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Original

Efficacy of Intravenous Human Immunoglobulin for Sepsis Patients

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We performed a comparative study of the ability of different immunoglobulin preparations to increase insulin-like growth factor (IGF)-1 and reduce the severity of sepsis in sepsis patients. Sixty-three sepsis patients showing high concentrations of soluble E-selectin (SES) were divided into three treatment groups: Group S, 26 patients administered freeze-dried sulfonated human immunoglobulin at 5 g/day for 3 days; Group I, 16 patients administered intact human immunoglobulin at 5 g/day for 3 days; and Group C, 21 untreated controls. IGF-1 concentrations were assayed prior to treatment (Day 0) and on Days 1, 3, 5 and 7, and comparisons were performed regarding SOFA (sequential organ failure assessment) score for various outcomes, including severity of sepsis and time connected to a respirator. In Group S, IGF-1 concentration increased significantly from Day 0 to Day 7 ($p<0.05$). However, no marked increases in IGF-1 concentration over time were seen in Groups I or C. Comparison of IGF-1 concentrations on Day 7, showed significantly higher levels in Group S than in Group I. SOFA scores tended to be lower in Group S than in Group I on Day 7. Respirator time was reduced in Group S compared with Group C ($p<0.05$). The three groups showed no significant differences in terms of mortality rate as of Day 28. Based on these findings, freeze-dried sulfonated human immunoglobulin appears likely to be effective in treating sepsis by enhancing anti-inflammatory activity via promotion of IGF-1 production.

Key Words: soluble E-selectin (SES), insulin-like growth factor (IGF), sepsis, human immunoglobulin

Introduction

In sepsis, prolonged systemic inflammatory response syndrome (SIRS) can reportedly lead to severe sepsis, with complications of organ failure and a high mortality rate[1]. Eliminating SIRS quickly by means of therapeutic intervention is essential[1]. Intravenous immunoglobulin (IVIg) preparations have been subjected to re-evaluation studies in Japan, and efficacy has been demonstrated in severe cases of infection that did not respond to a 3-day course of antibiotics[2]. In addition, by exerting immunostimulatory actions, IVIg has been shown to be effective in treating inflammatory diseases other than severe infections. In recent years, the plasma concentration of soluble E-selectin (SES) has been shown to reflect the extent of vascular endothelial cell damage and can provide a useful index for predicting the onset of organ failure[3)~6)]. Moreover, animal studies have shown that freeze-dried sulfonated human immunoglobulin (Ig), an IVIg, exerts anti-inflammatory activity by increasing production of insulin-like growth factor (IGF)-1 and inhibiting production of tumor necrosis factor (TNF). This anti-inflammatory activity is not seen with other Igs[2]. The present study, conducted in sepsis patients with high SES levels, investigated the efficacy of IVIg in eliminating SIRS and preventing organ damage via mediators such as IGF-1. We also investigated whether different IVIg preparations showed differences in efficacy.

Materials and Methods

Between April 2007 and March 2010, a total of 63 patients (35 men, 28 women) with high-SES (>29.7 ng/ml)[4] sepsis were brought to our emergency and critical care center. Patients were randomly allocated into three treatment groups to perform an

Table 1 Patient characteristics

	sIg (Group S)	iIg (Group I)	Control (Group C)
Respiratory diseases (e.g., pneumonia)	16	8	12
Digestive system diseases	8	4	4
Urological diseases (e.g., urinary tract infection)	1	1	0
Others	1	3	5
Total	26	16	21

Table 2 Characteristics of each group on Day 0

	sIg (Group S)	iIg (Group I)	Control (Group C)
Age (years)	70.96 ± 18.02	62.56 ± 16.17	65.95 ± 15.12
SES (ng/ml)	68.92 ± 45.11	84.15 ± 52.40	51.65 ± 51.71
IGF-1 (ng/ml)	79.94 ± 65.76	76.88 ± 49.97	73.48 ± 40.80
SOFA score	5.6 ± 2.7	6.8 ± 3.6	5.2 ± 2.9

(mean ± SD)

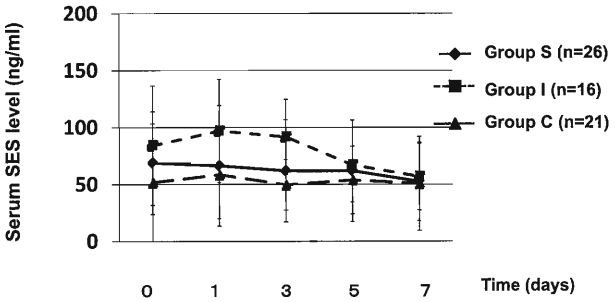

Fig. 1 Changes in serum levels of soluble E-selectin (SES)
Values are expressed as mean ± SD.
Closed diamonds, sIg (Group S); closed squares, iIg (Group I); closed triangles, controls.

