SERUM AMINO ACIDS (GLUTAMINE, GLUTAMATE, METHIONINE, AND ARGinine) FLUX AFTER CUTANEOUS THERMAL AND SMOKE INHALATION INJURIES IN RATS

Tang Y.-W.

Plastic and Reconstructive Surgery, Taichung Veterans General Hospital, Taichung, Taiwan

SUMMARY. Smoke inhalation injury remains a major co-morbid complication in burn patients. The mortality rate of patients with burns and smoke inhalation injury is reported to be much higher than that of patients with either injury alone. Patients with this combined injury need a more intensive and aggressive nutrition supply to help them survive. The present study, using rats, was designed to test amino acid flux in cases of combined injury (smoke inhalation injury and cutaneous burns). The purpose was to understand the amino acid flux after this combined injury. We tested four different amino acids, i.e. glutamate, glutamine, arginine, and methionine, and hypothesized that different amino acids would be affected differently. Our preliminary results showed that smoke inhalation injury alone did not cause a significant change in amino acid flux during the first five days after injury, but only some increase in arginine three days after injury. In the cutaneous burn and smoke inhalation injury group, all four amino acids decreased immediately and significantly. This result suggests that these four essential amino acids are all conditionally essential amino acids in this combined injury. However, in the simple smoke inhalation injury group, the amino acid supply was not as important, at least in the first five days after injury.

Introduction

The mortality of burn patients remains high in spite of the advances made in treating initial burn shock, controlling burn wound infection, and providing nutritional support. Smoke inhalation is recognized as a strong determinant of morbidity and mortality in burn patients, and most burn patients die because of associated inhalation injury. There are many reports on this combined injury (cutaneous burn and smoke inhalation injury = B & II) demonstrating that nutritional therapy plays the most important role in burn therapy. We have previously reported on serum amino acid flux after cutaneous major thermal injury. The present study, designed to test amino acid flux in cases of cutaneous burn injury and associated smoke inhalation, had two purposes. One was to establish an animal model in cutaneous burn and inhalation injury, and the other was to examine serum amino acid flux after B & II. Our hypothesis was that the plasma levels of amino acids would change after cutaneous burn injury and associated smoke inhalation, and that different amino acids would be affected differently. To the best of our knowledge, to date there have been no reports about amino acid flux after cutaneous thermal injury with smoke inhalation injury.

Materials and methods

Animals

Adult male Sprague-Dawley rats weighing 250-300 g each were obtained from the animal room of Taichung Veterans General Hospital (Taichung, Taiwan). The Committee for Laboratory Use and Care of Animals of Taichung Veterans General Hospital, Taichung, Taiwan, approved all studies. The animals were allowed at least three days to acclimatize to the animal room environment before use. The animal room was maintained at a constant temperature (25 °C) with a 12 h light/12 h dark cycle. The rats were fed a standard rat chow and given access to water ad libitum.

The animals were randomly divided into three groups: cutaneous thermal injury plus smoke inhalation injury (group 1), simple smoke inhalation injury (group 2), and non-burn (control, group 3). There were 20 rats in each group.

Smoke-generating instruments

Smoke was generated in a stainless steel combustion chamber, 12 x 12 cm in size and 16.7 cm in height, with a tight-fitting lid on the top, an air supply port near the bottom, and a smoke output port near the top. The lid was fitted with an adapter for a thermometer. The chamber was placed upon an electric hotplate for heating. Air was admitted via the air supply port at a rate of 12 ml per min (O2/CO2: 95:5). Smoke from the smoke exiting port was supplied to the intake port of the plastic animal chamber by means of a 25-cm steel tube and then 3 m of silicon tubing. The materials to be combusted to form smoke were 1 g cotton and 0.3 ml kerosene. These materials were
placed in a tinfoil dish 7 cm in diameter. To produce smoke, the chamber was heated to 200 °C, with air flowing as indicated previously, before the operative procedure for the inhalation was started.

The animal chamber was a plastic chamber, 330 x 250 x 154 mm (10 l), with a tight-fitting lid on the top and a smoke supply port on the lid. This port was connected to the smoke generator by way of the silicon tube. Smoke from the generator entered this animal chamber via the silicon tube. Smoke also leaked from the loosened lid of the animal chamber owing to over expansion of smoke.

Animal burn injury
The studies began between 8.30 and 9.30 each morning. The rats were anaesthetized intraperitoneally with 25 mg/kg phenobarbital sodium. They were shaved over the posterior back as well as caudally from the neck level. About 40% of the total body surface area (TBSA) was shaved.

