: 12GSP (5′-TCTGTTCTGGTAATCA TAGT-3′) for 1943insC or 15GSP (5′-GTGCTG TTTATTGCCATTTT-3′) for A5279G, and Super Script II RT reverse transcriptase (Invitrogen Japan, Tokyo, Japan). To detect the 1943insC mutation, a nested PCR was performed using the following primers: 12rPCR-UP (5′-CCCTATAGCATTTAC CCTCA-3′) and 12GPS for the first PCR, and 12mut-UP (5′-ACTTCTGTAGTGTGGGGggCC-3′; bold lower case characters are mismatched nucleotides) and 12 M-LW (5′-TTCATCATCATCTGGG-ATAC-3′) for the second PCR, introducing a new *Apa*I restriction site in the mutant PCR products, as a single PCR using the first or second PCR primer set failed to amplify authentic PCR products. The 1943insC mutant RT-PCR products would yield 19- and 221-bp fragments, whereas the wild-type products would not be digested (239 bp). To detect the A5279G mutation, PCR was performed with the following primers: 15 M-UP (5′-AAAAATCATCA GAGGGAAAG-3′) and 15mut-LW (5′-CTGGGT TCACAGCTGAcTAG-3′) introducing a *Spe*I restriction site in the wild-type PCR products. Thus, the wild-type RT-PCR products would yield 18- and 159-bp fragments, while the A5279G mutant products would not be digested (177 bp). These fragments were run on a 4% agarose gel and stained with ethidium bromide. We evaluated the allele-specific mRNA levels by the quantitative densitometric analyses using the NIH image software (version 1.62) (National Institutes of Health, Bethesda, MD, USA).

Preparation of mutant FV expression vectors

We prepared individual FV expression vectors bearing the identified mutations, 1943insC (FS592X; the initial Met residue is denoted amino acid +1) and A5279G (Y1702C), based on pMT2 containing a full-length cDNA of human FV (pMT2-FV). Both mutations were introduced individually into the pMT2-FV expression vector using the recombinant PCR method described elsewhere [14]. After recombinant PCRs, each DNA fragment encoding the 1943insC or A5279G mutation was isolated as *Bsp*36I-*Bsp*EI or *Bsp*MI-*Sna*BI fragments, and separately replaced into the appropriate position for the pMT2/FV expression vector. DNA sequencing confirmed that no unexpected mutation was found in any of the whole mutant inserts in either construct.

Transient expression of recombinant FVs in COS-1 cells

African green monkey kidney COS-1 cells were cultured in a 5% CO_2 humidified atmosphere at 37°C in Dulbecco modified Eagle medium (DMEM; Invitrogen) supplemented with fetal calf serum (10%), glutamine (1%), and antibiotics (penicillin and streptomycin, 100 IU mL^{-1} and 100 μg mL^{-1} respectively). Cells in 30-mm dishes were transfected with either wild type or individual mutant plasmids using the Fu-GENE6™ transfection reagent (Roche Diagnostics K.K., Tokyo, Japan) according to the manufacturer's instructions. After 48-h culture of the transfected cells in serum-free DMEM, conditioned media containing the secreted recombinant proteins were collected, then concentrated using Centrisart I (cut off MW 20000; Sartorius, Goettingen, Germany), and subjected to one-stage clotting assay as well as ELISA (Cedarlane Lab. Ltd) for recombinant FV antigen measurements as described above.

Results and discussion

Case report

The patient (individual II-1, Fig. 1) is a 39-year-old Japanese woman who had recurrent episodes of bleeding such as epistaxis, joint region haematoma and hypermenorrhea, which were treated with FV replacement therapy by transfusion of fresh frozen plasma. When the patient was 4 years old, she had been diagnosed as having coagulation FV deficiency, since laboratory tests revealed that the prothrombin time and the activated partial thromboplastin time were prolonged, and FV activity was below the measurable limit. There was no history of bleeding tendencies in her other family members tested, since FV activities in plasma of both her mother and a sibling were 65%, suggesting that they might be

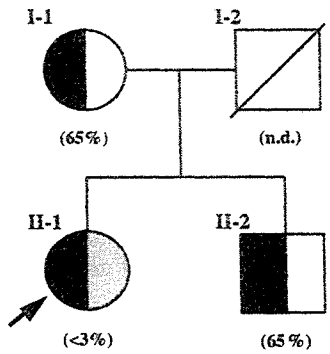

Fig. 1. Pedigree of the factor V-deficient family. The propositus is subject II-1 (arrow). Circle and square indicates male and female respectively. Values in parentheses represent plasma factor V activities (n.d., not done). Subjects with 1943insC and A5279G mutations are demonstrated with solid and shaded areas respectively.

heterozygous for FV-deficiency causing mutation. Consanguinity in the family was excluded.

