行政院國家科學委員會專題研究計畫成果報告

計畫名稱：L-glutamine 添加對燒燙傷老鼠營養素代謝及免疫反應之影響
Effects of L-glutamine Supplementation on Nutrient Metabolism and Immune Response in Thermal Injured mice

計畫類別：個別型計畫

計畫編號：NSC-90-2314-B-002-305

執行期間：90 年 8 月 1 日 91 年 7 月 31 日

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中華民國 九十一 年 十 月 一 日

中文摘要

本研究以燒燙傷老鼠的動物模式來探討 GLN 之添加對燒燙傷老鼠體內 nitric oxide(NO)產生，一般營養素代謝，及抗氧化酵素系統之影響，同時也探討 GLN 添加對燒燙傷後感染綠膿桿菌致死率之影響。本實驗分成兩個部分，以剛斷奶之雄性Balb/c mice為實驗對象，實驗一：將老鼠分成2組每組30隻，一組添加alanine + glycine (Ala + Gly)，一組添加 GLN, GLN 之添加量為 4% (w/w) ,二組除添加之胺基酸不同外其餘飲食成分均完全相同，此實驗飲食給予 4 週，4 週後將老鼠引致燒傷。在燒傷後之 1、2、3 天分別犧牲 10 隻，並取下肺肝腎等器官。實驗二：同樣將老鼠分成 2 組並餵食實驗飲食如實驗一，並於四週後引致體表燒傷，在燒傷後立即在燒傷部位之皮下注入約 10^6 之綠膿桿菌引致感染，觀察其致死率一週。結果顯示在燒傷之後不論 Gln 添加組或控制組，血漿中 glucose, lactate and NO 在第一天均顯著較第二、三天低，但 Gln 添加組或控制組在各不同時間點血中之 glucose, lactate, NO 或游離脂肪酸均無差異。在肝、腎、肺臟抗氧化酵素活性方面，兩組在各時間點酵素活性亦無差異。但本實驗觀察到 Gln 添加組在燒傷後感染綠膿桿菌之致死率顯著較控制組低。此結果顯示 Gln 之添加並未影響燒傷後醣類及脂質之代謝，也並未減輕燒傷老鼠之氧化壓力，但 Gln 添加可明顯改善燒傷感染之致死率。

關鍵字：燒傷、glutamine、營養素代謝、抗氧化酵素、致死率

Abstract

This study investigated the effect of glutamine (Gln) supplementation on nutrient metabolism and antioxidant enzyme activities in burned mice. Also, the survival rate of burned mice complicated with *Pseudomonas aeruginosa* was evaluated. There were 2 experiments in this study. Experiment 1: Sixty male BALB/c mice were assigned to 2 groups. One group was fed a control diet with casein as the protein source, while the other group was supplemented with 4% (w/w) Gln in addition to casein. The 2 groups were isonitrogenous. After 4 weeks, all mice received a 30% body surface area burn injury. Mice in each group were sacrificed for 3 consecutive days after the burn with 10 mice on each respective day. Plasma blood chemistry and antioxidant enzyme activities were analyzed. Experiment 2: Thirty mice were divided into 2 experimental groups, and were fed a control or Gln diet for 4 weeks as described in experiment 1. After 4 weeks, burn injury was induced, and mice were challenged with *Pseudomonas aeruginosa*. Survival of the burned mice was observed for 7 days. The results demonstrate that no differences in

plasma glucose, non-esterified fatty acids, and lactic acid concentrations were observed between the 2 groups on each respective day. Also, there were no differences in superoxide dismutase and glutathione peroxidase activities in tissue homogenates. The survival rate in Gln-supplemented group was significantly higher than the control group in burn mice with infection. These results suggest that Gln supplementation had no beneficial effect on glucose and lipid metabolism, nor had any effect on attenuating oxidative stress induced by burn injury. However, Gln supplementation improves survival of burned mice complicated with *P. aeruginosa* infection.

Key words: burn, glutamine, nutrient metabolism, antioxidant enzyme activity, survival rate

計畫緣由與目的

燒燙傷(thermal injury)是一種會增加氧化壓力之創傷，其在病理、生理上的變化包括嚴重的氮流失，代謝速率增加、賀爾蒙分泌之改變及免疫功能降低，另外燒燙傷也會促使活性氧自由基之產生，而使遠離燒傷部位之器官受損，傷口難以癒合易受細菌感染。有報告顯示在受傷及手術狀況下，血中 GLN 的濃度明顯下降，可能因而抑制免疫反應(immunosuppression)。嚴重燒燙傷時血中 GLN 之濃度會降低，而補充較高量 GLN 之燒傷老鼠其腸道細菌轉移(translocation)之比例較低。另外，依據臨床統計資料顯示綠膿桿菌(*Pseudomonas aeruginosa)*是目前院內感染之主要致病菌，燒燙傷病患亦最常被綠膿桿菌感染。目前關於 GLN 補充對燒傷影響的研究並不多，大多著重在蛋白質及 GLN 之代謝，並無關於 GLN 對燒傷感染方面之研究，亦無對體內氧化狀態之研究，故在本實驗中我們想以燒燙傷老鼠的動物模式，來探討 GLN 之添加對燒燙傷老鼠體內 nitric oxide(NO)產生，一般營養素代謝，及抗氧化酵素系統之影響，及 GLN 添加對燒燙傷後感染綠膿桿菌致死率之影響。