Cutaneous burn injury was induced by immersing the rats’ backs in boiling water for 20 sec. A standard template was used to restrict the burned area to 40% TBSA. Full-thickness burn injury was verified by histological slides. Immediately after burn injury, lactated Ringer’s solution (10 ml/100 mg) was injected intraperitoneally for resuscitation. This burn model has been thoroughly evaluated in our laboratory. The burn wounds were immediately treated with silver sulphadiazine cream and dressed with heavy gauze. At this point, all the rats were unconscious owing to the effect of the anesthesia.

Induction of smoke inhalation injury
After smoke was produced from the generator and had filled the animal chamber, rats in group 1 were put into the animal chamber immediately and left there for 5 min. We then took them out and let them breathe room air for 5 min. The rats were put into the animal chamber again for another 5 min. This procedure was repeated three times. The rats were then put back into cages and allowed to recover with room air in individual cages. They were then given water and food *ad libitum*. This method of inhalation injury had been verified by histological studies.

Group 2, the smoke inhalation injury group, underwent the same treatment as group 1, but the water was cold and maintained at 25 °C during anaesthesia. The rats were then put into the plastic chamber for smoke inhalation injury.

The sham controls were treated in the same manner as the study groups but the water was cold (25 °C) and the animal chamber was filled only with room air.

Preparation of plasma from burned rats
After injury, blood (0.5 ml) was collected at different times from the tail blood vessel on pre- and post-injury days 1, 2, 3, and 4. The blood samples were collected in chilled heparinized tubes and immediately centrifuged. Plasma from the same group of animals was pooled and stored at -140 °C in a nitrogen tank until used for the HPLC.

Analysis of amino acids by HPLC
We tested serum amino acids by HPLC.

Statistical analysis
Data are presented as mean ± SD. Statistical analysis was performed by either Student’s *t*-test or ANOVA. We used SPAA software (SPSS 6.1.3, SPSS Taiwan) on an IBM-compatible personal computer with Windows ME software. One-way analysis of variance was applied for comparisons between the groups (differentiated by days post-burn). Significant results were followed up with a Duncan post hoc test for paired groups. The level of statistical significance was set at *p* < 0.05.

Results
Ninety-six rats were obtained for this study. Fourteen rats died and were excluded. There were 20 rats in each study group.

Pathological studies
The pathological reports after smoke inhalation injury proved that this method would cause a significant pathological change: “Segmental loss of tracheal mucosal epithelial lining cells with inflammatory cell infiltration in the submucosa of the trachea and inflammatory exudates admixed with exfoliated epithelial cells in the lumen of the trachea were noted.”

Amino acid analysis
Four amino acids (glutamate, glutamine, arginine, and methionine) were tested in this study. The overall results of the control group are shown in Fig. 1. In this sham study group, all four amino acids showed a tendency to
decrease immediately after injury. Only arginine showed a slight increase after day 3 post-injury.

**Glutamate**

Plasma glutamate levels (Fig. 2) showed a quick drop in either inhalation injury or burns associated with inhalation (B & II) injury. However, the only significant change was noted in the inhalation injury group (day 3 post-injury). Levels dropped by 25.21% ($p < 0.05$). In general, there was no significant decrease in the first four post-injury days, and levels remained in the normal range after day 4 post-injury in both groups.

![Fig. 2 - Plasma glutamate profile showing non-significant decrease in both groups. The only significant change was noted in inhalation injury. * = significant change ($p < 0.05$).](image)

**Glutamine**

Plasma glutamine levels (Fig. 3) varied. In the inhalation group, there was no significant rise immediately after injury, although there was a 16-23% change. In the B & II group, levels dropped immediately after injury, but the changes were not significant.

![Fig. 3 - Plasma glutamine profile showing non-significant increase (inhalation group) and decrease (B & II).](image)

**Arginine**

In the simple inhalation group, plasma arginine levels (Fig. 4) showed a significant rise on day 3 post-injury day ≥7-30%, $p > 0.05$) and then dropped to near normal. However, in the B & II group, levels showed a significant decrease after day 3 post-injury (Ø32-36%, $p < 0.05$).

![Fig. 4 - Plasma arginine profile showing significant decrease (B & II) and increase (inhalation group). * = significant change ($p < 0.05$).](image)

**Methionine**

Plasma methionine levels dropped immediately and significantly in the B & II group (Ø30-39%, $p < 0.05$) (Fig. 5). However, there were no significant changes in levels in the inhalation injury group.