DNA sequencing

In order to identify causative FV gene mutation(s) in such an FV-deficient patient, we analysed nucleotide sequences of all 25 exons and exon–intron boundaries of the FV gene. Results from direct sequencing of the FV gene revealed that the patient had a C insertion in three consecutive cytosine nucleotides [^{589}Thr(ACC)–^{590}Gln(CAG)] in exon 12 at nucleotide positions 1940–1942 (1943insC), and an A–G transition in exon 15 at nucleotide position 5279 (A5279G) (Fig. 2). DNA samples from her mother and brother also showed heterozygosity for the 1943insC mutation, but no A5279G mutation (data not shown), which are consistent with the data of plasma FV activity, i.e. about half that of normal subjects; 1943insC is a novel mutation, which can cause a frame-shift resulting in a substitution of the amino acids after ^{590}Gln with two abnormal residues (^{590}Pro–^{591}Glu) followed by a stop codon (FS592X). The A5279G will cause the amino acid substitution Y1702C, which was previously designated FV Seoul 2 [15]. The A5279G FV gene mutation has also been found in Italian and Slovenian subjects [16,17], and is thought to be a very ancient and/or recurrent mutation. In this study, we demonstrated that this mutation also occurred in a Japanese subject, suggesting that the A5279G might be a hot-spot mutation rather than a founder mutation.

mRNA analysis (RT-PCR RFLPs)

We analysed the expression of mutant FV gene transcripts from the patient's platelets by mRNA-mediated PCR-RFLPs (RT-PCR RFLPs). For 1943insC (FS592X-FV mRNA), the nested RT-PCR followed by *ApaI* digestion yielded 239- and 221-bp bands, representing transcripts from the normal and mutant alleles, respectively, although the mutant signal was markedly reduced (Fig. 3a). For A5279G (Y1702C-FV mRNA), the RT-PCR products digested with *SpeI* yielded 159- and 177-bp bands, representing transcripts from the normal and mutant alleles respectively (Fig. 3b). Thus, both mutant transcripts were present in the patient's platelets. However, the FS592X-FV mRNA signal was markedly reduced to 12% of the wild type in the quantitative densitometric analysis, whereas the Y1702C-FV mRNA signal was more intense (250% of the wild type). These data suggest that the patient could be compound heterozygous for these mutations, and that her RNA surveillance system would eliminate most of the FV mRNA derived from the mutant allele encoding a premature termination by the frame-shift mutation, FS592X [18]. On the other

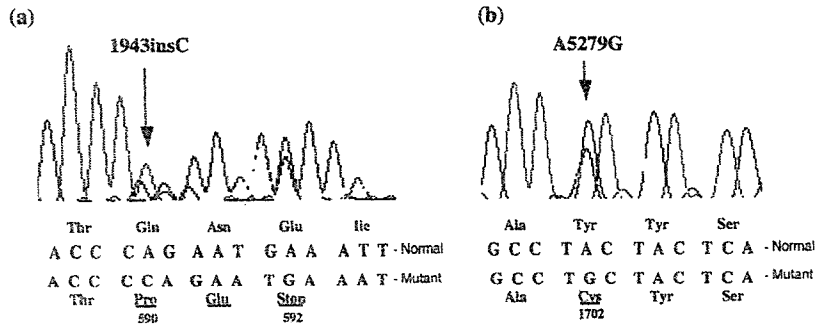

Fig. 2. Patient's nucleotide and amino acid sequences surrounding the mutations. (a) Nucleotide and amino acid sequences surrounding 1943insC. Arrow indicates mutation point. The mutation predicts an abnormal sequence of two amino acid residues and a stop codon. (b) Nucleotide and amino acid sequences surrounding A5279G. Arrow denotes mutation point. Patient's heterozygous sequencing pattern is shown.