材料與方法

本計劃以剛斷奶之雄性 Balb/c mice 為實驗對象，經一星期之適應期後，實驗一：將老鼠分成 2 大組，每大組各 30 隻，一組添加 alanine + glycine (Ala + Gly)，一組添加 GLN, GLN 之添加量為 4% (w/w) , Ala + Gly 之添加量為使兩組為等氮

量，Ala 為 4% Gly 為 2%，二組除添加之胺基酸不同外其餘飲食成分均完全相同，protein、fat、carbohydrate 約佔總熱量之 20%、12%、68%，此實驗飲食給予 4 週，4 週後引致燒傷。燒燙傷後禁食 24 小時，禁食 24 小時可造成老鼠類似燒傷病人之代謝異常情形。燒燙傷後第三天為異化作用之最高峰，為了解燙傷後三天內不同時間點異化作用之變化情形，故在燙傷後之 1、2、3 天分別犧牲 10 隻，以心臟採血，並摘下肺肝腎等器官。實驗二：同樣於四週後引致體表燒傷，並於燒傷部位之皮下注入約 10^6 之綠膿桿菌引致感染，觀察其致死率一週。燒燙傷後同樣禁食 24 小時，禁食 24 小時後繼續給予原飼料。燒燙傷之方式為將老鼠以腹腔注射麻醉後，剃掉背部的毛，將老鼠置於一底部開有一長方形空窗之隔熱模型中，將剃毛部位緊貼於空窗，再將此曝露處滴注 1ml 之 95%酒精，然後點火燃燒 15 秒，造成老鼠體表約 30%之三度燙傷，燙傷後立即給予腹腔注射生理食鹽水(10ml/100g BW)以補充水分。

結果與討論

本研究結果顯示在燒傷之後不論 Gln 添加組或控制組，血漿中 glucose, lactate and NO 在第一天均顯著較第二、三天低，但 Gln 添加組或控制組在各不同時間點血中之 glucose, lactate, NO 或游離脂肪酸(NEFA)均無差異（表 1）。由於燒傷後第一天禁食故血糖顯著較回復進食後低。研究顯示在燒傷時體內醣質新生作用大量進行，而葡萄糖之代謝即使在氧供應充足下，仍主要經由醣解作用(glycolysis)，故造成大量乳酸堆積，故在燒傷第二、三天後明顯觀察到血中乳酸值升高之情形。由於在本實驗中兩組在 plasma glucose, lactate, NEFA 濃度均無差異，顯示 Gln 之添加並未影響燒傷後醣類及脂質之代謝變化。

在肝、腎、肺臟抗氧化酵素 superoxide dismutase and glutathione peroxidase 活性方面，結果顯示兩組在各時間點兩種酵素知活性均無差異(表 2, 3)。由於 Gln 是 glutathione 之組成份之一，而 glutathione 是一強力之抗氧化劑，曾有實驗顯示 Gln 添加可使肝臟保留 glutathione 量較多，而使肝臟受損的情況較輕微。但在本實驗中 Gln 添加組抗氧化酵素活性並未降低，表示 Gln 添加並未減輕燒傷老鼠之氧化壓力。有可能是本實驗燒傷造成之氧化壓力太大，而 Gln 之添加尚不足以減輕氧化壓力。本實驗觀察到 Gln 添加組在燒傷後感染綠膿桿菌之致死率顯著較控制組低（圖 1），由於本實驗在營養素代謝及減輕氧化壓力上，並沒有看到 Gln 添加有正面之效果，Gln 添加組致死率較低是否因與發炎反應相關細胞激素分泌較少，或是增強了體內特異性與非特異性免疫反應，則有待目前正在進行中之今年度計劃繼續探討。

Table 1. Plasma glucose, non-esterified fatty acids (NEFA), lactate and nitric oxide (NO_2^-/NO_3^-), concentrations in mice 1, 2 and 3 days after the burn

	GLN			Control		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Glucose (mg/dL)	129.6 ±21.3#	200.0 ±21.5	184.4 ±44.9	131.4 ±19.4	217.8 ±21.7*	162.9 ±47.0
NEFA (mmol/L)	1.2 ±0.24	1.10 ±0.38	1.20 ±0.38	1.17 ±0.16	1.29 ±0.17	1.31 ±0.59
Lactate (mg/dL)	52.8 ± 9.6#	76.9 ± 9.1*	89.9 ± 14.3	55.7 ± 7.6#	74.0 ± 5.5*	97.6 ± 11.8
NO_2^-/NO_3^- (μmol/L)	29.2 ±10.9#	32.1 ±4.6	40.5 ±7.6	29.0 ±4.7#	34.9 ±3.5	39.1 ±9.2

Data are expressed as the mean ± SD.

