The Role of Intensive Insulin Therapy in Increasing Superoxide Dismutase (SOD) and Normalizing Hyperglycemia in Critically Ill Patients

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ABSTRACT

**Aim:** to find out the difference between intensive insulin therapy and conventional insulin therapy in influencing the increase of superoxide dismutase (SOD), decrease of cytokine production (TNF-α and IL-6), increase of albumin level, and occurrence of SIRS.

**Methods:** the study design was randomized pre and post control group design involving 40 adult patients admitted to the ICU of Sanglah Hospital, Denpasar. The study subjects were randomly allocated into two groups: the first group to receive intensive insulin therapy in which blood glucose was set at a level between 80 – 110 mg/dL; the second group to receive conventional insulin therapy, which was given if the blood glucose level exceeded 215 mg/dL and to be maintained at the level of between 180 – 200 mg/dL.

**Results:** this study showed: (1) There was a significant increase of SOD in the group receiving intensive insulin therapy as compared to the conventional insulin therapy (370.70 vs. 98.50 U/gHb, p=0.001), (2) There was no significant decrease in the TNF-α level, (3) There was a significant decrease of IL-6 level (10.25 vs. 2.02; p=0.023); (4) There was a significant decrease in the event of SIRS (10% vs. 45%, p=0.000) in the intensive insulin therapy group as compared to the conventional insulin therapy group.

**Conclusion:** increase of insulin dose in the intensive insulin therapy can maintain blood glucose at normoglycemic level between 80 – 110 mg/dL faster than that in the conventional insulin therapy. On the other hand, intensive insulin therapy can increase the SOD level, decrease IL-6 level, and decrease the events of SIRS in the ICU critically ill patients compared to conventional insulin therapy.

**Key words:** hyperglycemia, intensive insulin therapy, superoxide dismutase (SOD), critically ill patients, proinflammatory cytokine, SIRS.

INTRODUCTION

Hyperglycemia is a condition in which one’s fasting blood glucose level exceeds 110mg/dL and post prandial blood glucose exceeds 140 mg/dL. Carbohydrate constitutes the source of glucose and primary calorie used by the human body cells in energy-producing metabolism. However, if blood glucose level becomes excessive or reaches the condition of hyperglycemia, it may become a risk factor for increasing morbidity and mortality in critically ill patients treated in the ICU. At present the number of hyperglycemia cases is high enough both in developed and developing countries. Critically ill patients in the ICU tend to get hyperglycemia, the so-called stress diabetes or newly developed diabetes. This condition is caused by a number of anti-regulation hormones such as epinephrine, nor-epinephrine, catecholamine and glucagons produced through the hypothalamic-pituitary-adrenocortical (HPA) axis pathway. Hyperglycemia can increase reactive oxygen species (ROS) through enzymatic process, reaction of ox-phos and ADPH-oxidase, and through non-enzymatic process that generates gluco-oxidant and glycocylation of proteins and lipids (advanced glycation end-product [AGE]) resulting in increased production of superoxide anions. Oxydative stress is a major process that encompasses most of the pathological alterations in the diabetic vasculature. Meanwhile, super oxide dismutase (SOD) is an enzyme that functions as anti-oxidant only if superoxide ion is produced in the mitochondria. Over production of oxidative stress such as superoxide anions in mitochondria in hyperglycemia will be quenched by SOD and converted to hydrogen peroxide.
It has been known that there is a close relation between hyperglycemia and immune dysfunction, particularly related to infections. The primary dysfunction is due to phagocyte cells where the body tends to be susceptible to infection. The ROS in hyperglycemia may activate transcription factor of NF-kB and vascular cell adhesion molecule-1 (VCAM-1) which then stimulates production of inflammatory cytokines, such as TNF-\(\alpha\) and IL-1 as proximal cytokines.\textsuperscript{12-14} With its autocrine and paracrine effects, inflammatory cytokines may stimulate the other cytokines, such as IL-6 and many other mediators.\textsuperscript{15,21,22,37,47} Therefore, the inflammatory cascade systemically occurs resulting in reduced barrier function with increased permeability of endothelial cells.\textsuperscript{16,24,25} High glucose concentration also activates protein kinase C (PKC) by increasing formation of diacyl-glycerol (DAG). PKC activation to further increase the expression of transforming growth factor-\(\beta\) (TGF-\(\beta\)), believed to be able to cause thickening of capillary base-membranes.\textsuperscript{16-19}