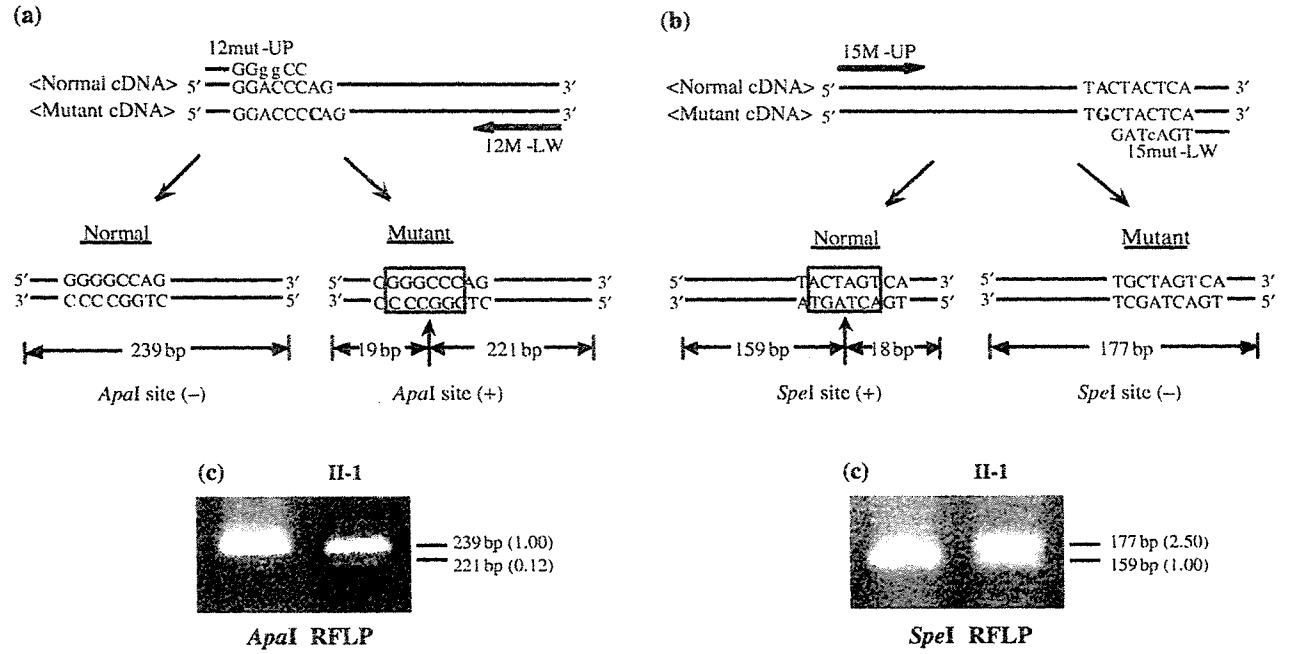

Fig. 3. Detection of mutant factor V mRNAs in patient's platelets. (a) Reverse transcriptase polymerase chain reaction (RT-PCR) products (239 bp) using primers 12mut-UP and 12M-LW were digested with *ApaI*, then electrophoresed on 4% NuSieve 3:1 agarose gel. Wild-type RT-PCR product migrated as an uncleaved 239-bp band, while FS592X (1943insC') RT-PCR product is represented by an *ApaI* cleaved 221-bp band. II-1, proband; C, control donor. Numbers in parentheses are relative amounts of signals measured by the quantitative densitometric analysis (wild type = 1.00). (b) RT-PCR products (177 bp) using primers 15M-UP and 15mut-LW were digested with *SpeI*, then electrophoresed on 4% NuSieve 3:1 agarose gel. Wild-type RT-PCR product migrated as *SpeI* cleaved 159-bp band, whereas Y1702C (A5279G) RT-PCR product is represented by an uncleaved 177-bp band. II-1, proband; C, control donor. Numbers in parentheses are relative amounts of signals measured by the quantitative densitometric analysis (wild type = 1.00).

hand, both the FV antigen and activity in her plasma were below the detectable limit, suggesting that the mutant Y1702C-FV might be impaired during the post-transcriptional process of protein synthesis and/or in secretion. Indeed, it has also been previously reported that the FV allele bearing the Y1702C mutation was expressed normally at the mRNA level, but not at the protein level in plasma [15].

Expression of wild type and mutant recombinant FVs in COS-1 cells

It is important to determine the patient's phenotype on Met1736Val polymorphism, as it will exert a great influence on the expression level of the recombinant FV [19]. Sequence analysis revealed that the patient was homozygous for 1736Met, which is the same phenotype encoded in the pMT2-FV, and thus its influence may not be revealed in expression experiments for her Y1702C-FV.

We investigated the effects of the identified mutants on FV secretion by conducting transient transfection experiments in COS-1 cells, which do not express endogenous FV. We observed that the wild type recombinant FV proteins were secreted efficiently into culture media with an adequate specific activity (0.94), but that the mutant Y1702C-FV showed an impaired secretion (1.8% of the wild type) and inadequate FV procoagulant activity (0.56) (Fig. 4). These data tend to support the conclusion that the Y1702C mutation could be causative for the FV deficiency as reported previously [15]. Indeed, plasma levels of FV activity in her mother and brother, who had only the Y1702C mutation in heterozygous, were reduced to 65% of normal. The 1702Y is a highly conserved amino acid not only in FV molecules among various species, but also in human FVIII and ceruloplasmin [15]. Moreover, an X-ray crystal structure analysis of wild type FV has demonstrated that the FV Y1702C mutation leads to the disappearance of two hydrogen bonds with P1618, and that its structure was significantly altered by a new hydrogen bond bridge formed between this cysteine and one of the other free cysteines [15]. These data suggest that 1702Y may play an important role in maintaining the structure and function of the FV molecule.

