

LITERATURE REVIEW

Ulinastatin as a Potential Alternative Therapy for Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis

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Abstract

Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) is an emergency skin disease with high mortality rates despite the incidence rate is not great. Patients with SJS/TEN will experience reduced quality of life due to the pain experienced when eating, drinking or urinating. There is not a specific treatment for patient with SJS/TEN so far. Corticosteroids and intravenous immunoglobulin (IVIG) are the most commonly used therapies yet there are still controversies due to their positive and negative effects. Ulinastatin, a trypsin inhibitor and an anti-inflammatory compound that is made from human urine is a therapeutic option for SJS/TEN. Ulinastatin may provide anti-inflammatory effect by inhibiting TNF- α production, thereby reducing the risk of septic complications and lowering mortality. Until now, the known side effects are nausea, vomiting and allergic to gelatin. Although it has a promising potential, the effectiveness of ulinastatin is still poorly studied. Further research is needed regarding the usage of ulinastatin on patients with SJS/TEN.

Keywords: Stevens-Johnson Syndrom, Toxic Epidermal Necrolysis, Ulinastatin

Introduction

Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN) is one of the most uncommon diseases. It is an acute skin reaction in which skin undergoes inflammatory reaction. The reaction stated as SJS if the skin lesion below 10% of total body area. The lesion can progress becoming TEN with lesion over 30% of total body area. Its incidence in the Eastern part of the world was approximately 8 case per one million population in one year. In Indonesia, based on the data from Cipto Mangunkusumo Hospital Jakarta, SJS/TEN incidence among hospitalized patient from 2009 to 2011 were 29 people (unpublished data). Although it has a small incidence, Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis can attack all races in all ages, with men and women ratio 3:5 and had high mortality rate. There were 5 to 12 percent SJS patients and 30 percent TEN patients died.

SJS/TEN's patients may have a lowered quality of life. This is due to the involvement of mucosa on mouth which causes pain and difficulty in eating or drinking. Patients who have rashes on the genital area may have dysuria or inability to secrete urine. To cope with those problems, patients are given many kind of treatments such as. However, there is no

definitive treatment for SJS/TEN. The usage of corticosteroid and intravenous immunoglobulin (IVIG), which are widely used for this disease, are still controversial.

Corticosteroid has been used as the first line of drug for SJS/TEN, but there is controversy about its usage. Based on research done by Kakourou *et al*, corticosteroid will shorten fever duration compare to SJS/TEN without corticosteroid administration. On the other hand, Ramussen's research shows that the usage of corticosteroid will prolong the healing time. This drug has also been related to increased mortality rate, sepsis and prolonged treatment.

Although it is known for its antibody activity which may inhibit or prevent apoptosis, IVIG administration as an alternative for SJS/TEN has an inconsistent result. A few research show a contrast result for its role, for example a study done by Trent *et al* showed that IVIG may decrease mortality rate in TEN patient, however Bachot *et al* showed that patients' mortality rate, especially the elderly, increased by administrating IVIG.

Many studies have been conducted to find the cure for SJS/TEN, but most of the researches are focused on corticosteroid and IVIG instead of alternative treatments which may effectively treat SJS/TEN, such as ulinastatin. Ulinastatin is a compound derived from human urine.

Ulinastatin works by inhibiting inflammatory response which may cause apoptosis in SJS/TEN.⁷

Research on ulinastatin has been conducted in developed countries as a treatment option for many inflammatory diseases such as pancreatitis and hemorrhagic shock.⁹ However, we found only few studies regarding to ulinastatin's role in SJS/TEN.

Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis (SJS/TEN)

Definition and Classification

Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis is an acute idiopathic skin reaction on mucocutaneous which is usually triggered by consumption of certain drugs. The characteristic of this disease is erythema on the skin and mucosa layer which followed by necrosis and shedding of the skin. If this disease is not treated as soon as possible, patient's life may be threatened as it involved various human body system.³

Usually, Stevens-Johnson Syndrome is often known as a variant of major erythema multiforme. Toxic Epidermal Necrolysis is a variant of Stevens-Johnson Syndrome. These two conditions can occur simultaneously although more than 50% of TEN lesion developed directly from erythema to necrosis

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followed by epidermal shedding. Due to this reason, the classification of SJS/TEN is based on the total area of the skin loss:³

- | | | |
|------------------|---|---------------------|
| 1. Less than 10% | : | SJS |
| 2. 10%-30% | : | SJS/TEN overlapping |
| 3. More than 30% | : | TEN |

Risk Factors

People more than 40 years old has a higher risk to develop SJS/TEN. Women are more prone to this disease than men with the ratio 0,6.³ Other risk factors are Human Immunodeficiency Virus (HIV), collagen abnormality and cancer. Human Leukocyte Antigen (HLA) also show a correlation with the development of SJS/TEN.³

Etiology

The most prevalent etiology is hypereactive response to drugs such as carbamazepine. However, there are other causes such as infections and malignancies. SJS/TEN can also be idiopathic

Pathophysiology

The pathophysiology of SJS/TEN has not been fully understood. Several studies have discussed about the mechanism underlying this disease such as Gell and Coombs Classification. Gell-Coombs Classification stated that there are four types of body responses to infectious agents:

1. Type I: Anaphylactic reaction (mediated by IgE); eg. urticaria.
2. Type II: IgG/IgM or cytotoxic mediated reaction (complement activation); eg. thrombocytopenia.
3. Type III: Immune complex-antibody response.
4. Type IV: cellular immunity mediated reaction; eg. SJS/TEN.

SJS and TEN are included into type IV hypersensitivity, especially type IVc. There are four type of type IV hypersensitivity based on cytokine and cell type, those are type IVa (via monocyte), IVb (via eosinophils), IVc (via CD4 and CD8 T cell activity), and IVd (neutrophils mediated).

Other factors which may lead to SJS/TEN are HLA genes, cytotoxic signals, and immune system molecules.³ Human Leukocyte Antigen gene play a role in antigen-processing presentation and cytotoxic protein may lead to SJS/TEN by apoptosis, chemoattractant and pro-inflammatory substances activation via Fas-FasLigand (Fas-FasL) interaction, perforin/granzyme B and granulysin.¹³ Tumor Necrosis Factor (TNF)- α , interleukin (IL)-10, interferon (IFN)- γ and other cytokine and chemokine may increase in SJS/TEN. TNF- α is also found in epidermis keratinocyte and play a

role in Fas-FasL regulation together with IFN- γ and IL-15.

Reactive Oxygen Species (ROS) also play a role in giving contribution to SJS/TEN via oxidative stress. Free radical can trigger oxidative stress and cause intracellular and cell membrane damage. The damage lead to release of pro-apoptotic molecules (FasL and TNF- α) which cause apoptosis and necrosis.⁵

Inflammation process in Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis

After a susceptible person exposed with the causal agents of SJS/TEN (mainly drugs), hypersensitivity reaction will emerge from his/her body. When the antigen enters the immune system, our immune system may respond. The immune response does not depend on the size of the molecule. All molecules can lead to immune responses. Antigens will bind to other macromolecules on the surface of the cell or in plasma to form hapten-carrier complex. The complex then become a multivalent complex and secreted out of the cell in the form of Antigen-Presenting Cell (APC). These complexes are going to trigger the activation of T lymphocyte, B cell or Mast Cell.

There is another theory besides the hapten-carrier complex theory. This theory is called the

p-i concept or “direct pharmacological interaction of drugs with immune receptors”. In this theory, if drugs occupy the specific position of T cell receptor, it may stimulate cytokine production, proliferation and cytotoxic effect from the T cell. As the result, immune response is activated. The p-i concept can cause cross sensitivity towards several drugs such as carbamazepine.²¹

Those two immune response may be two different concepts. However drugs such as carbamazepine may trigger both responses. SJS/TEN symptoms may be affected by several risk factors: genes, keratinocytes, and cytokines such as perforin/granzyme B, Fas-FasL receptor interaction, granulysin and TNF- α .²¹