Currently, insulin is considered to be the most rational anti-diabetes medicine because it is a hormone that is produced in the human cells and has an anabolic function. However, it is frequently assumed to be able to cause serious complications such as hypoglycemia.\textsuperscript{19,20,23} Recently, many studies have been done on the treatment of hyperglycemia, in particular in diabetes, but their results have not been empirically maximal yet.\textsuperscript{23,27} Besides, there is still a debatable issue about the exact level of blood glucose to reach with the insulin therapy. Several results of studies have shown that tight blood glucose control may improve clinical outcome of hyperglycemia in hospital.\textsuperscript{26,28-30} Up to now, critically ill patients with hyperglycemia in the ICU have been treated with a standard procedure in which insulin is infused only if blood glucose level reaches \(\geq 200 – 225\) mg/dL.\textsuperscript{29,30}

The present study is a comparative study on the effectiveness of intensive insulin therapy for decreasing and maintaining blood glucose at the level between 80 – 110 mg/dL and of conventional insulin therapy for maintaining blood glucose at the level between 180 – 200 mg/dL, carried out on critically non-surgical ill patients (medical critical illness) with hyperglycemia being treated in the ICU.

METHODS

Study Subjects, Design, and Procedures

The study subjects consisted of adult patients, aged 20 to 60 years, admitted to the medical ICU, and were categorized as eligible for inclusion. Written informed consent was obtained from each closest family member, because the patients were unable to give consent. The protocol and consent forms were approved by the institutional review board of the Udayana University Medical Faculty, Sanglah Hospital Denpasar on January 14, 2008. The study design was experimental research using pre and post test control group design on intensive insulin therapy group and conventional insulin therapy group. The study was carried out between January and June, 2008. In 6 months period of ICU admission, of a total 40 patients, 20 patients were randomly allocated to receive intensive insulin therapy and the other 20 patients got conventional insulin therapy. In the conventional group, continuous insulin infusion (50 IU of Actrapid Novo in 50 ml of 0.9\% sodium chloride with the use of a pump (Perfusor Compact, B Braun Germany) was started only when blood glucose level exceeded 215 mg/dL and was adjusted to maintain a blood glucose level of between 180 and 200 mg/dL. When the blood glucose level fell below 180 mg/dL, the insulin infusion was tapered off and eventually stopped.

In the intensive group, insulin infusion was started when the blood glucose level exceeded 110 mg/dL and was adjusted to maintain normoglycemia (80 to 110 mg/dL). The dose of insulin infusion was 1 to 4 IU per hour, and blood glucose level measured at one to four hour intervals. When patients were hemodynamically stable, enteral or mixed with parenteral feeding was started, aimed at a total of 25 to 30 kcal per kilogram body weight.

Data Collection

Baseline data of the study subjects/patients, including those of clinical characteristics, were obtained from patients’ records. These data were scored according to the Acute Physiology and Chronic Health Evaluation (APACHE II) system.

On admission to the ICU, blood and urine samples were soon collected. Test on IL-6, and TNF-\(\alpha\) made use of blood without anti coagulant at the amount of 9-10 cc. Test on SOD used anti coagulant EDTA at the amount of 3-5 cc. Tests on TNF-\(\alpha\), IL-6, and SOD were done to the two groups before insulin therapy was started and on the seventh day after insulin therapy. Tests on TNF-\(\alpha\), and IL-6 by Elisa method using Quatikine kit. Meanwhile, test on SOD was done by Eliza’s method using Ransol kit. Test on blood glucose was done every 1-4 hours to adjust insulin therapy titration by hexokinase method. Beckman Coulter CX7 was used to check blood glucose of patients being admitted to the ICU, and MEDISAFE READER was used to control blood glucose in the ICU that had been equalized with Beckman Coulter CX7.
After seven days of monitoring, events of hypoglycemia, systemic inflammatory response syndrome (SIRS), and mortality rate were recorded and analyzed.

**Statistical Analysis**

Based on the data obtained from the present study, we hypothesized a significant increase of superoxide dismutase (SOD) level after intensive insulin therapy compared to that of the conventional insulin therapy. The outcome variables were compared with application of Student’s t-test and Fisher’s exact test. The data are presented as means ± SD.

**RESULTS**

Blood glucose control pre-test, severity of illness, and the baseline characteristics of all patients with prolonged critical illness (intensive care unit [ICU] who stayed for one week is shown in table 1.