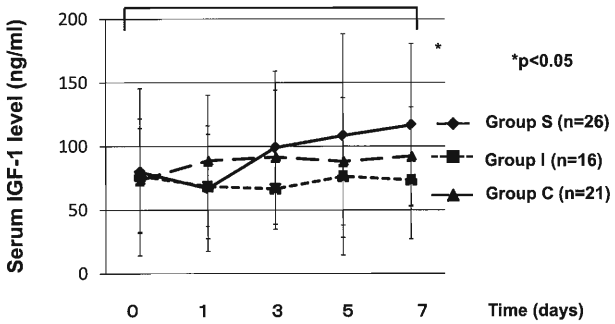

Fig. 2 Changes in serum levels of IGF-1
Values are expressed as mean ± SD.
Closed diamonds, sIg (Group S); closed squares, iIg (Group I); closed triangles, controls.
*$p < 0.05$ vs. sIg on Day 0.

open-label comparative study by the envelope method: Group S, 26 patients administered freeze-dried sulfonated human Ig (Venilon-I®, Teijin Pharma, Tokyo) at 5 g/day for 3 days; Group I, 16 patients administered a different intact human Ig (Venoglobulin-IH®, Mitsubishi Tanabe Pharma; Osaka or Glovenin-I®, Takeda Pharmaceutical, Osaka) at 5 g/day for 3 days; or Group C, 21 patients not administered any Ig. Blood samples were collected on the day on which treatment was started (Day 0) and on Days 1, 3, 5 and 7. Comparisons were performed regarding the serum SES level, serum IGF-1 level and SOFA (sequential organ failure assessment) score for evaluating the severity of sepsis. Surviving patients were compared with regard to the time connected to a respirator. Statistical testing for significance was performed using the t-test. Assay of IGF-1 concentration in this study was performed after obtaining approval (No. 984) from the Institutional Review Board of Tokyo Women's Medical University, and all patients provided written informed consent prior to enrollment in the study.

Results

Tables 1 and 2 show patient background data. Mean patient age and SOFA score on Day 0 did not differ significantly between groups (Table 2). Figure 1 shows the temporal profiles of serum SES levels in each group. SES levels on Day 0 were 68.92 ± 45.11 ng/ml in Group S, 84.15 ± 52.40 ng/ml in Group I and 51.65 ± 51.71 ng/ml in Group C. No significant differences were found among the three groups in terms of SES concentration on Day 0. Figure 2 shows temporal profiles of serum levels of IGF-1 as a function of whether Ig was administered. In Group S, IGF-1 level increased significantly from 79.94 ± 65.76 ng/ml on Day 0 to 116.67 ± 63.94 ng/ml on Day 7 ($p < 0.05$). However, no marked increases in IGF-1 level over time were seen in Groups I or C. In addition, comparison of serum IGF-1 levels on Day 7 showed that levels were significantly higher in Group S (116.67 ± 63.94 ng/ml) than in Group I (76.86 ± 44.97 ng/ml, $p < 0.05$) or Group C (92.23 ± 38.65 ng/ml, $p < 0.05$) (Fig. 3). Changes in SOFA

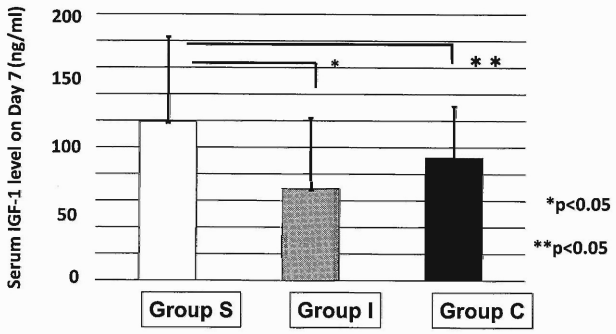

Fig. 3 Serum levels of IGF-1 on Day 7
Values are expressed as mean ± SD.
*p<0.05 vs. iIg (Group I); **p<0.05 vs. control (Group C).

