Nutritional Support in 1990's

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ISI-5 Effect of Administered Human Growth Hormone on Protein Metabolism in Septic Rats

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It is well known that growth hormone (GH) is anabolic agent which effects on the metabolism of lipid, carbohydrate, and protein^{1,2)}. Especially GH stimulates protein metabolism and accumulates positive nitrogen balance. Although nitrogen sparing effect of GH has been examined for healthy subjects, burned patients and the patients with elective surgery^{3,4,5)}, there are only a few papers described for the effect on septic condition^{6,7)}.

In order to estimate the effect of recombinant human growth hormone (HGH) on protein metabolism in septic condition, two experimental studies were designed using sepsis models induced by cecal ligation and induced by administration of endotoxin to rats.

Experimental Design I

Wistar male rats weighing about 230 g were used.

After 24 hours of fasting, silastic catheter was inserted into the superior vena cava via the right jugular vein for administration of TPN solution. Rats were divided into 4 groups. Group I (n=10) underwent sham operation. In Group II (n=14), Group III (n=7) and Group IV (n=10), sepsis was induced by cecal ligation without puncture according to Wichterman's method. HGH was administered continuously every day together with total parenteral nutrition (TPN) infusion to Group III and Group IV. In Group III, a 100 mU/day dose of HGH was given to each rat and a 200 mU/day dose of HGH was given to Group IV rats. All rats were maintained in individual metabolic cages and received TPN during the following 4 days. Each rat received 180 kcal/kg·day of non-protein calories, 1.1 g/kg·day of nitrogen (N), minerals and vitamins.

Urine was collected every day for determination of total N and 3-Methylhistidine (3-MH) excretion. All rats were sacrificed on Day 5. Arterial blood samples were obtained for measurement of blood chemistries. All values were expressed as mean SD, and compared by student's t-test and analysis of

Fig. 1 : Cumulative nitrogen balance of septic rats induced by cecal ligation.

Group I : Control group, non septic rats
 Group II : septic group without GHG
 Group III : Septic group with 100 mU of GHG
 Group IV : Septic group with 200 mU of GHG

Fig. 2 : Cumulative nitrogen balance of septic rats induced by administration of endotoxin (LPS)

Group I : Control group, non septic rats
 Group II : Septic group with 1 mg/100 g B.W. of LPS
 Group III : Septic group with 2 mg/100 g B.W. of LPS
 a : non GHG, b : 200 mU of GHG

variance (ANOVA). Significance was designated at the 95 % confidence level.

Results

I. Protein Metabolism: Cumulative N balance during 4 day's course of TPN is shown in **Fig-1**: Group-I, 1264 ± 355 mg/kg; Group-II, 117 ± 693 mg/kg; Group III, 92 ± 735 mg/kg; Group-IV, 1001 ± 279 mg/kg. Remarkable improvement was seen in Group-IV comparing with Group-II ($P < 0.01$), however no improvement was seen in Group-III.

The cumulative urinary excretion of 3-MH in Group I was 6.2 ± 0.9 mg/kg and in Group II, Group III and Group IV were 12.0 ± 2.2 mg/kg, 13.4 ± 2.9 mg/kg and 10.5 ± 2.3 mg/kg, respectively. Urinary excretions of 3-MH were significantly higher in all sepsis groups than in sham-operated group, however no significant difference was seen between Group II and Group IV.

II. Blood Chemistries: Serum albumin levels were significantly lower in all sepsis groups than in sham operated groups. The blood urea nitrogen (BUN) in group IV was significantly lower than that in Group II.

The serum levels of blood glucose, insulin, free fatty acid and ketone bodies in Group II and Group III were significantly higher than that in Group I, however there was no difference among sepsis groups. The serum level of GHG was not detected in Group I and Group II.

These data showed that although exogenously administered GHG was effective on protein metabo-

lism in septic rats, high dose of HGH was required to improve N balance. According to this results, we examined the effects of exogenously administered high dose of HGH on protein metabolism in different severity of septic conditions induced by administration of endotoxin (LPS).

Experimental Design II

Wistar male rats weighing about 230 g were used and they received 4 days TPN as mention as preveously. Rats were devided to three groups. Group I was control Group without administration of endotoxin. Endotoxin (LPS, E. Coli 025: B6, Sigma Chemical Co.) was administered continuously on 2nd, 3rd and 4th day of study together with TPN infusion to Group II and Group III.

In Group II, a 1 mg/100 g body weight dose of LPS was given to each rat and a 2 mg/100 g body weight dose of LPS was given to Group III rats.

Each group was devided to two subgroups with or without administration of HGH.

A 200 mU/day dose of HGH was administered continuously together with TPN infusion.

Urine was collected on the 3rd and 4th day to determine total nitrogen and 3-MH, excretion.

Whole body protein kinetics was measured on the 4th day of the study using a primed continuous infusion of ^{15}N glycine over 24 hrs. according to the Picou & Taylor-Roberts method.