**Superoxide Dismutase (SOD) Level in the Intensive Insulin Therapy and Conventional Insulin Therapy**

The study result showed that the mean level of SOD in the pre-test phase of intensive insulin therapy was 1,084.90 ± 193.43 U/gHb, while it was 1,049.40 ± 166.58 in the pre-test phase in the conventional insulin therapy group. At seventh day post-test, SOD level of the intensive insulin therapy group was 1,455.60 ± 180.25 U/gHb; while it was 1,147.90 ± 165.42 U/gHb in the conventional insulin therapy group. Result of analysis of paired sample test showed that there was a significant increase in the mean level of SOD at the pre and post-test of intensive insulin therapy on both groups (p=0.001). The result of the analysis of t-test showed that there was a significant difference between the level of SOD at both post-test intensive insulin therapy and conventional insulin therapy, in which p=0.001. Meanwhile, the increase in mean level of SOD (ΔSOD) in intensive insulin therapy group was 370.70 ± 163.35 U/gHb, and it was 98.50 ± 96.14 U/gHb in conventional insulin therapy group.

It can therefore be concluded that there was over production of superoxide ion in hyperglycemia. In order to reduce the negative effect of oxidative stress, a huge amount of SOD must be needed. Intensive insulin therapy leads to the decrease of ROS level and increase of SOD to reach the normal level.

**Table 1. Characteristics of study subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intensive insulin therapy (n=20) (mean ± SD)</th>
<th>Conventional insulin therapy (n=20) (mean ± SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex : Male</td>
<td>13</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>: Female</td>
<td>7</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.250 ± 14.029</td>
<td>41.860 ± 13.804</td>
<td>0.318</td>
</tr>
<tr>
<td>APACHE II Score</td>
<td>17.300 ± 2.849</td>
<td>16.900 ± 2.614</td>
<td>0.646</td>
</tr>
<tr>
<td>pre-test Glucose</td>
<td>214.700 ± 48.043</td>
<td>224.150 ± 66.293</td>
<td>0.609</td>
</tr>
<tr>
<td>pre-test SOD</td>
<td>1,084.900 ± 193.433</td>
<td>1,043.400 ± 166.588</td>
<td>0.538</td>
</tr>
<tr>
<td>pre-test TNF-α</td>
<td>10.266 ± 7.220</td>
<td>8.159 ± 6.299</td>
<td>0.332</td>
</tr>
<tr>
<td>pre-test IL-6</td>
<td>23.218 ± 9.636</td>
<td>20.352 ± 10.172</td>
<td>0.366</td>
</tr>
</tbody>
</table>

The APACHE II, or Acute Physiology and Chronic Health Evaluation, score denote the severity of illness, with severely ill patients having high score

**Table 2. SOD level in intensive insulin therapy and conventional insulin therapy**

<table>
<thead>
<tr>
<th>SOD Level</th>
<th>Intensive Insulin Therapy (n=20), (Mean ± SD)</th>
<th>Conventional Insulin Therapy (n=20), (Mean ± SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Test</td>
<td>1,084.900 ± 193.433</td>
<td>1,049.400 ± 166.566</td>
<td>0.538</td>
</tr>
<tr>
<td>Post Test</td>
<td>1,455.600 ± 180.252</td>
<td>1,147.900 ± 165.423</td>
<td>0.001</td>
</tr>
<tr>
<td>Δ SOD</td>
<td>370.700 ± 163.353</td>
<td>98.500 ± 96.143</td>
<td>0.001</td>
</tr>
</tbody>
</table>
**TNF-α Level in Intensive Insulin Therapy and Conventional Insulin Therapy**

Result of the study showed that level of TNF-α at the pre-test intensive insulin therapy group was 10.26 ± 7.22 pg/mL and in the pretest conventional insulin therapy group it was 8.15 ± 6.29 pg/mL. At the seventh day post-test, TNF-α in intensive insulin therapy group was 7.28 ± 2.77 pg/mL, while it was 8.78 ± 4.48 pg/mL in the conventional insulin therapy group. On the basis of paired sample test, there was no significant difference in both intensive insulin therapy group (p=0.078) and conventional insulin therapy group (p=0.713). Furthermore, the result of t test done to the level of TNF-α of the two groups showed no significant decrease of the level of TNF-α (p=0.211). Result of t test on the mean level (Δ TNF-α) of TNF-α also showed no significant difference between the two groups (2.98 vs. 0.63, p=0.977).

