全靜脈營養輸入特殊胺基酸對敗血症老鼠營養素代謝及免疫反應之影響

Effects of parenterally infused specific amino acids on nutrient metabolism and immune response in septic rats

中文摘要

第一部份：全靜脈營養輸入精胺酸對敗血症老鼠營養素代謝、胺基酸組成及發炎反應介質之影響

本研究的目的在探討全靜脈營養(TPN)溶液中添加 Arginine(Arg)，對敗血症發生之早期及晚期營養素代謝及免疫功能之影響。本實驗使用體重 200-250g 之雄性 Wistar 大白鼠，進行頸靜脈插管後將老鼠分爲 Arg 組及 Glycine (Gly)組，完全由 TPN 供給營養，添加量分別爲 3.15 公克 Arg/公升及 5.43 公克 Gly/公升 TPN 溶液，如此可使兩組給予之必需胺基酸完全相同，並使兩組輸入之 TPN 爲等熱量等氮量，TPN 溶液之熱量濃度爲 1 kcal/mL，氮供應量爲 270mg/kg 體重/天，氮與熱量比爲 1：143，TPN 輸入 5 天後以盲腸穿刺及結紮手術(CLP)引發敗血症。在敗血症後的 2、4、6 及 24 小時各時間點犧牲老鼠，取腹腔動脈血及腹水供分析用。並收集 TPN 最後兩天及 CLP 後 24 小時之尿液做氮平衡之分析。結果顯示在氮平衡方面，CLP 前 Arg 組及 Gly 組呈現正氮平衡，而 CLP 後兩組之間均呈現負氮平衡，但 2 組間皆無統計上之差異。在血中胺基酸組成方面，Arg 組血中 Arg 濃度顯著比 Gly 組高。在支鏈胺基酸(BCAA)方面，在 CLP 後 2 小時 Gly 組之 valine 濃度比 Arg 組高，但在其他各時間點 2 組的 BCAA 濃度皆無差異，CLP 後 2 及 24 小時時血中 alanine(Ala)的濃度在 Arg 組皆比 Gly 組高，表示在 CLP 後 Arg 組肌肉組織分解較明顯，且分解所產生之 BCAA 快速轉變成 Ala，而 Ala 經 Alanine cycle 可轉變成葡萄糖供身體在異化作用時使用，所以認爲 Arg 組的敗血症老鼠肌肉組織的異化作用較 Gly 組明顯。在淋巴球之 T 細胞分佈方面，Arg 組之 CD4/CD8 比例在 24 小時時比 Gly 組高。腹水中之 cytokine 部份在 CLP 後 6 小時 interleukin-1 β 及 tumor necrosis factor - α 的濃度 Gly 組高於 Arg 組，interleukin-6 的濃度在 24 小時時 Gly 組亦是高於 Arg 組。血中及腹水中 nitric oxide 的濃度在 CLP 後 4 小時時 Arg 組皆比 Gly 組高且有統計上之差異。此結果顯示與添加 Gly 組相較，TPN 溶液中添加 Arg 可減少敗血症時腹水中 cytokine 之分泌，應可因而減輕敗血症老鼠之局部發炎反應，亦可促進敗血症老鼠的細胞性免疫能力，因此 Arg 的添加對於提升敗血症老鼠免疫力具有正面效果，但並無法防止敗血症引致之肌肉組織分解及負氮平衡。

