COMPARATIVE PLASMA PHENYLALANINE AND GLUTAMATE PROFILES IN SCALDED RATS TREATED WITH MEBO OR COPPER (I) NICOTINATE COMPLEX

COMPARAISON DES TAUX PLASMATIQUES DE PHÉNYLALANINE ET DE GLUTAMATE CHEZ DES RATS ÉBOUILLANTÉS TRAITÉS LOCALEMENT PAR MEBO OU COMPLEXE CUIVRE (I)- ACIDE NICOTINIQUE

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SUMMARY. The time profile of total proteins and albumin in line with two amino acids, Phe and Glu, are of great importance due to their indicative value of the healing and/or deterioration of burn wounds. In this experiment, rats exposed to scalding that produced a second-degree burn were divided into four groups: control, placebo, MEBO ointment, and copper (I) nicotinate complex ointment (an antioxidant anti-inflammatory) for four weeks. The three treatments showed noticeable similarities in the Phe profiles, with a slight drop 24 hours post burn, a peak elevation by the second week, and a slight, insignificant decline by the third week, with the placebo group showing highest values over normal (P< 0.05). In the Glu profile, large differences were observed: two set profiles, one representing MEBO and copper (I) nicotinate showed one peak in the second week, and the other representing placebo showed two peaks in the first and third weeks. Albumin and total protein time profiles showed the same trend throughout the whole experiment, and are in line with a reformed metabolic state parallel to wound closure. These results reveal that MEBO and copper nicotinate have comparable effects on wound healing in scald burns, and that plasma Glu profile is a better systemic indicator reflecting the regularity and steadiness of the healing process in MEBO and copper complex treatment.

Keywords: MEBO, Cu (I) nicotinate complex, scald burn, phenylalanine, glutamate, plasma protein

RÉSUMÉ. Les évolutions temporelles de la protidémie, de l’albuminémie ainsi que de Phe et Glu sont de bons prédicteurs de l’évolution vers la cicatrisation ou non des brûlures. Dans cette étude expérimentale, des rats ayant subi une brûlure du 2ème degré par ébouillantement ont été divisés en 4 groupes : contrôle, placebo (P), pansements au MEBO ou au complexe cuivre (I) – acide nicotinique (CuNic) pendant 4 semaines. Les trois groupes traités avaient des profils de Phe superposables, avec une légère diminution initiale, un pic dans la 2ème semaine et une augmentation non significative durant la 3ème quand le groupe P avait des valeurs élevées (p<0,05). Les profils de Glu étaient plus disparates : un (MEBO et CuNic) avec un pic en 2ème semaine, les autres avec 2 pics (1ère et 3ème semaines). Ces profils étaient aussi observés avec les protides et l’albumine et correspondaient à des évolutions cicatricielles et métaboliques. MEBO et CuNic ont des effets comparables sur la cicatrisation après ébouillantement, au mieux explorés par l’évolution de Glu.

Mots-clés : MEBO, complexe cuivre- acide nicotinique, ébouillantement, phénylalanine, acide glutamique, protéines plasmatiques

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Introduction

Following severe burns, the body responds on two levels: a local level where thermal exposure causes tissue damage and release of a vast amount of reactive oxygen species which cause lipid peroxidation, hence the broadening and deepening of the affected area, and a level that is mediated by the remote organs, especially the liver, which produces acute phase proteins and modulating inflammatory response. The most prominent protein of plasma, albumin, decreases drastically after severe burn injury. Hypoalbuminaemia persists, attributed to a change in rate of metabolism and degradation or to hemodynamic disturbances, as these changes could adopt indicative profiles that reflect the physiologic response to thermal injury and phases of healing. Burns, especially severe II degree and higher, were found to cause increased resting energy expenditure, negative nitrogen balance and increased plasma free amino acid concentration. These effects were documented long ago, and found to be ascribed mainly to a hypermetabolic state that leads to increased proteolysis in peripheral tissue as well as increased hepatic uptake, the process that provides free amino acids for the liver gluconeogenesis machinery which is activated by several hormonal changes such as catecholamines and glucocorticoids. Most plasma free amino acid profiles have been shown to be insignificant in relation to survival in burn and trauma patients, except for phenylalanine. The effects of burn injury may extend to converting some nonessential amino acids into essential ones. That is why glutamic acid was found to become essential amino acid in cases of skin burns where the burns are severe or they affect the majority of body surface area, generally because it is consumed at high rates for gluconeogenesis.

Materials and methods

Ninety-two female albino rats weighing about 120 grams were used. The animals were segregated into five groups as follows: a positive control group made up of 10 animals, which were sacrificed 24 hours after induced burn to represent the acute phase of injury; three groups of 24 animals each which received placebo, copper (I) nicotinate complex and MEBO ointment, respectively; ten negative control animals representing the starting point of the profile. Standardized dorsal skin burn was induced in the interscapular area by a modified scald model by applying hot water at 70°C for 12 seconds on the shaved area, using laboratory test tubes of fixed diameter. The second-degree burn covered 10% total body surface area. Copper (I) nicotinate complex was obtained as a gift from FARCO pharmaceutical company, Alexandria Egypt. Copper complex was mixed with medical vaseline, bought from the Al Gomhuryia Company, Sawah Al Amiriya Cairo, Egypt. MEBO ointment was purchased from local markets.