**IL-6 Level in Intensive Insulin Therapy and Conventional Insulin Therapy**

The study result showed that level of IL-6 in the pre-test intensive insulin therapy group was 23.21 ± 9.63 pg/mL, and it was 20.35 ± 10.17 pg/mL in the conventional insulin therapy group. The result of Kolmogorov-Smirnov’s normality test showed that the two groups were in normal distribution. The result of t test showed that there was no significant difference between the two groups (p=0.366). At the seventh day post-test, the post-test IL-6 level in intensive insulin therapy was 12.96 ± 7.81 pg/mL, while the post-test IL-6 level in the conventional insulin therapy group was 18.32 ± 7.11 pg/mL. The data also showed that there was a decrease in the level of IL-6, both in intensive and conventional insulin therapy group. With paired samples test, it was found that there was a significant decrease in the level of IL-6 in intensive insulin therapy group (p=0.001), but not in conventional insulin therapy group (p=0.411). Result of t test on the post-test IL-6 level showed there was a difference between the two groups. The t test on the decrease of the mean value of IL-6 level (Δ IL-6) in the two groups showed a significant difference (10.25 vs. 2.02; p=0.023).

The most likely reason of the fact that the two methods of insulin therapy gave different results is because in the conventional insulin therapy group blood glucose was maintained at the level of hyperglycemia (180 – 200 mg/dL), in intensive insulin therapy it was maintained at normal limit (80 – 110 mg/dL), so production of oxidative stress in conventional insulin therapy group was still higher than in intensive insulin therapy group. Therefore, the production of inflammatory mediator was higher in conventional insulin therapy group. The result of t test analysis showed that there was a significant difference (10.25 vs. 2.02; p= 0.023) of the mean level score of IL-6 (ΔIL-6) in the two groups. Based on the finding that there was a decrease of IL-6 level before and after intensive and conventional insulin therapy, and for the purpose of finding out whether there was influence on glucose level, SOD, TNF-α, albumin, insulin therapy infusion, and APACHE II score on ΔIL-6, analysis of univariate general linear model was done. Result of the latter analysis showed that infusion of insulin therapy was significantly correlated with cytokine IL-6 production (p=0.037).

**Table 3. TNF-α Level in Intensive Insulin Therapy and Conventional Insulin Therapy**

<table>
<thead>
<tr>
<th></th>
<th>Intensive Insulin Therapy (n=20) (Mean ± SD)</th>
<th>Conventional Insulin Therapy (n = 20) (Mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>10.266 ± 7.220</td>
<td>8.159 ± 6.299</td>
<td>0.332</td>
</tr>
<tr>
<td>Post</td>
<td>7.280 ± 2.777</td>
<td>8.786 ± 4.489</td>
<td>0.211</td>
</tr>
<tr>
<td>Δ TNF-α</td>
<td>2.986 ± 7.171</td>
<td>3.108 ± 17.361</td>
<td>0.977</td>
</tr>
</tbody>
</table>

**Table 4. IL-6 Level in Intensive Insulin Therapy and Conventional Insulin Therapy**

<table>
<thead>
<tr>
<th></th>
<th>Intensive Insulin Therapy (n=20) (Mean ± SD)</th>
<th>Conventional Insulin Therapy (n = 20) (Mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>23.218 ± 9.630</td>
<td>20.352 ± 10.172</td>
<td>0.336</td>
</tr>
<tr>
<td>Post</td>
<td>12.960 ± 7.814</td>
<td>18.329 ± 7.114</td>
<td>0.001</td>
</tr>
<tr>
<td>Δ IL-6</td>
<td>10.258 ± 11.171</td>
<td>2.023 ± 10.758</td>
<td>0.023</td>
</tr>
</tbody>
</table>

**The Event of SIRS in Intensive Insulin Therapy and Conventional Insulin Therapy**

During the study two patients (10%) of the intensive insulin therapy group got into SIRS, while of the conventional insulin therapy group nine patients (45%) became SIRS. Analysis by Fisher’s exact test showed a significant decrease in the number of patients entering SIRS in the intensive insulin therapy group compared to conventional insulin therapy group (p=0.001). Besides, the result of the analysis also showed that the odd ratio of the event of SIRS was 0.136 in which the trust interval was 0.025 – 0.748. Meanwhile, the relative risk to the event of SIRS in the two groups was 0.222 in which the trust interval was 0.055 – 0.902.
This event occurred because in the conventional insulin therapy group, blood glucose was maintained at the level of hyperglycemia. This may inhibit production of reactive oxygen mixture as oxidative stress, which can activate NF-kB and stimulate production of pro-inflammatory cytokine such as TNF-α, IL-1, and IL-6. Moreover, hyperglycemia may cause decrease in phagocytic function of phagocyte cells.