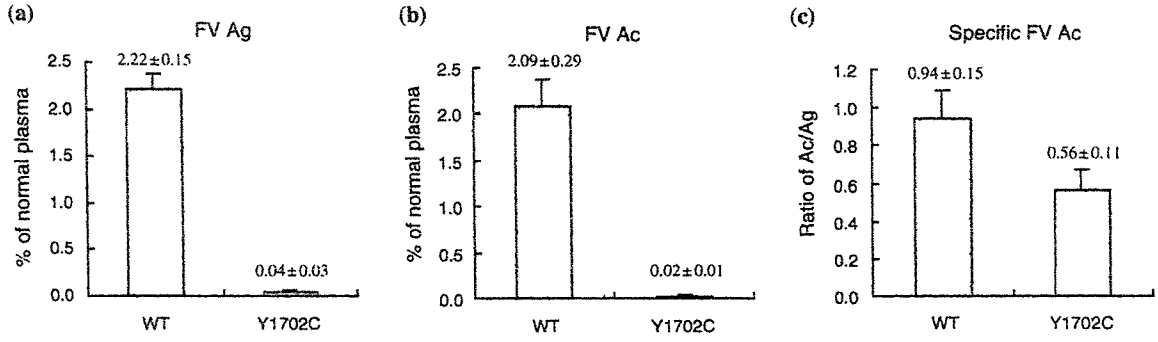

Fig. 4. Transient expression of wild-type factor V (FV) and 1702C mutant FV in COS-1 cells. Plasmids containing wild type (pMT2/FV) or mutant (pMT2/FV-Y1702C) FV cDNA were transiently transfected in COS-1 cells using FuGene reagent. Antigen and activity levels of recombinant FVs were measured in conditioned media 48 h after transfection (a, FV antigen; b, FV activity; c, FV-specific activity). Bars represent mean values ± SD of three independent experiments, each performed in duplicate. FV levels were expressed as percentage of normal pooled plasma from 25 healthy individuals.

On the other hand, recombinant FS592X-FV molecule was not detected in cultured media of the transfected COS-1 cells (data not shown). The transcripts of FS592X-FV were detected in the patient's platelets, but were found to be markedly reduced compared with normal allele transcripts (Fig. 3a). Moreover, as the FS592X-FV is a truncated molecule in the A2 domain, it would not be processed normally as reported for other mutant coagulation factors [20,21].

Conclusion

In this study, we investigated the molecular basis of a severe coagulation FV deficiency in a Japanese woman and identified two distinct mutations (1943insC/FS592X and A5279G/Y1702C) in her FV gene. The data indicated that both mutant FV molecules would be impaired, at least in part, during the post-transcriptional process of protein synthesis and/or in secretion. Taken together with the above observations, it seems to suggest that each mutation could be separately responsible for severe FV deficiency, while this phenotype is due to the in-trans combination of the two defects.

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Review Article

Intravenous Immunoglobulin Therapy for Acquired Coagulation Inhibitors: A Review

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Abstract

Intravenous immunoglobulin (IVIG) therapy has been used for autoimmune diseases and disorders involving autoantibodies, including coagulation inhibitors. In this review, we have evaluated the efficacy and safety of IVIG therapy for acquired coagulation inhibitors, including factor VIII inhibitor, and for acquired von Willebrand syndrome on the basis of 44 reports published between 1965 and 2005. Among 35 patients with factor VIII inhibitor, we estimated the efficacy of IVIG therapy alone (which includes complete remissions and partial responses with a clinical benefit) to be 30% (11 cases), whereas the response to combination therapy with IVIG plus immunosuppressive agents (eg, corticosteroid, cyclophosphamide) seemed to be better (approximately 70%, 33/45 cases) than with IVIG therapy alone. In acquired von Willebrand syndrome, the efficacy of IVIG therapy was estimated to be 30%. The response to IVIG therapy appears to occur rapidly, and coagulation inhibitors seem to be neutralized immediately. Moreover, severe complications or side effects rarely occur during IVIG treatment. IVIG therapy thus may be considered one choice for treating acquired coagulation inhibitors, although its efficacy improves when used in combination with immunosuppressive agents.
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Key words: Intravenous immunoglobulin therapy; Acquired coagulation inhibitors; Autoimmune disease; Factor VIII inhibitor; von Willebrand syndrome

