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Effects of taurine on polymorphonuclear phagocytosis activity in burned patients

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Summary. This study determines the effects of taurine (Tau) on phagocytosis of polymorphonuclear neutrophils (PMN) isolated from normal subjects (n = 41) and severely burned patients (n = 20). Phagocytosis was measured by nitroblue of tetrazolium (NBT) reduction in samples with and without latex bead stimulation. Taurine was added at doses of 0.2, 0.4, 0.8 and 1.6 mM to stimulated samples. In control cells there were statistically significant increases in phagocytosis after addition of Tau 0.8 mM and 1.6 mM to as compared to samples without Tau addition (295 ± 23% and 330 ± 35% vs. 248 ± 18%; mean ± S.E.; $p < 0.05$). A statistically significant increase in phagocytosis was observed in cells from the burned population after addition of Tau 1.6 mM (288 ± 38% vs. 198 ± 13%; mean ± S.E.; $p < 0.05$). No changes in phagocytosis were found in cells from a subgroup of burn patients (n = 13) followed over 7, 15 and 21 days. These results indicate that taurine supplementation in vitro at doses of 0.8 to 1.6 mM improves the phagocytic capacity of neutrophils in healthy subjects and in patients with severe burn injury, mainly when neutrophil function is unaltered.

Keywords: Phagocytosis – Taurine – Neutrophils – NBT

Introduction

Taurine (Tau), a sulfur amino acid with a molecular mass of 125 daltons, is the major end product of methionine-cysteine metabolism. Taurine is present in several food sources (Laidlaw et al., 1990), is a normal constituent of animal (O'Donnell et al., 1981) and human diets (Erbersdobler et al., 1984), and is found in almost all mammalian tissues. Taurine levels in plasma, subject to high variation, are not a good indication of the status of this amino acid in the body. Deposits found in the leukocytes or platelets are more stable and are superior for this purpose (Vinton et al., 1986).

Among several actions (Gaull et al., 1979), taurine has protective effects on cell membrane stabilization and it has been implicated in the immune response because of its ability to modulate antimicrobial function (McLoughlin et al., 1982). The polymorphonuclear neutrophils (PMNs) are the main proinflammatory cells activated in the initial host response to sepsis or surgical trauma. Taurine constitutes 76% of the free amino acid pool (Fukuda et al., 1982); human leukocytes contain 26 mM of taurine and human neutrophils 22 mM, while the average extracellular concentration in human serum is 50 μM (Anderson et al., 1988). One reason for its abundance in the neutrophil cytosol is to trap chlorinated oxidants (Marcinkiewicz et al., 1998) or free radicals during the oxidative burst induced by phagocytosis. The majority of studies on the interrelationship between taurine and neutrophil function have centered upon the modulation of this oxidative pathway.

The ability of phagocytes to reduce the almost colorless compound nitroblue tetrazolium (NBT) to dark blue formazan reflects the increased oxidative metabolism of these cells during phagocytosis. By means of this property, the quantitative NBT reduction assay provides rapid information on the intracellular superoxide anion produced during the oxidative burst and is the basis for measuring phagocytosis.

Neutrophil function is impaired after thermal injury and this could increase the risk of infection (Bjornson et al., 1981). The reason for this impairment has not been elucidated and the exact mechanisms involved in the process are not known.

The aim of the present study was to determine the effects produced by taurine on the phagocytic function of polymorphonuclear neutrophils in vitro, as measured by the NBT reduction test. The work was carried out in samples from healthy subjects and severely burned patients up to 21 days after injury.

Materials and methods

NBT assay

Polymorphonuclear cells were isolated in all samples by Polymorphrep™ (d = 1.113), a sodium metrizoate/dextran solution. The quantitative NBT test was carried out on aliquots of 250 μL (2.5 $\times$ 105 cells) mixed with 20 μL phosphate buffer (PBS; pH 7.4) in non-stimulated samples or with 20 μL of latex bead suspension (particle diameter 1.094 μ, Sigma: LB-11) in stimulated samples. A volume of 250 μL of 0.1% nitroblue of tetrazolium (Sigma: N-6876) was added to all samples. After 30 min of incubation at 37°C the reaction was stopped, tubes were centrifuged at 3000 $\times$ g during 30 min, supernatants were discarded and the reduced NBT was extracted with dioxan (Sigma). Supernatant optical density (O.D.) was measured at 525 nm using dioxan as a blank control, as described (De la Fuente et al., 1991). The results, expressed in percentage, were calculated considering the OD of the non-stimulated sample to be 100% and dividing this value by the OD of the latex-stimulated sample.