第二部份：全靜脈營養輸入 Glutamine 對敗血症老鼠血中胺基酸組成及發炎反應介質之影響

重症病人在使用全靜脈營養(TPN)支持時常易發生感染而引發敗血症，敗血症發

生時會使體內營養素代謝異常，體組織分解及免疫調節物質分泌不正常，造成多重器官衰竭。Glutamine(GLN)是一個大量存在於體內的非必需氨基酸，研究顯示在術後或異化性疾病發生時血中及組織中 GLN 的濃度會顯著減少，而給予 GLN 的補充可減緩異化作用的發生。以往的研究顯示 TPN 溶液添加 GLN 可促進蛋白質合成，對腸道重量，敗血症引致之腸道組織傷害及存活率有幫助，並無關於 GLN 添加對 TPN 併發敗血症後免疫反應、及除蛋白質代謝外其他營養素代謝的報告。因此本研究的目的在探討 TPN 溶液中添加 GLN，對敗血症發生之早期及晚期營養素代謝及免疫功能之影響。本實驗使用體重 200-250g 之雄性 Wistar 大白鼠，進行頸靜脈插管後將老鼠分爲 GLN 組及 Glycine (Gly)組，完全由 TPN 供給營養，GLN 組爲在 TPN 溶液中添加 2%之 GLN，Gly 組則以與 GLN 組等氮量之 Gly 添加以做爲控制組，兩組除胺基酸組成不同外其餘組成均完全相同。TPN 輸入 5 天後以盲腸穿刺及結紮手術(CLP)引發敗血症。在敗血症後的 2、4、6 及 24 小時各時間點犧牲老鼠，取腹腔動脈血及腹水供分析用。並收集 TPN 最後兩天及 CLP 後 24 小時之尿液做氮平衡之分析。結果顯示在血中胺基酸組成方面，GLN 組血中 GLN 濃度顯著比 Gly 組爲高。在支鏈胺基酸(BCAA)方面， Gly 組血中 valine 之濃度在 2、4 及 6 小時時皆比 GLN 組高，isoleucine 則在 2 及 6 小時時比 GLN 組高。Gly 組血中 alanine 濃度隨著敗血症後時間的增加而增加，在 4 及 24 小時均比 GLN 組爲高。在氮平衡方面，引致 CLP 之後 24 小時，GLN 組負氮平衡之現象均顯著較 Gly 組減輕。由於異化作用時肌肉組織中 BCAA 會釋出，提供胺基供 Ala 合成及醣質新生之用，由本實驗結果可推斷 Gly 組肌肉組織 BCAA 之釋出較多，Ala 合成亦較多，表示 Gly 組肌肉組織之分解較 GLN 組嚴重。血漿游離脂肪酸及三酸甘油酯的濃度在 6 小時時 Gly 比 GLN 組高，顯示 Gly 組脂肪組織之分解在敗血症早期較 GLN 組爲多。在淋巴球之 T 細胞分佈方面， CD4/CD8 之比例在 CLP 後之各個時間點 GLN 組均有較 Gly 組高之趨勢，但只有在 CLP 後 4 小時 GLN 組顯著較 Gly 組爲高。腹水中之 cytokines 部份 interleukin(IL)-6， IL-1 及 TNF-a 不論在各時間點 Gly 組或 GLN 組間均無差異。腹水中的 nitric oxide 濃度在 CLP4 小時後 Gly 組顯著較 GLN 組高。這些結果顯示與 Gly 組相較，GLN 有抑制 nitric oxide 分泌的現象，但是對發炎部位 cytokines 之分泌卻沒有抑制之作用。本研究結果顯示，與添加 Gly 組相較，TPN 溶液中添加 GLN 可減輕敗血症老鼠脂肪組織及肌肉組織之分解，並可使敗血症引致之負氮平衡嚴重程度減輕。GLN 之添加雖然對於發炎部位細胞激素分泌沒有抑制的作用，但是可增加細胞性免疫能力。

英文摘要

(1)Effects of parenteral infusion with arginine on plasma amino acid profiles and inflammatory mediators in septic rats