1. Introduction

Intravenous immunoglobulin (IVIG), a highly purified immunoglobulin G (IgG) fraction derived from pooled human plasma, is currently one of the most widely used plasma components in the world [1,2]. It was originally introduced as replacement therapy for patients with primary immunodeficiency disorders. In 1981, Imbach et al reported a serendipitous observation that a high-dose infusion of IVIG (2 g/kg of body weight infused over 5 days) was able to transiently increase the platelet count in children with idiopathic thrombocytopenic purpura (ITP) [3]. With the encouragement of this and other reports on ITP [4], the clinical applications of IVIG have increased markedly over the past 25 years to include many autoimmune diseases. IVIG has been shown to be efficacious in clinical trials for graft-versus-host disease [5], myasthenia gravis [6], Guillain-Barré syndrome [7], Kawasaki disease [8], and chronic inflammatory demyelinating polyneuropathy [9]. It has also been used to treat immune neutropenia and for coagulation inhibitors [10-12], but its efficacy and safety have not been firmly established.

Coagulation inhibitors, antibodies against individual clotting factors, interfere with blood coagulation. The most common coagulation inhibitor is factor VIII inhibitor, an antibody against factor VIII that neutralizes the coagulant activity of factor VIII. Factor VIII inhibitor develops in patients with hemophilia A as an alloantibody after replacement therapy or spontaneously as an autoantibody in nonhemophilic patients [13], including postpartum patients and those with autoimmune disease, malignancy, or diabetes [14]. Once developed in such patients, factor VIII inhibitor poses a serious problem for the management of bleeding episodes, because any infused factor VIII will be rapidly neutralized and will not be available to induce hemostasis [15]. Although IVIG therapy has been used as one of the immunotherapies

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for eradicating coagulation inhibitors, such an indication is considered off label [2].

The aim of this review is to examine the efficacy and safety of IVIG therapy in patients with acquired inhibitors against factors VIII, IX, or V, and in patients with acquired von Willebrand disease. Cases with lupus anticoagulant were not included in this review. An electronic search of the Medline/PubMed database from 1965 to 2005 was performed to identify relevant articles. This search yielded 108 citations, 72 of which were considered appropriate and reviewed. The bibliography of each review paper was examined to identify articles that may have been missed by our electronic searches.

2. History

In 1983, Nilsson et al reported an interesting observation [11]. A patient with severe hemophilia B and factor IX inhibitor was treated with extracorporeal protein A–Sepharose adsorption to remove the inhibitor, followed by the administration of factor IX concentrate and cyclophosphamide. This procedure produced a 15-fold increase in factor IX inhibitor on one occasion but did not cause any increase of the inhibitor titer on another occasion, when 5 g of IVIG was also given to the patient to restore the reduced IgG level. The investigators suggested that the administration of IVIG appeared to suppress antibody synthesis in hemophilia B patients with factor IX inhibitor.

Three groups of investigators reported the use of IVIG in the management of factor VIII inhibitors in 1984 [12,16,17]. IVIG therapy combined with vincristine produced a transient disappearance of acquired factor VIII inhibitor along with a slow rise of factor VIII activity in a 13-year-old boy with autoimmune disease [16]. IVIG therapy was ineffective in 2 patients with hemophilia A inhibitor [17]. Sultan et al [12] reported that IVIG therapy (0.4 g/kg body weight per day for 5 days) resulted in the rapid, marked, and prolonged suppression of factor VIII inhibitor in 2 patients with acquired factor VIII antibody (autoantibody) but that it had little or no effect in 2 hemophilic patients with factor VIII antibody (alloantibody). They showed by in vitro experiments that IVIG preparations were able to neutralize the anti–factor VIII activity of the patients' plasma and the IgG fraction of the patients' sera. Many articles were subsequently published on the effect of IVIG on acquired factor VIII inhibitors, as is discussed later.

3. Possible Mechanisms of Action

The rapid rise in the platelet count in ITP following IVIG administration is thought to occur through binding to and blocking Fcγ receptors on macrophages, thereby preventing the removal of antibody-coated platelets by the reticuloendothelial system in the spleen and liver [4]. This mechanism, however, does not appear to explain the effect on coagulation inhibitors.