On day 5, rats were sacrificed and arterial blood samples were obtained for measurements of blood chemistries.

Results

I. Protein Metabolism: The cumulative N balance of the latter 2 days of the study was shown in Fig. 2: Group Ia (n=10: Control group and non HGH rats) ; $986.5 \pm 150.3 \text{ mg/kg}$, Group I b (n=10: control group, HGH rats) ; $1183.5 \pm 201.9 \text{ mg/kg}$, Group II a (n=8: 1 mg/100 g B.W. of LPS, non HGH rats) ; $584.9 \pm 52.2 \text{ mg/kg}$, Group II b (n=8: 1 mg/100 g B. W. of LPS, HGH rats) ; $825.9 \pm 100.8 \text{ mg/kg}$, Group III a (n=8: 2 mg/100 g B. W. of LPS, non HGH rats) ; $373.1 \pm 37.9 \text{ mg/kg}$, Group III b (n=7: 2 mg/100 g B. W. of LPS, HGH rats) ; $398.2 \pm 45.8 \text{ mg/kg}$. Group I showed the best N balance and Group III showed the worst. The cumulative N balances of HGH rats were significantly higher than that of non HGH rats in Group I and in Group II, wherease in Group III there was no difference between in HGH rats and in non HGH rats.

The cumulative urinary excretion of 3-MH showed the highest excretion in Group III of the three groups. However, HGH was not effective on decreasing of urinary excretion of 3-MH.

II. Blood Chemistries: The serum levels of total protein and albumin showed the lowest level in Group III of the three groups, however there was no significant difference between HGH rats and non HGH rats.

The serum levels of BUN showed the highest level in Group III. In Group II, the level of the HGH rats was significantly lower than that of the non HGH rats ($p < 0.05$). The serum glucose levels of HGH rats were higher than that of non HGH rats, however there was no significant difference. There was no difference in serum riglyceride levels among each group, however the serum levels of free fatty acid in Group I and Group II were significantly higher in HGH rats than in non HGH rats. ($p < 0.05$, $p < 0.01$ respectively).

III. Whole Body Protein Kinetics: The whole body protein turnover rate, breakdown rate and synthesis rate were the most accelerated in Group III of the three groups. The whole body protein turnover rate and breakdown rate were no significant difference between HGH rats and non HGH rats. The whole body protein synthesis rate was shown in Fig-3. In Group I and Group II, the whole body

Fig. 3 : Whole body protein synthesis rate of septic rats induced by administration of endotoxin (LPS)

protein synthesis rate of HGH rats was significantly higher than that of non HGH rats, whereas in Group III there was no difference between HGH rats and non HGH rats (Fig. 3).

Discussion

The goal of nutritional support is an improvement of protein metabolism. It is very hard to gain nitrogen retaining in critically ill patients, in spite of receiving high energy and nitrogen. Especially patients fail to retain positive N balance in acute phase of septic condition. Shaw reported that net protein catabolism was accelerated when nitrogen more than 1.5 g/kg per day was administered to the patients with sepsis⁸⁾. In septic condition, whole body protein kinetics are accelerated. HGH increases tissue amino acid incorporation and results in stimulation of protein synthesis. There are many reports about the effect of HGH on protein metabolism in burned, traumatized and surgical patients. However there are only a few papers for septic condition.

In our studies, high dose of HGH (200 mU/body) was required to improve N balance in rats with mild septic condition. The dose is as same as 0.8 IU/kg·day (0.32 mg/kg·day).

In our experimental design II, exogenously administered high dose of HGH failed to improve nitrogen retaining effect for the rats with severe sepsis induced by 2 mg/100 gB. W. dose of LPS administration. Why does not work HGH on protein metabolism in severe septic condition? A several mechanisms can be speculated. The first mechanism is thought that HGH stimulate positive nitrogen balance as a direct action of lipolysis, however fat could not be mobilized in severe sepsis. In our studies, serum FFA levels in severe sepsis were not significantly different between in HGH rats and in non HGH rats. The second mechanism is considered that the sensitivity of HGH receptor may be altered or decreased in severe septic condition as like as mentioned about insulin receptor. The third mechanism is speculated that although HGH stimulates the production of insulin-like-growth factor-1 (IGF-1) by liver and results in acceleration of protein synthesis as an indirect action, IGF-1 may be not produced by liver in severe septic condition. Dahn reported that IGF-1 production was inhibited in human sepsis administering of 4 mg/day (10 IU/day) of HGH for 48 hours⁹⁾.

In our studies, we have no data to explain this mechanism. Farther studies are necessary.

Conclusion

- 1) High dose of HGH was required to improve nitrogen balance (N. B.) in septic rats. In severe sepsis, N. B. was not improved, in spite of administration of high dose of HGH.
- 2) HGH had no effect on decreasing urinary excretion of 3-methylhistidine.
- 3) Nitrogen sparing effect of HGH results from a increased whole body protein synthesis rather than a decreased whole body protein breakdown.
- 4) Careful consideration should be taken for administration of HGH in septic patients.

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