Gene Involvement in SJS/TEN

Wei *et al.*, in his research stated that gene plays a role in SJS/TEN pathophysiology. The research showed that in vitro, carbamazepine and its derivatives can interact with HLA-B*1502 without cellular metabolism or antigen-processing presentation. The binding between HLA/peptide with the drugs may cause the drug to be taken to Cytotoxic T cell and trigger immune reaction.^{15,23}

Keratinocytes

Keratinocytes have an important role in SJS/TEN pathogenesis. Lesion on the skin

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shows that the metabolites which cause SJS/TEN are from the skin.¹³ Enzymatic reaction also occurs in keratinocyte, such as cytochrome P450 react with carbamazepine on SJS/TEN patient's epidermis.²⁴ T cell migrates to keratinocyte and induce a reaction which is triggered by perforin/granzyme B and Fas-FasLigand receptors (CD95 receptor). After that, hypersensitivity type IVc occurs and causes SJS/TEN to destroy keratinocytes.^{20,24}

In SJS/TEN patients, certain drugs can cause cellular immunity to react and phagocytes the drugs. After phagocytosis, the drug will be presented by HLA-B which a class I Major histocompatibility complex (MHC) to CD8+ cytotoxic T cells.^{13,25} Nassif *et al* showed many cytotoxic T cells inside the patient's blisters. T lymphocyte would then triggers apoptosis from perforin/granzyme B pathway.

Perforin/Granzyme B

Perforin and granzyme pathway are initiated by perforin which open the way for granzyme to cause cell death. Perforin binds to target cell membrane. Granzyme B enters via endocytosis. After entering the cell, granzyme will bind to mannose-6-phosphate receptor/insulin-like growth factor II. When perforin gives a signal to release granzyme, granzyme would trigger caspase-3 via mitochondria and cause DNA fragmentation

and apoptosis. granzyme B also triggers apoptosis from the division of BH3-Interacting domain death agonist (Bid). Bid will cause cytochrome c enter the cytosol and apoptosis occur.²⁵

Fas Receptor and Fas Ligand Interaction

Keratinocyte may induce FasL expression via pro-inflammatory cytokines and the ligand is going to bind to Fas receptor. Fas receptor then recruits Fas-associated death domain protein (FADD) and pro-caspase 8. Pro-caspase would activate caspase 8 to trigger caspase pathway and DNA degradation. This would lead to keratinocyte apoptosis.

Granulysin

Granulysin may induce apoptosis in SJS/TEN patient from different pathways. Granulysin is a pro-apoptotic protein which causes cytotoxicity at cell and expressed in high amount.⁷ It is found more than perforin/granzyme B and soluble FasL in SJS/TEN patients' blister (2-4 times more). Granulysin can trigger apoptosis pathway by destroying mitochondria. The destroyed mitochondria then release cytochrome-c and DNA fragmentation leading to apoptosis. In addition, binding of granulysin to cell membrane can trigger influx of calcium into intracellular and apoptosis.

Tumor Necrosis Factor (TNF)- α

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TNF- α is involved in apoptosis and necrosis pathway by activating caspase, Fas-FasL expression, and nitric oxide (Reactive Oxygen Species) production. Fas-FasL complex induces extrinsic pathway of apoptosis and Reactive Oxygen Species (ROS) induces intrinsic pathway. In short time, both eventually lead to activation of caspase. Activated caspase degrades DNA and nucleoproteins, nuclear matrix component, and cytoskeleton, leading to fragmentation of cells and death. On the other hand, these molecules can lead to decline in cell membrane integrity and pore permeability in long time. Leak of mitochondrial matrix lead to cell rupture and necrosis.^{31,}

Ulinastatin

General Description

Ulinastatin is also called urinary trypsin inhibitor (UTI), HI-30, or bikunin. It is acid glycoprotein with a molecular weight of 67,000 kDa. Ulinastatin is a serine protease inhibitor multivalent Kunitz type, found in human urine and blood. The compound consists of 143 amino acid residues and has two homologous domains Kunitz type.

Ulinastatin is produced by hepatocytes in the form of precursors associated with α 1-microglobulin. In hepatocytes, ulinastatin pairs with one or two of the three evolutionarily

related heavy chain (HC) 1, HC 2, HC 3 through chondroitin sulfate chains forming protein structure.