Several hypotheses on the mechanisms of action of IVIG on factor VIII inhibitor have been put forward. Sultan et al and Kazatchkine and Kaveri postulated that anti-idiotypic antibodies present in IVIG preparations neutralize factor VIII autoantibodies [12,18]. $F(ab')_2$ fragments from IVIG preparations inhibited anti–factor VIII activity in $F(ab')_2$ fragments from the patient's plasma. Anti–factor VIII $F(ab')_2$ fragments were specifically retained on an affinity column of Sepharose-bound $F(ab')_2$ from IVIG, indicating that a direct interaction occurred through the antibody-binding sites of both immunoglobulins [19]. Anti-idiotypes against various autoantibodies were shown to be present in pooled normal human polyspecific immunoglobulin. In addition, IgG prepared from elderly donors and multiparous women was reported to contain a higher frequency of neutralizing antibodies against factor VIII autoantibodies [20]. It is puzzling that such an in vitro antibody-neutralizing effect was not always demonstrated, even though in vivo administration of IVIG produced a marked reduction of the inhibitor titer [21,22].

The fall in inhibitor titer following IVIG therapy without simultaneous immunosuppressive treatment appears to be rapid (within several days) in most cases [12,23,24] but is slow (more than 10 days) in others [22,25]. There must be slow effects of IVIG on autoantibody production. In addition to its direct and immediate action on antibodies, IVIG has been proposed to suppress antibody formation by B-cells, a process mediated through the down-regulation of Fcγ receptors [26]. Furthermore, IVIG may induce T-cell suppressor activity [27]. These observations taken together suggest that IVIG exerts its effect on the inhibitor titer through more than one mode of action.

4. Efficacy

4.1. Against Factor VIII Inhibitor

We extensively reviewed the international literature published from 1965 to 2005. The typical IVIG dosage used for treating factor VIII inhibitor was 0.4 g/kg per day for 5 consecutive days.

The efficacy criteria (ie, the response to IVIG therapy) were as follows [28]: Complete remission (CR) was defined as the disappearance of the inhibitor, partial response (PR) was defined as a decrease in the inhibitor titer by at least 25% of the baseline value, and failure was defined as other than CR and PR.

In Table 1, we present all of the cases in which the efficacy of IVIG treatment alone was evaluated [12,22-25,28-40]. The response to IVIG therapy alone was failure in 11 cases (31.4%) and PR in 21 cases (60.0%), but with a subsequent clinical benefit in only 8 patients. Finally, 3 patients (8.6%) achieved CR. The efficacy of IVIG therapy alone, which includes CR and PR with a clinical benefit, among these 35 patients was estimated to be 31.4% (11 cases). In most cases of CR or PR, the response to IVIG treatment was rapid, and factor VIII inhibitor seemed to be neutralized immediately.

We summarize the responses to combined therapy with IVIG plus immunosuppressive agents in Table 2 [21,25, 28,32,35,38-52]. The response to IVIG plus steroid and/or cyclophosphamide therapy was better than to IVIG treatment alone. CR was achieved in 19 (73%) of 26 patients who were treated with IVIG plus steroid. In addition, 14 (74%) of 19 patients who received IVIG plus steroid and

Table 1.
Evaluable Patients from the Literature with Acquired Factor VIII Inhibitor Who Were Treated with Intravenous Immunoglobulin (IVIG)*