Dosage and regimen were as follows: copper nicotinate complex powder was estimated to equal 3mg cu / kg body weight / day. The three treatments consisted of applying 1 gm of ointment to cover the wound once daily. Blood sampling was done by sacrificing 4 animals from each group once a week for 4 weeks. Blood was heparinized and centrifuged at 3000 rpm for 15 minutes to separate plasma. Total protein was measured by the biuret test, according to STANBIO kit cat No. 0250, and albumin was measured by the bromocresol green method according to Diamond Diagnostics, Cairo, Egypt. Glutamic acid and phenylalanine analysis was performed by paper chromatography adopted from TLC separation procedure. The chromatograph was made visible by immersing the paper in ninhydrine solution, then drying it in an oven and picturing it by UV light at λ= 302nm in dark room UVP. The chromatograph was later analyzed using software “doc-it”. Statistical analysis was performed by ANOVA, then for specific results, by post-hoc Tukey’s HSD test for multiple group pairwise comparisons. The data obtained were analysed, and graphs were created using “Origin pro 8”.

Results

Groups have been abbreviated as follows: P denotes the Vaseline (placebo) group, C denotes the copper (I) nicotinate complex group and M denotes the MEBO treatment group. Table I and Fig. 1 show that plasma total protein concentration de-
increased significantly 24 hrs post burn, from normal 8.04±1.09 to 5.07±0.18 g/dl (P<0.01). In the P group this decrease continued through the first week (4.87±0.24), then increased significantly from the second week until the fourth week with concomitant significantly lower values than the M or C groups (P<0.01). The M and C groups showed a significant increase over the acute value from the first week of treatment (5.22±0.29, 5.58±0.27 g/dl, P<0.05). Both treatment groups reached close-to-normal values by the fourth week (7.26±0.36, 7.76±0.38 g/dl), albeit they were still significantly higher than normal (P<0.05). Albumin plasma concentration (Table I and Fig. 1) decreased dramati-
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cally from 3.54±0.17 to 1.82±0.09 g/dl at 24 hrs post burn: about one third of the plasma albumin had vanished (P<0.01). This decrease continued in the first week (1.66±0.08) then started to increase insignificantly in the second week (1.87±0.09) in the P group. There was then a significant increase compared to the 24 hrs point in the 3rd and 4th weeks (P<0.01). The difference between total protein and albumin collective profiles is that, except for the P group, plasma albumin was close to normal, while the total protein collective profile for the three treatments showed nearly normal values by the fourth week. Table II and Fig. 2 show a distinctive profile for Glu acid in plasma after burn as follows: in the first week of treatment there was a small, insignificant difference between the M and C group, while the P group have almost the same value (P<0.05). In the third week, the MEBO group exhibited significantly lower values than the placebo and Copper complex groups. All groups showed close to normal values in the fourth week (P<0.05).

Table III and Fig. 3 show that the Phe profile is similar in the three groups, with significantly

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<th>Table III - Plasma free Glu</th>
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Results are recorded as mean ±SD

Letters indicate significant difference between treatment groups and 24 hours post burn

* Significant difference between treatment group and zero time/control group

![Fig. 1 - Plasma protein](image1)

![Fig. 2 - Time profile of Plasma Phe](image2)
higher values for the P group (P<0.05). The M and C group profiles show one peak by the second week (62.94±2.83, 93.6±1.12 nmol/ml respectively), then a decline by the third week towards normal (62.9±1.83, 63±1.85nmol/ml) with insignificant differences between them (P<0.05), while the P group profile maintains higher significant values with a peak in the third week (67.3±2.32 nmol/ml, P<0.05). By the fourth week, the P group still shows far higher values than both the M and the C group (62.97±2.85, 60.66±1.24, 61.56±1 nmol/ml respectively, P<0.05).

Discussion

Results of the experiment at 24 hrs and through the first week post burn correspond with high rates of hypoproteinemia and hypoalbuminemia, where albumin deficiency clearly affects total protein level as it is the main constituent of blood protein. As has been documented during the acute phase of burn wound, a state of increased vascular permeability and leakage persists, proportional to the severity of the wound.16 This could be associated with decreased liver synthesis of plasma protein even in minor burns, or due to increased extravasation of albumin to the wounded areas and hence decreased compensation in humans and rats.17,18,19 Our results showed great agreement with those documented results. Moreover, the profiles of the three groups showed a difference in rate of return back towards normal in favour of the MEBO and Copper (I) Nicotinate treatments over the placebo treatment, in accordance with a gradual rehabilitating liver synthesis rate of protein and decreased extravasation as the wound closes. This logical profile of total protein and albumin was not reflected in the Phenylalanin or glutamate profile, which not only showed faster rates of return back to normal in the C and M groups, but also revealed delayed healing or sustained proteolytic state in the three groups, albeit the MEBO and Copper Complex treated groups showed great improvements around the fourth week, surpassing that of the placebo. This is in accordance with the documented facts that blood free amino acids reflect the balance between protein synthesis and proteolysis, in light of the fact that muscles persist in a protein catabolic state for almost 9 months after burn in humans.20,21 In this context Glu showed a unique profile in the placebo group, where a setback was observed in the middle of the treatment course which might reflect an unsteady healing process. Besides, glutamate is not as involved in muscle protein turnover as phenylalanine, which may explain why the end point of glutamate is closer to normal than that of phenylalanine. Meanwhile, phenylalanine is not produced by the mammalian body22,23 while glutamate is. This may account for the semi-hyperbolic profile of Phe and the rapidly reached steady state of Glu, especially in MEBO and Copper complex treated animals.

Conclusion

Free plasma Phe is a good indicator of the healing process and the rehabilitation of synthesis/hydrolysis balance of body protein, while the Glu profile might be more a flashing indicator of the steadiness of the healing process of the wound and whole body in terms of normal metabolism rather than synthesis/hydrolysis balance. MEBO or Copper (I) nicotinate treatments cause the Phe and Glu profile to curve more towards normal and decrease the slope of the curve line, and both affect the time of healing with differences that reflect the more powerful effect of MEBO.
BIBLIOGRAPHY