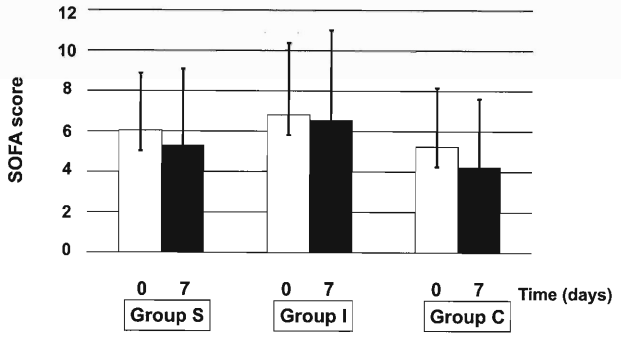

Fig. 4 Changes in total SOFA score
Open bar, Day 0; closed bar, Day 7.
Values are expressed as mean ± SD.

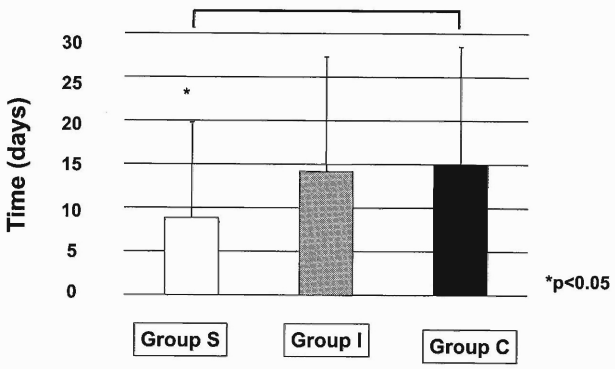

Fig. 5 Mean duration of mechanical ventilation for each group
Values are expressed as mean ± SD.
*p<0.05 vs. controls (Group C).

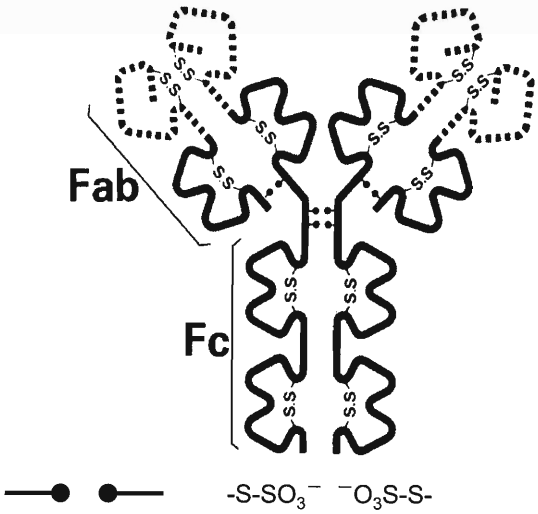

Fig. 6 Structure of sulfonated immunoglobulin
Sulfonated immunoglobulin has the interchain disulfide bond selectively sulfonated.

score accompanying administration of each Ig in Groups S and I were also compared on the basis of Day 0 and Day 7 scores. Figure 4 shows that, although no significant differences were present, scores tended to be lower in Group S than in Group I on Day 7. Finally, comparison was performed regarding the time surviving patients in each group were connected to a respirator. Mean respirator times were 8.84 ± 10.95 days in Group S, 14.18 ± 13.12 days in Group I and 14.94 ± 13.51 days in Group C. Although no significant differences were seen between Groups S and I, respirator time tended to be shorter in Group S. However, time on a respirator was significantly shorter in Group S than in Group C ($p<0.05$; Fig. 5). On Day 28, the three groups showed no significant differences in terms of mortality rate.

Discussion

Igs are produced by plasma cells, and are thought of as antibodies having the function of host defense[7]. IVIg preparations include purified IgG, and have been used primarily as immunostimulants in patients with severe infections of neutropenia. IVIg is thought to act by binding of the specific antibodies contained therein to antigens, resulting in expression of activity in preventing infections as a result of functions such as opsonization activity, complement activation, neutralization of toxins and viruses and antibody-dependent cytotoxicity[2)~7)]. In Japan, administration of IVIg at 5 g/day for 3 days with antibiotics has been reported as effective in the treatment of severe infections[2)8)]. However, Okajima et al. investigated a freeze-dried sulfonated Ig (Venilon-I®) in animal studies and showed expres-

sion of not only activity in preventing infections, but also anti-inflammatory activity in regard to sepsis[2].