No.	Reference	Sex/Age, y	Associated Disease	IVIG Dosage, g/kg per d	Inhibitor Titer, Bethesda U		Response	Clinical Outcome
					Before	Nadir (d†)		
1	Hudak et al [29]	F/40	Postpartum	0.5 × 5 d	16	<1 (105)	CR	Sustained remission
2	Schwartz et al [25]	M/68	CLL	1 × 2 d	1	0 (14)	CR	Sustained remission
3	Schwartz et al [25]	F/83	Diabetes	1 × 2 d	0.9	0 (61)	CR	Sustained remission
4	Sultan et al [12]	M/62	Idiopathic	0.4 × 5 d	25,000	550 (3)	PR	No clinical benefit‡
5	Sultan et al [12]	F/29	Postpartum	0.4 × 5 d	10,500	1000 (3)	PR	No clinical benefit
6	Zimmermann et al [30]	F/64	Idiopathic	0.5 × 8 d	75	10 (25)	PR	Clinical benefit‡
7	Zimmermann et al [30]	F/70	Idiopathic	0.5 × 8 d	51	3.8 (9)	PR	Clinical benefit
8	Newland et al [22]	F/71	Diabetes	0.4 × 5 d	50	20 (45)	PR	Clinical benefit
9	Heyman et al [31]	M/64	Idiopathic	0.4 × 5 d	47	28 (17)	PR	No clinical benefit
10	Nishida et al [23]	F/39	Idiopathic	0.4 × 5d	115	17 (3)	PR	No clinical benefit
11	Schwerdtfeger et al [32]	F/31	Postpartum	0.5 × 5 d	420	104 (6)	PR	No clinical benefit
12	Sultan et al [33]	M/78	NA	0.4 × 5 d	42	20 (30)	PR	No clinical benefit
13	Sultan et al [33]	M/72	Carcinoma	0.4 × 5 d	38	10 (5)	PR	Transient benefit
14	Schwartz et al [25]	M/54	Alcoholism	1 × 2 d	1228	208 (7)	PR	No clinical benefit
15	Schwartz et al [25]	F/72	Idiopathic	1 × 2 d	880	570 (48)	PR	No clinical benefit
16	Schwartz et al [25]	F/25	Idiopathic	1 × 2 d	280	1.9 (57)	PR	Clinical benefit
17	Schwartz et al [25]	F/38	Postpartum	1 × 2 d	102	56 (22)	PR	Clinical benefit
18	Schwartz et al [25]	M/77	Carcinoma	0.4 × 5 d	39	24 (3)	PR	No clinical benefit
19	Schwartz et al [25]	M/60	Griseofulvin	0.4 × 5 d	29	18 (19)	PR	No clinical benefit
20	Crenier et al [28]	M/65	Cardiomyopathy	0.4 × 5 d	120	72 (30)	PR	No clinical benefit
21	Crenier et al [28]	M/74	Bronchitis	0.4 × 5 d	24	12 (7)	PR	No clinical benefit
22	Michiels et al [24]	F/31	Postpartum	0.5 × 5 d	12	1 (11)	PR	Clinical benefit
23	Lafferty et al [34]	F/42	SLE	0.4 × 5 d	500	185 (NA)	PR	Clinical benefit
24	Walsh et al [35]	F/72	Cholecystitis	30 g × 1 d	6	NA	PR	Clinical benefit
25	Hiller et al [36]	M/57	Surgery	30 g × 5 d	24	20 (2)	F	Transient benefit
26	Casas et al [37]	M/70	Lymphoma	0.4 × 7 d	8.6	35 (NA)	F	Transient benefit
27	Sultan et al [33]	M/45	Vasculitis	0.4 × 5 d	25	28 (NA)	F	NA
28	Pignone et al [38]	F/66	RA	0.4 × 6 d	13	26 (7)	F	NA
29	Hauser et al [39]	F/29	Postpartum	0.4 × 5 d	10	110 (NA)	F	NA
30	Mateo et al [40]	F/82	CLL	0.4 × 5 d	9.5	10 (30)	F	NA
31	Schwartz et al [25]	M/64	Diabetes	1 × 2 d	452	340 (6)	F	No clinical benefit
32	Schwartz et al [25]	F/83	LA	0.4 × 5 d	102	96 (5)	F	No clinical benefit
33	Schwartz et al [25]	F/48	Idiopathic	1 × 2 d	59	46 (2)	F	No clinical benefit
34	Schwartz et al [25]	M/73	Carcinoma	0.4 × 5 d	42	108 (5)	F	No clinical benefit
35	Schwartz et al [25]	M/62	Idiopathic	1 × 2 d	1.4	1.4 (11)	F	No clinical benefit

*CR indicates complete remission; CLL, chronic lymphocytic leukemia; PR, partial response; NA, not available; SLE, systemic lupus erythematosus; F, treatment failure; RA, rheumatoid arthritis; LA, lupus anticoagulant.

†Number of days after starting IVIG treatment.

‡Subjective evaluation by the doctors in charge.

cyclophosphamide reached CR. Only 2 cases of treatment with IVIG plus cyclophosphamide were reported, and these patients achieved CR [52]. Conversely, 18 (75%) of 24 patients treated with steroid plus cyclophosphamide instead of IVIG achieved CR. This degree of efficacy is consistent with the report by Green et al [45]. In these reports, however, the evaluation of efficacy depended on the patients' symptoms (ie, improvement of bleeding tendency), because the disappearance of inhibitors was not followed up.

Thus, the overall efficacy of IVIG therapy alone is almost 30%, whereas that of a combination therapy with IVIG plus steroid and/or cyclophosphamide is approximately 70%.

Recent reports have described patients with acquired factor VIII inhibitors who rapidly responded to immunosuppressive regimens including rituximab, a monoclonal antibody against $CD20^+$ B-cells [53,54]. These data suggest that immunosuppressive therapy using rituximab could become a powerful tool against coagulation inhibitors.

4.2. Acquired von Willebrand Syndrome

Acquired von Willebrand syndrome is a rare bleeding disorder with laboratory findings similar to those of congenital von Willebrand disease. According to an international registry, acquired von Willebrand syndrome is primarily associated with lymphoproliferative diseases, immunologic and cardiovascular disorders, and solid tumors. The prevalence of acquired von Willebrand syndrome in these underlying disorders is still unknown.