Significantly different from days 2 and 3 of the same group

* Significantly different from days 1 and 3 of the same group

† Significantly different from the corresponding day of the control group

Table 2. Glutathione peroxidase activity in tissue homogenates 1, 2 and 3 days after the burn

	GLN			Control		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Lung	0.37 ±0.11	0.37 ±0.11	0.31 ±0.05	0.36 ±0.12	0.32 ±0.08	0.29 ±0.10
Liver	1.43 ±0.40	1.33 ±0.29	1.44 ±0.12	1.60 ±0.15	1.58 ±0.22	1.61 ±0.21
Kidney	0.77 ± 0.13	0.84 ± 0.12	0.68 ± 0.10	0.92 ± 0.34	1.16 ± 1.00	0.78 ± 0.15

Data are expressed as the mean ± SD. (U/g tissue)

Table 3. Superoxide dismutase activity in tissue homogenates 1, 2 and 3 days after the burn

	GLN			Control		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Lung	7.74 ±2.31	6.69 ±2.39	6.91 ±2.55	8.47 ±2.63	8.25 ±3.19	8.41 ±3.71
Liver	12.35 ±5.09	7.97 ±3.08	7.93 ±1.89	9.63 ±3.19	9.00 ±2.78	11.16 ±4.15
Kidney	6.41 ± 1.55	7.15 ± 1.21	6.14 ± 1.47	5.68 ± 1.15	6.08 ± 1.40	6.48 ± 1.11

Data are expressed as the mean ± SD. (U/g tissue)

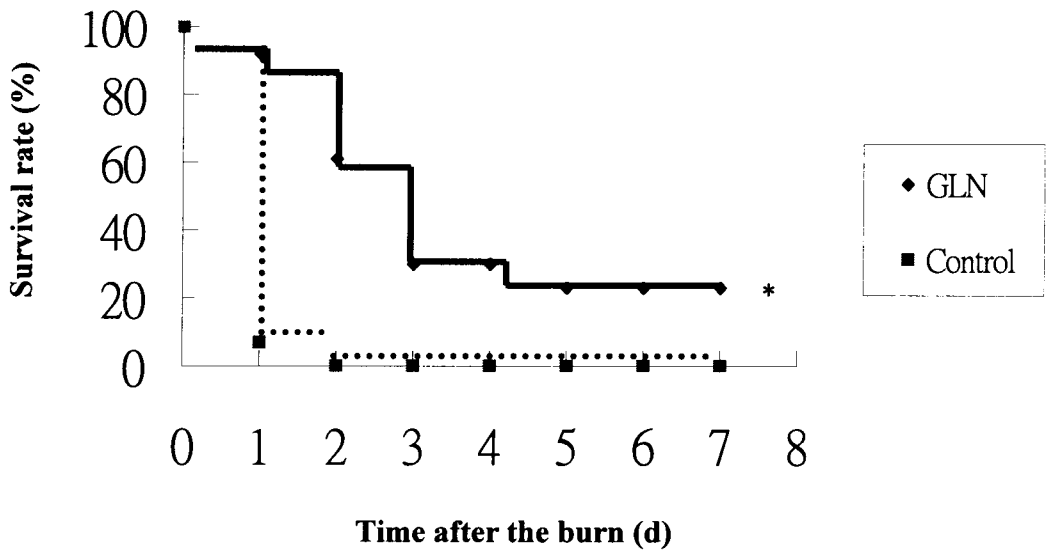

Fig. 1 Survival curves of burned mice challenged with 1x LD_{50} *P. aeruginos*. The Gln group were significantly higher than those of the control group ($p < 0.001$).

計畫成果自評

本計畫均遵照當初之實驗設計進行，本計畫之結果最近已整理完成，正在準備投稿國外期刊。

References

1. Tredget EE, Yu YM. The metabolic effects of thermal injury. World J Surg 1992;16:68-79.
2. Till GO, Beauchamp C, Menapace D, et al. Oxygen radical dependent lung damage following thermal injury of rat skin. J Trauma 1983;23:269-77.
3. Till GO, Hatherill JR, Tourtellotte WW, et al. Lipid peroxidation and acute lung injury after thermal trauma to skin. Am J Pathol 1985;119:376-84.
4. Willmore DW. The effects of glutamine supplementation in patients following elective surgery and accidental injury. J Nutr 2001;131:2543S-2549S.
5. Parry-Billings M, Evans J, Calder PC, Newsholme EA. Does glutamine contribute to immunosuppression after major burns? Lancet 1990;336:523-525.
6. Holder IA. The pathogenesis of infection owing to *Pseudomonas aeruginosa* using the burned mouse model: experimental studies from the Shriners Burns Institute, Cincinnatti. Can J Microbiol 1985;31:393-402.
7. Hong RW, Rounds JD, Helton WS, Robinson MK, Wilmore DW. Glutamine preserves liver glutathione after lethal hepatic injury. Ann Surg 1992;215:114-119.