Pharmacodynamics

At the time of the inflammatory reaction, ulinastatin separates from IaI family protein through proteolytic cleavage by neutrophil elastase. Several serine proteases such as trypsin, thrombin, chymotrypsin, kallikrein, plasmin, elastase, cathepsin, and factors IXa, Xa, Xia, and XIIa can be inhibited by ulinastatin. Ulinastatin has also been reported to suppress the expression of urokinase-type plasminogen activator (uPA) through inhibition of protein kinase C (PKC).

In humans, ulinastatin has a protective effect against ischemic reperfusion injury in the liver, kidney, heart, lung, and stomach. Giving prepump administration (5000 U/kg) ulinastatin repair cardiopulmonary bypass-induced hemodynamic instability and lung disorders through attenuation of production-release of IL-6 and IL-8.

Ulinastatin has only few side effects. The side effects include nausea, vomiting, and diarrhea. At the injection site, ulinastatin may cause angialgia, redness, and itching. Allergic reactions can also occur.

Pharmacokinetics

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Study on pharmacokinetics of ulinastatin still very limited. Techpool Bio-Pharma reported that provision of 300,000 IU/10 ml ulinastatin intravenously (IV) to male subjects, the concentration in the blood decreases linearly within 3 hours. Its half-life is 40 minutes. After 6 hours of the initial administration, it has been excreted in the urine as much as 24%.

Clinical Usage

In the market, the price of ulinastatin can be quite high, reaching U.S. \$ 130 for 10 mg. The unit dose is 1,000,000 IU for every 5 mg solution of ulinastatin, depending on the concentration of the stabilizer component that may be added. Ulinastatin can be administered into the human body intravenously with a maximum dose of 300,000 U/day. On maintenance therapy, the dose used is 2,500-5,000 U/kg.

To anticipate the expensive price on the market, ulinastatin can be made in the laboratory by centrifugation and stabilization from human urine using methods by Proksch and Routh.³³ Urine was centrifuged (1000g for 15 min) and the supernatant purified to obtain pure ulinastatin concentration. Gelatin is used to stabilize the compound.

Giving ulinastatin in patients SJS/TEN reported to give good results.⁴⁸ Erythema, fatigue, and

fever showed obvious improvement within 24 hour of starting ulinastatin therapy. The skin lesion resolved between 4 to 7 days compared 10 to 12 days without ulinastatin. Commonly used dosage is about 7500 U/kg/day IV for 4-7 days after the appearance of skin lesions. After the lesions disappear, it reduced to 2500-5000 U/kg/day as maintenance therapy until full recovery. Ulinastatin exhibit anti-inflammatory activity and reduce the rate of infiltration of neutrophils and the release of elastase and chemical mediators.³⁴

Use of ulinastatin reported to have side effects such as nausea and vomiting, but it is not life threatening. However, gelatin allergies can sometimes appear after repeated administration of ulinastatin.⁴⁶

Ulinastatin as Anti-Inflammatory for SJS/TEN

In vitro tests, ulinastatin is reported to inhibit the production of TNF- α and IL-1 in Lipopolysaccharides (LPS)-stimulated human monocytes and HL60 cells or bronchial epithelial cells which were stimulated by LPS or neutrophil elastase.¹⁷ Aosasa *et al* stated that ulinastatin block TNF- α production by inhibiting the translation of secreted TNF- α , rather than transcription of its mRNA as in

other trypsin inhibitor compounds. This is believed to be the differentiator between ulinastatin with trypsin inhibitor compounds generally.⁴⁵