IVIG was also effective in stopping bleeding in acquired von Willebrand syndrome [55]. Several groups reported that acquired von Willebrand syndrome associated with systemic lupus erythematosus [56], monoclonal gammopathy [57-60], malignant lymphoma [61], and prostatomegaly [62], and of undefined origin [63,64] responded well to IVIG therapy. Some patients were successfully treated with the combination of IVIG and desmopressin, but the effect was transient

Table 2.
Responses of Patients with Acquired Factor VIII Inhibitor to Immunosuppressive Agents with or without Intravenous Immunoglobulin (IVIG) Therapy

	IVIG + Pr (26 Cases)			IVIG + Pr + Cy (19 Cases)			Pr + Cy (24 cases)		
Reference	CR	PR	F	CR	PR	F	CR	PR	F
Green et al [41]	1								
Carreras et al [21]	1								
Heyman et al [31]			1†						
OiSullivan et al [42]					1				
Pirner et al [43]					1				
Lionett et al [44]	1								
Pignone et al [38]							1		
Green et al [45]							5		5
Hauser et al [39]							1		
Mateo et al [40]	1								
Schwartz et al [25]	1	1							
Crenier et al [28]	1			1					
Lafferty et al [34]					1				
Sohngen et al [46]							2		
Bossi et al [47]	4		1	8		1	3		
Gandini et al [48]	1								
Dykes et al [49]	4	1	2						
Grunewald et al [50]				2			4		
Mazzucconi et al [51]	3	1							
Delgado et al [52]	1			3	1		2		1
Total	19	3	4	14	4	1	18		6

*Pr indicates prednisolone or dexamethasone; Cy, cyclophosphamide; CR, complete remission; PR, partial response; F, treatment failure.
†IVIG dosage: 0.4 g/kg per d for 2 d.

in most cases. According to data from an international registry, the efficacy of IVIG therapy in acquired von Willebrand syndrome was estimated to be 30% (21/63 patients) [65,66]. Of note, however, is that in most cases the efficacy of IVIG was subjectively evaluated (ie, a good response means to stop bleeding) by the doctors in charge. This efficacy is similar to that for treatment with desmopressin (38/119) or with immunosuppressive agents (23/66), but corticosteroids alone were effective in only 19% of patients (12/63).

4.3. Other Coagulation Inhibitors (Factor V or IX Inhibitor)

Patients with inhibitors against factor V or IX are extremely rare. Only one report described acquired factor IX inhibitor developing in a patient with autoimmune polymyositis [67]. Single-agent therapy with IVIG was effective in suppressing inhibitor synthesis and in stopping bleeding. Another report described acquired factor V inhibitor developing in an 82-year-old female patient following abdominal surgery [68]. Nine-day treatment with IVIG (0.4 g/kg per day) was partially effective in suppressing the inhibitor titer and improving the patient's hemorrhagic diathesis.

5. Safety

Adverse reactions to IVIG therapy are usually mild and self-limited: headache, back pain, low-grade fever, myalgia, and chills. The IVIG preparations currently in clinical use are also assumed to carry virtually no risk of transmitting infectious agents. Rarely, however, serious complications can occur. In recent years, thromboembolic complications have occasionally been reported in patients who received IVIG. Stroke, acute myocardial infarction, and deep vein thrombosis were estimated to occur at an incidence of 3% to 5% [69]. Thromboembolism appeared to develop mainly in patients who had other risk factors, such as an advanced age, being bedridden, and a history of thromboembolism. What triggers thromboembolic complications? During 5 courses of treatment with IVIG (24-54 g/day), the plasma IgG concentration was noted to increase 4-fold, and plasma viscosity increased to beyond the normal range [70]. It appears that increased blood viscosity after high-dose IVIG infusion is responsible for thromboembolism. Slow infusion of IVIG (a daily dose of 0.4 g/kg in not less than 8 hours) has been recommended to prevent thromboembolism [71].

Interestingly, our own review of the literature revealed no thromboembolic complications in 80 patients with acquired factor VIII inhibitor who had received IVIG. It is tempting to speculate that the presence of a coagulation inhibitor may counteract thrombosis formation.

6. Discussion

In general, treatments of acquired coagulation inhibitors are divided into 2 approaches: One is to stop the present bleeding events, and the other is to remove inhibitors by immunomodulative therapy. In cases of acute bleeding in patients with factor VIII inhibitors, conventional management consists of human factor VIII concentrate or desmopressin for low inhibitor levels (<5 Bethesda U) and porcine factor VIII or bypass therapy (eg, recombinant activated