![Fig. 5 - Plasma methionine profile showing immediate and significant decrease (burn & inhalation injury group). * significant change ($p < 0.05$).](image)

**Discussion and conclusions**

Inhalation injury from smoke and the noxious products of combustion in fires is associated with over half of the burn deaths in our burn unit. In a large-area burn with associated inhalation injury, the mortality rate is double that of a large-area cutaneous burn alone. The post-burn metabolism is considered to be the same as that of the simple cutaneous burn. The initial change in plasma amino acid profiles demonstrates muscle protein lysis and
hyperaminoacidemia, reflecting a change in the inter-organ and inter-tissue fate of amino acid metabolism. Amino acids play an important role in adaptation to burn trauma, as do neoglucogenesis, acute phase protein synthesis, and tissue repair. These metabolic changes are accompanied by profound alterations in plasma concentrations of hormones and various inflammatory mediators, which suggest that these may be important regulatory factors for post-burn amino acid metabolism. Metabolic effects in smoke inhalation injury show the same response as in major trauma when it is associated with airway inflammation.

The present study is a continuation of our investigations on the kinetic aspects of four different amino acid fluxes in rats with cutaneous burn and smoke inhalation injuries. It has been reported that, in either cutaneous burn or inhalation injury, glutamine levels either increased or decreased. Glutamate levels increased in the initial days post-burn. Arginine levels rose after either burn or acute lung injury. Methionine is known to be beneficial to endothelial cells after acute lung injury, and has been variously reported with no change, increase, and decrease in levels. However, in burn patients, the requirement of methionine was reported to have increased. Regardless of increases or decreases in plasma levels, it has been suggested that these four amino acids are conditionally essential amino acids in critically ill or burn patients.

The present results showed that in the sham control group, all tested amino acids (glutamate, glutamine, arginine, and methionine) showed a minor depression but no significant change. These results are probably due to the stress to animals. After injury, the levels of these four amino acids changed. In general, in simple inhalation injury, glutamate, glutamine, and methionine levels showed a non-significant decrease (Δ5-15%), and only arginine had a significant rise in the initial third day post-burn. However, when there was inhalation injury associated with cutaneous burn, all four amino acids showed an immediate and significant drop in levels of 15-25% compared with that of the group with inhalation injury alone.

Arginine disposal rate increased in severely burned patients compared with that of healthy adults, but the synthesis did not increase. It has been suggested that arginine is a conditionally indispensable amino acid in the support of parenterally fed, severely burned patients. The inhalation group in our study showed an increase in plasma arginine levels during the initial two days, and then a drop. Arginine levels also showed an immediate and marked decrease in the B & II group. This may have been due to arginine flux decrease during the early phase of inhalation injury and to endogenously synthesized nitric oxide (NO). NO, a product of certain cytokine-activated cells, affects rates of apoptosis, a mechanism of programmed cell death. Arginine plays a central role in the normal function of several organs, the immune system, and especially the T-cell function. Arginine is also important in the diet of burn patients because it promotes wound healing, reduces post-burn immune suppression, and enhances anabolism of protein.

Methionine is a sulphur-containing essential amino acid that participates in the metabolism of all macromolecules. Its many functions in the cell include biosynthesis of the membrane, control of gene expression, and cytoprotection. Metabolites of methionine are necessary for continual maintenance of membrane structure and integrity, a process that entails loss and regeneration of phosphatidylcholine at the outer layer of surface membranes. Methionine is also integral to metabolic pathways that regulate gene expression at various levels: chromatin structure, gene sequence, transcription, post-transcriptional processing of RNA, and protein synthesis. Methionine kinetics is profoundly altered by burn injury, and consequently the nutritional requirements for sulphur-containing amino acids are significantly increased. It has also been noted that methionine levels are decreased by smoke inhalation injury.

Many reports have shown that the metabolic rate increased with marked amino acid efflux after smoke inhalation injury, but only when this injury was associated with secondary bacterial infection - pneumonia, ARDS, etc. In early pulmonary injury after smoke inhalation injury, exposure did not accentuate pulmonary dysfunction during the subsequent 5-day study in a report by Traber. The results of Traber’s next study, which used a combination of a 40% cutaneous burn with smoke inhalation injury, showed significant changes in both haemodynamic and metabolic effects. This combined injury was associated with hypoproteinaemia, presumably a consequence of the great volume of resuscitation fluid and a greater exudative surface, i.e. burn wound plus airway. In this study, we gave all animals the same amount of resuscitation fluid, and the changes in B & II (marked decreases) were thus probably due to an increase of plasma amino acids influx to the liver or other visceral organs, to form acute phase protein or decrease the rate of muscle protein lysis.