The concept of ulinastatin work in driving the inflammation

A group of researchers demonstrated the performance of in vitro ulinastatin inhibits TNF- α -stimulated LPS. LPS binds to soluble LPS-binding protein, and both bind to the cluster of differentiation (CD) 14 to be presented to the LPS receptor Toll-like receptor 4 (TLR4). TLR4 activates several signaling pathways, involving extracellular-signal-regulated kinase (ERK) 1/2. LPS also activates c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), which increases the translation and stability of TNF- α . ERK 1/2 itself acts as a pathway that controls the induction of post-transcription TNF- α . Suppressing this pathway is likely to be the cause of disturbance in translation or secretion thus inhibited the production of TNF- α . However, it remains unknown which specific pathways involved until now.⁷ Furthermore, ulinastatin decreases the metabolism rate involving the regulation of arachidonic acid, such as thromboxane B2 production, which plays a role in the pathogenesis of sepsis. Inamo et al report concludes therapeutic mechanism of

ulinastatin in SJS/TEN includes the stabilization of lysosomal membranes, inhibition of apoptosis, and suppression of TNF- α activity.

Comparison with Other SJS/TEN Therapy

Corticosteroid Therapy

Corticosteroids are often used as a primary treatment in case of SJS/TEN.²⁸ These compounds inhibit the function of APC and macrophages, as well as affect the inflammatory response by reducing the synthesis of inflammatory mediators such as prostaglandins, leukotrienes, and platelet-activating factors. The end result, impaired immune function caused by cytotoxic T lymphocytes and macrophages are suppressed and inflammation can be inhibited.

Corticosteroid therapy remains controversial, there is the possibility increasing risk of complications of sepsis and gastrointestinal bleeding.²⁹ Side effects allegedly caused by corticosteroids can induce bacterial or viral infections or hypercoagulable state in acute phase.⁷ Administration of corticosteroid in 48 hours or more since onset of disease and administration of glucocorticoids in patients with TEN does not provide a therapeutic effect. A retrospective study concluded that SJS treatment with steroids can significantly prolong the healing process.²⁸ Halebian et al mentioned that the mortality rate among the

advanced TEN patients with steroid therapy (46 ± 6 years, $n = 15$) against non-steroid (37 ± 7 years, $n = 15$) is 2:1, indicating the danger of corticosteroids for patients with TEN.

Intravenous Immunoglobulin Therapy (IVIG)

IVIG therapy was first introduced by Viard et al in 1998. This treatment resists the CD95 (Fas) receptor which induces keratinocyte apoptosis. IVIG is produced from blood plasma, fractionated and purified to obtain a product containing 90-98% IgG, IgA bit, IgM, CD4, CD8, HLA, and cytokines.¹⁶

Appropriate dose for this therapy is still unclear. Viard et al provide protocol infusion dose 0.7 g/kg/day for 4 consecutive days.¹⁶ This dose is highly modified, ranging from 1.6 g/kg/day to 3 g/kg/day for 3 days. Variability due to lack of consistent and controlled, randomized comparative studies.

Various studies have reported positive results in this therapy. However, there are also experiments stating poor results of IVIG therapy. Brown et al and Bachot et al did not demonstrate significant improvement in patient mortality with TEN. Elderly patients and those with impaired kidney function are also reported to have no therapeutic effects.⁵⁵ Prins et al suspected cause of patient's diversity response

is a variety of anti-Fas activity is higher than any IVIG is used.

Ulinastatin Compares with Corticosteroid and IVIG

SJS/TEN usually treated using corticosteroid and IVIG. However, both of them still controversial, providing unstable results. Corticosteroid also reported to induce gastrointestinal bleeding in some cases. Report from Inamo et al using ulinastatin giving better result compares to corticosteroid and IVIG. Fever completely resolved after 24 to 36 hours and the mean hospital stay was 11.0 days for patients treated by ulinastatin. IVIG gives an average 2.5 days to resolve the fever and 11.0 days for corticosteroid treatment. The mean hospital stay for both later treatment was 12.2 days.⁵²

Conclusion

Currently, there is good evidence to suggest that there may be a bidirectional relationship between T2DM and ALD. Evidence in the literature has shown that ALD may influence the development of T2DM and vice versa.

However, there is much less information regarding the effects of these two conditions on liver function when they occur together. This is a notable gap that is of significant importance, given the potential for worse liver outcomes occurring in these patients in the face of epidemics of both conditions in Australia. As such, further research in the area needs to occur in order to develop well-defined strategies to prevent and manage the combined effects of T2DM and ALD should they occur concurrently.

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