<table>
<thead>
<tr>
<th></th>
<th>SIRS</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensive Insulin Therapy</td>
<td>Yes</td>
<td>2</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Conventional Insulin Therapy</td>
<td>Yes</td>
<td>9</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>11</td>
<td>29</td>
<td>40</td>
</tr>
</tbody>
</table>

Analysis on the predicted risk of SIRS in intensive insulin therapy group and conventional insulin therapy group (Table 6) showed an odd ratio of the event of SIRS in the two groups was 0.136 with the trust interval 0.025 – 0.748. Meanwhile, the relative risk of SIRS in the two groups was 0.222 with a confidence interval 0.055 – 0.902.

<table>
<thead>
<tr>
<th>Score</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odd Ratio (Intensive/Conventional)</td>
<td>.136 .025 .748</td>
</tr>
<tr>
<td>Relative Risk</td>
<td>.222 .055 .302</td>
</tr>
<tr>
<td>Relative Risk</td>
<td>1.636 1.073 2.497</td>
</tr>
</tbody>
</table>

DISCUSSION

Cell damage is induced by reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radical. The main source of ROS in vivo is aerobic respiration. In hyperglycemia, certain complex mechanism, enzymatic and non-enzymatic processes give rise to the increase in ROS production. ROS is a lead actor of diabetic complication. The main damage to cells results from the ROS-induced alteration of macromolecules such as poly-unsaturated fatty acid in membrane lipids, essential protein and DNA. Under normal condition, ROS are cleared from the cells by the action of super oxide dismutase (SOD) as anti-oxidant enzyme. SOD plays an extremely important role in the protection of all aerobic life-systems, including that of man. SOD catalyzes the dismutation of superoxide into hydrogen peroxide. Hyperglycemia-induced mithochondrial superoxide over production tends to decrease the amount of SOD, causing the increase in demand as shown in the result of a study published by Colack et al in 2007.

Insulin treatment of hyperglycemic patients can decrease over production of superoxide anions and concomitantly decrease SOD consumption and lead to increasing SOD level. In the present study, we have found that a significant increase of SOD level occurred in intensive insulin therapy as compared to the conventional insulin therapy.

In this study we also have found that intensive insulin therapy gave only a slight effect on circulating TNF-α, and no significant effect was seen between two groups. This might be due to the difficulty of finding the appropriate time to detect the existence of TNF-α, the instability of TNF-α in blood, and other risk factors such as central obesity, alcohol consumption, and other uncontrollable genetic factors. Besides, TNF-α production is influenced by many factors including stressor from underlying diseases causing hyperglycemia, the treatment of which has not yet been perfectly known.

The present study showed a significant decrease of circulating IL-6 between the two groups, because in the conventional insulin therapy group blood glucose was maintained at hyperglycemia level, in intensive insulin therapy while blood glucose was maintained at normal level, so production of oxidative stress in the conventional insulin therapy group remained higher than in the intensive insulin therapy group. Therefore, the production of inflammatory mediator was also higher in the conventional insulin therapy group.

CONCLUSION

Result of the present study, involving treating patients by intensive insulin therapy, showed significant improvements in both molecular biological markers and clinical outcomes related to hyperglycemic state. Intensive insulin therapy of hyperglycemia resulted in decrease in the production of superoxide anions as ROS to acceptable lower level. As a result, therefore, the level of SOD enzyme increases. It also had effect to decrease the production of inflammatory cytokine like IL-6 that can decrease the event of SIRS and improve permeability of blood vessel, so the patients’ clinical outcomes become better. Besides, no significant
difference was found with regard to the complications of hypoglycemia, morbidity and mortality with the intensive insulin therapy compared with the conventional insulin therapy.

Despite some of its limitations, this study has demonstrated the importance of maintaining adequate glycemic control in critically ill patients, which apparently is very promising for further management of hyperglycemia.

REFERENCES

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