We considered the question why pulmonary damage, induced by smoke inhalation injury and proved by histological study, did not induce significant changes in plasma amino acid levels. Traber reported that there was no change in protein leakage from the pulmonary microvasculature, either in burns associated with smoke inhalation injury or in inhalation injury alone. Another study by Traber showed that although pulmonary dysfunction was not accentuated in the first five days after inhalation injury, this may have been because this type of injury did not cause a critical condition that changed all the metabolic effects.

Another question was whether these metabolic effects would be manifested after five days in animals with smoke inhalation injury. There have been many other reports that
amino acid levels were markedly decreased when lung injury was associated with infection and its sequential complications, such as ARDS, etc. We believe the answer to the above question is negative in our study. It could be positive only if we had implanted some bacteria into the airway.

When both injuries are combined, victims suffer from a more severely stressful critical condition. The combined injuries cause more dramatic metabolic effects than those of one injury. In the present study, amino acid levels showed a sudden and dramatic 25-36% drop compared with those of inhalation injury alone.

To the best of our knowledge, to date, there have been no other studies on the effect of burn injury combined with smoke inhalation injury on indices of amino acids in the five-day post-burn period. The precise mechanisms responsible for the unchanged plasma amino acid levels in the early post-inhalation injury period remain undefined, but have been attributed to tissue toxicity of the chemicals in the inhaled smoke. Most reports have shown that systemic effects after thermal and inhalation injury are primarily caused by systemic or pulmonary sepsis and are observed only in severest cases of inhalation injury. Our results are consistent with these clinical studies.

In summary, we found that all four amino acids (glutamate, glutamine, arginine, and methionine) raised the rate of disposal in either inhalation injury alone or combined with cutaneous burn injury. Their consumption markedly increased when patients suffered from both inhalation injury and cutaneous burn injury. Therefore, with the limited endogenous production rate and the increased irreversible disposal of these four amino acids, it is further apparent that glutamine, glutamate, arginine, and methionine are conditionally indispensable amino acids in the support of parenterally fed patients with combined severe burn and inhalation injuries. When the feeding modality is parenteral nutrition, a performed regimen of these four amino acids appears to be obligatory. However, in the case of smoke inhalation injury alone, supplying these essential amino acids is probably not mandatory during the initial days after injury. Nevertheless, further study on nutritional supplementation after smoke inhalation injury is needed.

RÉSUMÉ. Les lésions causées par l’inhalation de fumée restent une importante complication co-morbide dans les patients brûlés. Selon les données de la littérature, le taux de mortalité des patients atteints de brûlures et de lésions dues à la fumée est beaucoup plus élevé par rapport à celui des patients atteints d’un seul des deux types de lésions. Pour survivre, les patients qui présentent cette lésion associée ont besoin d’un apport nutritionnel plus intensif et plus agressif. Avec l’emploi des rats, l’auteur de cette étude voulait tester le flux des aminoacides dans les cas de lésions associées (inhalation de fumée et brûlures cutanées) dans le but de comprendre le flux des aminoacides à la suite de la lésion associée. Il a testé quatre aminoacides différents, c’est-à-dire glutamate, glutamine, arginine et méthionine, dans l’hypothèse que les divers aminoacides réagiraient en manière différente. Les résultats préliminaires démontrent que les lésions dues seulement à l’inhalation de fumée n’ont causé aucune modification significative dans le flux des aminoacides pendant les cinq premiers jours après la lésion, mais seulement un certain incrément dans l’arginine trois jours après la lésion. Dans le groupe de brûlures cutanées et inhalation de fumée, tous les quatre aminoacides diminuaient immédiatement et en manière significative. Ce résultat suggère que ces quatre aminoacides essentiels soient tous des aminoacides conditionnellement essentiels dans cette lésion associée. Cependant, dans le groupe de seule inhalation de fumée, l’apport des aminoacides n’était pas si important, au moins pendant les premiers cinq jours après la lésion.

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Address correspondence to: Dr Yu-Wen Tang, Plastic and Reconstructive Surgery, Taichung Veterans General Hospital, Taichung, Taiwan. Home address: 38, Lane 19, Fu-Kong Road, Shi-Ton Chi, Taichung 40764, Taiwan. Tel.: office: 886 4 2374 1214; home: 886 935 745 120; e-mail: a001@vghtc.gov.tw or newvin@hotmail.com

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DEPARTMENT OF EMERGENCY & CRITICAL MEDICINE NIPPON MEDICAL SCHOOL
1-15 Sendagi, Bunkyo-ku, Tokyo 113-8603 Japan
Tel.: +81-3-5814-6199 / Fax +81-3-3821-5102 / E-mail: kaido@nms.ac.jp

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