This study was designed to investigate the effect of preinfusion with total parenteral nutrition (TPN) supplemented with arginine (Arg) or glycine (Gly) on plasma amino acid pattern and inflammatory-related cytokines in septic rats. Fifty-six rats, with internal jugular catheters, were divided into 2 groups. The two groups received isonitrogenous isocaloric TPN supplemented with 270mg of nitrogen per kilogram per day as either Arg or Gly. TPN provide 270kcal/kg body weight and the kcal/nitrogen ratio in TPN solution is 143:1. TPN was maintained for 5 or 6 days. On day 5, sepsis was induced by cecal ligation and puncture (CLP). After CLP for 2, 4, 6, and 24h, rats were sacrificed, respectively, to investigate the metabolic changes in septic stage between the Arg and Gly groups. The results demonstrated that CLP resulted in a negative nitrogen balance, but there were no difference in nitrogen balance between the two septic groups. Plasma amino acid profiles demonstrated that Arg levels were significantly higher in the Arg groups than the Gly groups. Compared with early septic stage, plasma concentrations of branch chain amino acid (BCAA) were reduced, whereas alanine level were increased in late septic stage, regardless the animals were supplemented with Arg or Gly. No significant difference in plasma BCAA levels between the two septic groups were seen at various time points, except that plasma valine levels were significantly higher in the Gly group than in the Arg group at 2 h after CLP. However, plasma concentrations of alanine were significantly higher in the Arg group than in the Gly group. Since BCAA released from muscle protein in the catabolic stage, and rapidly converted to alanine to offer energy substrate for the body, the higher alanine level in the Arg group may indicate that the catabolic reaction of septic rats preinfused with Arg is more obvious than those preinfused with Gly. Plasma interleukin (IL)-1b levels were undetectable. No difference in plasma concentrations of IL-6 as well as tumor necrosis factor-a were observed between the Arg and Gly groups at various time points. However, IL-1b and tumor necrosis factor-a concentration in peritoneal lavage fluid at 6 h after CLP in the Gly group were significantly higher than the Arg group. T-lymphocyte population in blood revealed that CD4+/CD8+ ratio was significantly higher in the Arg group than the Gly group at 24 h after CLP. These results suggest that compared with Gly infused groups, Arg infusion resulted in a lower inflammatory-related cytokines in early septic stage in the location of the injurious stimulus, and CD4+/CD8+ ratio was enhanced after CLP for 24 h. However, TPN with Arg demonstrated no beneficial effect in preventing tissue protein breakdown.

(2)Effects of parenteral infusion with glutamine on plasma amino acid profiles and inflammatory mediators in septic rats

The effects of total parenteral nutrition (TPN) enriched with glutamine on plasma amino acid profiles and immune response were evaluated in septic rats. Sixty male Wistar rats weighing 200-250 g were used and were divided into two experimental groups. The all rats in the experimental groups received TPN solution at an energy level of 270 kcal/kg BW. The TPN solutions were isonitrogenous and identical in nutrients composition except for the differences in amino acid content. One experimental group received 2% glutamine (GLN), whereas the other group received glycine (Gly) instead. TPN was maintained for 5 or 6 days according to the sacrificed schedule of the rats. On day 5, sepsis was induced by cecal ligation and puncture (CLP). After CLP for 2, 4, 6, and 24 h, rats were sacrificed, respectively, to investigate the metabolic changes in different septic stage between the GLN and Gly groups. The results demonstrated that plasma GLN levels in GLN group was significantly higher than in Gly group at various time points. Plasma valine levels in Gly group was significantly higher at 2, 4, 6h after CLP, and isoleucine were higher at 2, and 6h after CLP than in GLN group. Plasma alanine levels were increase in accordance with the time after CLP in Gly group, and the alanine levels were significantly higher in Gly group than in GLN group at 4 and 24h after CLP. Since branch-chain amino acids (BCAAs) were released in catabolic condition and may consequently converted to alanine to offer energy substrate for other organs. The higher plasma BCAA and alanine level observed in Gly group may indicate that a greater extent of catabolic reaction was happened in septic rats preinfused with Gly than in GLN group.

Sepsis resulted in a negative nitrogen balance in both groups, however, the nitrogen loss was attenuated when GLN was administered. Plasma non-esterified fatty acid and triglyceride concentrations were significantly higher in Gly group than in GLN group at 6 h after CLP. The results in T lymphocyte population revealed that CD4/CD8 ratio had a tendency to be higher in the GLN group than in the Gly group, but statistically significant difference was only observed between the Arg and Gly groups at 4h after CLP. Interleukin (IL)-6 concentration in peritoneal lavage fluid (PLF) were significantly higher, whereas IL-1b was lower in 24 h after CLP than other time points, regardless the rats were preinfused with GLN or Gly. There were no significant difference in IL-1, IL-6, tumor necrosis factor-a concentration in PLF at various time points between Gly and GLN groups. Nitric oxide (NO) levels in PLF in Gly group were higher than in GLN group at 4 h after CLP. These results suggest that compared with Gly groups, TPN with GLN inhibited NO secretion, however, GLN had no effect on cytokine secretion in PLF.

In summary, the present study demonstrated that compared with Gly, TPN with GLN supplementation ameliorated the catabolic reaction of muscle protein, and negative nitrogen reaction was improved. In addition, GLN supplementation enhanced cell-mediated immune response in the early stage of sepsis, however, GLN had no effect on cytokine secretion in the location of injurious stimulus.