Phagocyte function study

The study was approved by our hospital Ethical Committee. Two groups were included. The control group consisted of 41 healthy volunteers from the hospital staff, 15 men and 26 women, 37.4 ± 13.2 years old, who had a complete hemogram as part of the hospital's standard health control program. The burn group contained 20 patients with severe thermal injury [more than 12% body surface area; (BSA)], 18 men and 2 woman, 41.7 ± 18.8 years old. In 11 patients, 12–30% of the BSA was affected, in 6 others, 30–50% and in 3, more than 50%. Samples were obtained within the first 24 hours in all patients and at 7, 15 and 21 days after hospital admission in 13 of them. All patients survived the burn and were ultimately discharged.

In both cases and controls, 6 aliquots of isolated cells were prepared for each individual studied: one non-stimulated with 20 μL phosphate buffer (pH 7.4); one stimulated with 20 μL of latex bead suspension and 4 latex-stimulated with the addition of Tau (0.2, 0.4, 0.8 and 1.6 mM) at levels between 2 and 20 times over our established physiological range (31–82 μmol/L).

Statistical analysis

For statistical purposes we used the Wilk's normality test, analysis of variance for repeated measurements (ANOVA), the Wilcoxon test for paired samples and the Spearman correlation analysis (SPSS 8.0 software). P-values of less than 0.05 were considered significant.

Results

Burn patients presented normal initial mean values for hemoglobin (13.54 ± 3.14 g/dL), hematocrit (39.53 ± 9.68%) and RBC count (4.50 ± 1.13 $\times$ 10^{12}/L), and a high leukocyte count (12.24 ± 6.27 $\times$ 10^{9}/L). Eight patients presented neutrophilia (83.2 ± 3.8 %) and one neutropenia. Plasma levels of cholesterol (120 ± 39 mg/dL) and triglycerides (110 ± 51 mg/dL) were within the normal range (<200 and <150 mg/dL respectively).

Calculation of the reproducibility of the NBT assay gave a CV of 8.2% for a pool of stimulated samples. The purity of the PMN isolates was between 95 and 100%. In the normal subjects, O.D. results were 0.057 ± 0.024 for unstimulated and 0.134 ± 0.06 for stimulated cells (248 ± 15%; mean ± S.E.), values that are within our normal range (140–360%; 25th to 75th percentiles).

Results of Tau addition to stimulated cells in normal patients are shown in Table 1. Statistically significant differences were observed in the stimulated samples vs. stimulated plus Tau 0.8 mM and 1.6 mM ($p < 0.05$). Results expressed in percentage term (mean ± S.E.)

Table 1. Phagocytosis results (NBT reduction test)

Tau addition (mM)	Control (O.D.)	Control (%)	Burn (O.D.)	Burn (%)
Without	0.134 ± 0.06	248 ± 18	0.129 ± 0.05	198 ± 13
0.2	0.157 ± 0.09	297 ± 28	0.153 ± 0.11	238 ± 37
0.4	0.146 ± 0.07	283 ± 23	0.152 ± 0.10	234 ± 37
0.8	0.158 ± 0.08*	295 ± 23*	0.159 ± 0.07*	241 ± 18
1.6	0.188 ± 0.11*	330 ± 35*	0.193 ± 0.09*	288 ± 38*

* Statistically significant differences vs. samples without added taurine ($P < 0.05$)
O.D values: mean ± S.D. and % values: mean ± S.E.

Table 2. Results of phagocytosis after taurine addition. Burn patients after 21 days of injury

Taurine addition (mM)	Day 0	Day 7	Day 15	Day 21
Without	198 ± 13	246 ± 108	179 ± 18	270 ± 47
0.2	238 ± 37	253 ± 139	160 ± 25	221 ± 19
0.4	234 ± 37	261 ± 129	182 ± 28	259 ± 22
0.8	241 ± 18	280 ± 169	187 ± 28	241 ± 45
1.6	288 ± 38*	322 ± 184	214 ± 30	308 ± 78

Calculation of results: OD increase vs. OD in the unstimulated samples (without latex); O.D values: mean ± S.D. and % values: mean ± S.E. (n = 20); * Statistically significant differences vs. samples without added taurine ($p < 0.05$)

were: without Tau, 248 ± 18%; Tau 0.2 mM, 297 ± 28%; Tau 0.4 mM, 283 ± 23%; Tau 0.8 mM, 295 ± 23% and Tau 1.6 mM, 330 ± 35%.