Freeze-dried sulfonated human Ig is an Ig in which the disulfide bonds (S-S bonds) in the IgG molecules have been selectively sulfonated (Fig. 6). The physicochemical properties are considered to be the same as those of other intact Igs. However, Okajima et al. demonstrated that freeze-dried sulfonated human Ig expresses anti-inflammatory activity by interaction of the Fcγ portion with receptors on the surface of sensory neurons, stimulating those neurons and thus promoting production of IGF-1 and inhibiting production of TNF[2]. In addition, simultaneous animal studies demonstrated that these activities are observed only with sulfonated human Ig, and activation is not seen with other intact Igs[2]. These characteristics of sulfonated human Ig are very intriguing.

To date, the pathogenesis of severe sepsis has been thought to be as follows. In addition to the tissue damage caused by the pathogens themselves, stimulation by factors such as endotoxin causes monocytes to produce large amounts of TNF, an inflammatory cytokine. TNF acts on damaged cells, activates the caspase group of proteolytic enzymes, and as a result causes apoptosis. These processes are thought to promote neutrophil accumulation, damage vascular endothelial cells via neutrophil elastase and radical oxygen species, cause circulatory damage, increase apoptosis in damaged tissues, and in the end cause organ dysfunction and complete the pathogenesis of severe sepsis[1)2)9)~11)].

With the objective of supporting those earlier animal study findings, we decided to perform studies aimed at elucidating whether IVIg expresses the same anti-inflammatory activity clinically, and whether all IVIg preparations express similar anti-inflammatory activity. We thus focused on IGF-1 as the target molecule for evaluating anti-inflammatory activity. IGF-1 is a peptide that belongs to the IGF group, having a molecular weight of 7,500. Formerly called somatomedin, IGF-1 is now considered to play important roles in the growth, differentiation and maintenance of the function of cells as a factor that acts via growth hormone[12)~14)]. Research has elucidated that, in the presence of noxious stimuli, vascular endothelial cells and sensory nerves are activated by substances produced as a result of tissue damage, and that calcitonin gene-related peptide (CGRP) is released from the sensory nerve endings[15)16)]. In addition, the endothelial nitric oxide synthase (eNOS) becomes activated, and anti-inflammatory activity is expressed via cyclooxygenase 1 and prostaglandin (PG)I_2 and PGE_2[15)16)]. Furthermore, in this process, production of IGF-1 is promoted by CGRP and PGE_2, and IGF-1 inhibits the previously mentioned caspase 3 and endothelial monocyte-activating polypeptide-II, a leukocyte chemotactic factor, thereby resulting in expression of anti-inflammatory activity by inhibiting leukocyte accumulation and apoptosis[15)~19)]. We surmised that measuring changes in serum IGF-1 levels would enable measurement of the therapeutic effects of anti-inflammatory activity in severe sepsis. Moreover, we have provided support for this concept by reporting that the change in serum IGF-1 level in patients with septic DIC can serve as a marker that reflects the efficacy of treatment[20].

The experimental subjects of the present study consisted of 63 patients with high-SES (>29.7 ng/ml)[4] sepsis who were brought to our emergency medical care center. E-selectin is a leukocyte adhesion factor on the cell membrane of vascular endothelial cells, and earlier research revealed that plasma levels of the soluble form, SES, are elevated at the time of TNF-induced damage to vascular endothelial cells[4]. In addition, Suga[5] and Kobayashi et al.[21] from our department reported that SES is useful for judging subsequent organ damage in sepsis patients and predicting outcomes. The high-SES patients investigated in the present study can thus all be considered as severe sepsis cases. Based on the earlier results generated in animal studies, we randomly allocated subjects into three treatment groups to perform an open-label comparative study using the envelope method.

The three treatment groups were compared with regard to temporal changes in serum IGF-1 level and SOFA score (used to evaluate the severity of sepsis). Groups were also compared in terms of the

time patients were connected to a respirator, which reflects the clinical status of the most characteristic symptoms of sepsis: acute lung injury (ALI); and acute respiratory distress syndrome (ARDS). Serum IGF-1 level, which had been measured over time, was significantly elevated in Group S on Day 7 compared with Day 0 (start of treatment), whereas no significant elevations were seen in Group I (administered another intact Ig) or Group C (not administered any Ig). These results can be explained on the basis that in Group S, the increase in serum IGF-1 level was due to drug administration and reflects the therapeutic effects of the drug. In addition, even when comparing all three groups on Day 7, the serum IGF-1 level was significantly higher only in Group S, a result supported by Okajima et al.[2] in animal studies. This result indicates that anti-inflammatory activity was generated only in Group S. In addition, SOFA score, used to evaluate the severity of sepsis, tended to be decreased in Group S following administration of freeze-dried sulfonated human Ig. This result appears to reflect alleviation of the organ damage and expression of a therapeutic effect that improves the sepsis. In an earlier study[20] from our institution, we reported that the improvement rate for multiple organ failure was significantly higher in a group administered freeze-dried sulfonated human Ig than in a group of intact human Ig and not administered any Ig, supporting the present results.