At day 0 (first 24 hours) O.D. results in the burn group were 0.057 ± 0.024 for unstimulated and 0.129 ± 0.05 for stimulated cells. Results of Tau addition (O.D.) showed statistically significant differences in stimulated samples vs. stimulated plus Tau 0.8 mM and 1.6 mM ($p < 0.05$) (Table 1). When results were expressed in percentage term, statistically significant increases in phagocytosis were observed in samples with Tau 1.6 mM ($p < 0.05$). Subsequently, we created a subgroup of patients with phagocytosis levels in the normal range and studied the effects of taurine addition on day 0. At a dose of 1.6 mM, statistically significant increases were observed with respect to samples without taurine (300 ± 31% vs. 220 ± 15%; $p < 0.05$). The addition of taurine to samples collected at 7, 15 and 21 days showed no significant differences vs. samples without Tau (Table 2; n = 13).

The Spearman analysis showed a no correlations for phagocytosis versus triglycerides ($r = -0.071$), cholesterol ($r = -0.395$) or burned area ($r = 0.407$).

Discussion

The causes of the changes in neutrophil function after injury are not completely understood but appear to be multifactorial. Two main lines of thought have attempted to explain these changes; one is focussed on the presence of serum inhibitors (microenvironment) that directly influence neutrophil function (Bjornson et al., 1978), and the other on intrinsic cellular defects (Dahlgren et al., 1999; Holmes et al., 1967). With regard to the first, it has been described that hyperlipidemia can affect phagocytosis by modifying membrane lipid composition (Masuda et al., 1986). We explored this hypothesis in our samples from burn patients with normolipidemia, but found no statistical association between phagoytosis and cholesterol or triglyceride serum concentrations. Hence we believe that neutrophil function was not affected by normolipidemia plasma status.

Focussing more specifically on burn injury, it is paradoxical that both depressed neutrophil activity (mainly chemotaxis) and increased neutrophil function have been reported as secondary phenomena in non-infectious processes involving active inflammation (Bogomolski-Yahalom and Matzner, 1995). It is accepted that there is a general activation of circulating neutrophils after thermal injury, followed by impairment of neutrophil microbicidal mechanisms. The contributions of numerous authors over the years illustrate this duality (Bjerknes et al., 1990; Zapata-Sirvent and Hansbrough, 1993). Also, data obtained from various sources clearly point to a multifactorial cause for the change in phagocytic response. Our results, evidencing a highly individualized phagocytic response in burn patients, are in keeping with this concept.

Another related fact is that burn patients show an increased demand for sulphurated amino acids (Martenson et al., 1987). This increased demand coincides with the time of greatest resynthesis of scar proteins. In parallel, during the first hours there is an increase (of unknown origin) in total antioxidant capacity, which does not correlate with the severity or extension of the burn (Farriol et al., 2001). All this evidence suggests that the neutrophil response in this phase is dictated by multiple factors (Rosenthal et al., 1996; Rodeberg et al., 1997).

In practice, all the components of phagocytosis (chemotaxis, adhesion, aggregation, granular content, degranulation processes, respiratory burst and bacterial killing) are not completely evaluated due to the complexity of this task. Assessment of neutrophil functional capacity with the NBT test is simple and perfectly valid (Niculescu et al., 1995). We chose this method because of its ease of execution and excellent cost-effectiveness (i.e. regarding the information provided), and our results in burn patients coincide with those reported (Zapata-Sirvent et al., 1993) using flow cytometry.

With regard to taurine, it is known that because of its antioxidant capacity it protects cells from self-destruction during processes that generate oxidants. Its effects on phagocytosis may be secondary to its membrane-stabilizing effects or to inhibition of apoptosis (Wang et al., 1996), but the major function of taurine in leukocytes is to trap chlorinated oxidants to produce the long-lived taurine cloramine (Odajima and Yamazaki, 1998). An extensive review explaining the role of taurine in host defense has been published (Stapleton et al., 1998). Supplementation with taurine appears to result in a rise of bacterial phagocytosis with impairment of oxidative pathways. However, the optimum levels of intra and extracellular oxidants and antioxidants are unknown.

The results obtained with the simple, fast ex vivo method used in the present study demonstrate that taurine doses of around 1 mM produced a positive effect on the activation of phagocytosis as a general anti-inflammatory mechanism. Hence, we believe that dietary supplementation with taurine in the post aggression phase of severe burn injury could improve immunological defense.

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