In septicemia, the lung is the major target organ of the body due to the presence of vascular endothelial cells. With the objective of evaluating the effects of each Ig on the status of ALI/ARDS, we compared the three patient groups in terms of the time patients had to be on a respirator. Respirator time tended to be shorter in Group S than in the other two groups; in particular, respirator time was significantly shorter in Group S than in Group C. Deguchi et al.[22] used the PO_2/FiO_2 (P/F) ratio to evaluate the effects of treatment on ALI/ARDS, reporting that administration of freeze-dried sulfonated human Ig improved the P/F ratio compared with the pretreatment ratio. That finding is evidence of expression of a therapeutic effect by freeze-dried sulfonated human Ig, similar to our present findings of reduced time the patient had to be on a respirator.

In this study, the three groups showed no significant differences in terms of mortality rate as of Day 28. We suspected that mortality rate might more strongly reflect other factors such as side effects, rather than sepsis, and more detailed examination of this issue is warranted in the future.

The present clinical study has elucidated that production of IGF-1 is promoted in sepsis patients administered freeze-dried sulfonated human Ig. This finding can be thought to represent clinical proof of a cascade of events that has been identified in earlier experiments[15)~19)]. Acting via sensory nerves, freeze-dried sulfonated human Ig promotes production of CGRP and thus production of IGF-1. In the treatment of sepsis, failure of initial therapy can lead to progression to severe sepsis in some patients, accompanied by organ damage, septic shock accompanied by a drop in blood pressure, and finally a serious, life-threatening state. We think that the anti-inflammatory activity expressed by freeze-dried sulfonated human Ig and documented in the present study, when combined with conventional treatments for sepsis, will help to prevent worsening of the severity of sepsis cases in the future.

Conclusion

In comparison with other intact Igs, freeze-dried sulfonated human Ig appears likely to prove effective in treating sepsis by expressing anti-inflammatory activity via promotion of IGF-1 production.

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敗血症に対する静注用免疫グロブリン製剤の有効性の検討

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今回，我々は当救命救急センターへ搬送された敗血症症例を対象にインスリン様成長因子-1（insulin-like growth factor-1：IGF-1）の産生効果や重症度改善効果などを各種免疫グロブリン製剤間で比較検討し，その有用性について比較検討した．

可溶性 E-selectin（soluble E-selectin：SES）濃度の高値な敗血症症例に対し，乾燥スルホ化人免疫グロブリン群（5 g/day：3 日間）(S 群）26 例，intact 型人免疫グロブリン群（5 g/day：3 日間）(I 群）16 例，非投与群（C 群）21 例の 3 群に分け，投与前（第 0 日），投与後 1, 3, 5, 7 病日の IGF-1 濃度を定量し，敗血症の重症度として SOFA score，呼吸器装着期間などを比較検討した．その結果，IGF-1 値は，S 群において第 7 病日において，第 0 病日の値に比して有意な上昇を認めた($p<0.05$)．しかし，他の 2 群については経時的に IGF-1 値の顕著な上昇は認められなかった．また，第 7 病日の各群における血中 IGF-1 値について比較したところ，S 群は他の 2 群に対し，有意差をもって高値を呈した($p<0.05$)．さらに，各免疫グロブリン投与に伴う SOFA score の変化について比較検討した．その結果，S 群では，統計的有意差はないもののⅠ群に比較し，第 7 病日で減少を認めた．また，各群の生存例に対し，人工呼吸器装着期間について，検討した．S 群は，Ⅰ群とは有意差こそなかったが，人工呼吸器装着期間の短縮を認めた．一方 C 群に対しては，有意差をもって人工呼吸器装着期間の短縮が認められた($p<0.05$)．なお，3 群を比較した場合，第 28 日病日死亡率については有意差はみられなかった．

以上より乾燥スルホ化人免疫グロブリンは，IGF-1 の産生を亢進させ，抗炎症作用を発揮することにより敗血症治療において治療効果が期待される．