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Short communication

Effects of L-carnitine on morphology and cellular parameters of hen erythrocytes

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Abstract

The aim of our study was to determine the influence of L-carnitine (L-CAR) on the cellular parameters of hen erythrocytes during a 48 hour exposure to L-CAR at concentrations of 25, 50 and 100 µg/mL in nutrient-deficient medium. Cell morphology, haemolysis, caspase 3/7 activity and glucose uptake (GU) were determined. The results showed a lower percentage of apoptotic cells and decreased haemolysis of erythrocytes treated for 48 hours at all the concentrations of L-CAR. The amino acid at 50 µg/mL inhibited the activity of proapoptotic caspase 3/7; however, it increased GU. In contrast, caspase 3/7 level was increased but GU was decreased in erythrocytes treated with 100 µg/mL of L-CAR when compared to the control. It may be hypothesized that reduction of apoptotic changes in hen erythrocytes may result from increased GU.

Key words: L-carnitine, erythrocyte morphology, caspase 3/7, hemolysis, apoptosis

Introduction

L-carnitine (L-CAR) (β -hydroxy- γ -trimethylammonium butyrate) is an amino acid synthesized by microorganisms, plants and animals (Bremer 1983). Little is known about the protective effects of L-CAR on the morphology and cytophysiological parameters of avian erythrocytes in nutritional deficiencies. The purpose of our study was to determine the influence of L-CAR on the morphology, hemolysis, caspase 3/7 activity and glucose uptake in hen erythrocytes incubated in nutrient-deprived culture medium.

Materials and Methods

L-carnitine hydrochloride (L-CAR; $\geq 98\%$ purity; Sigma-Aldrich, Germany), was diluted in RPMI 1640 to make three concentrations: 25, 50 and 100 µg/mL. Blood was taken from 10 green-legged partridge hens (*Gallus gallus domesticus*) from the wing vein. Erythrocyte suspension (10 replicates) in RPMI 1640 with 120 U penicillin (Polfa Tarchomin, Poland) without L-glutamine or HEPES and without FBS-Foetal Bovine Serum) was exposed to the appropriate concentrations of L-CAR for 48 hours at 41.2°C. After incubation erythrocyte hemolysis was determined using the spectrophotometric method and cell morphology was

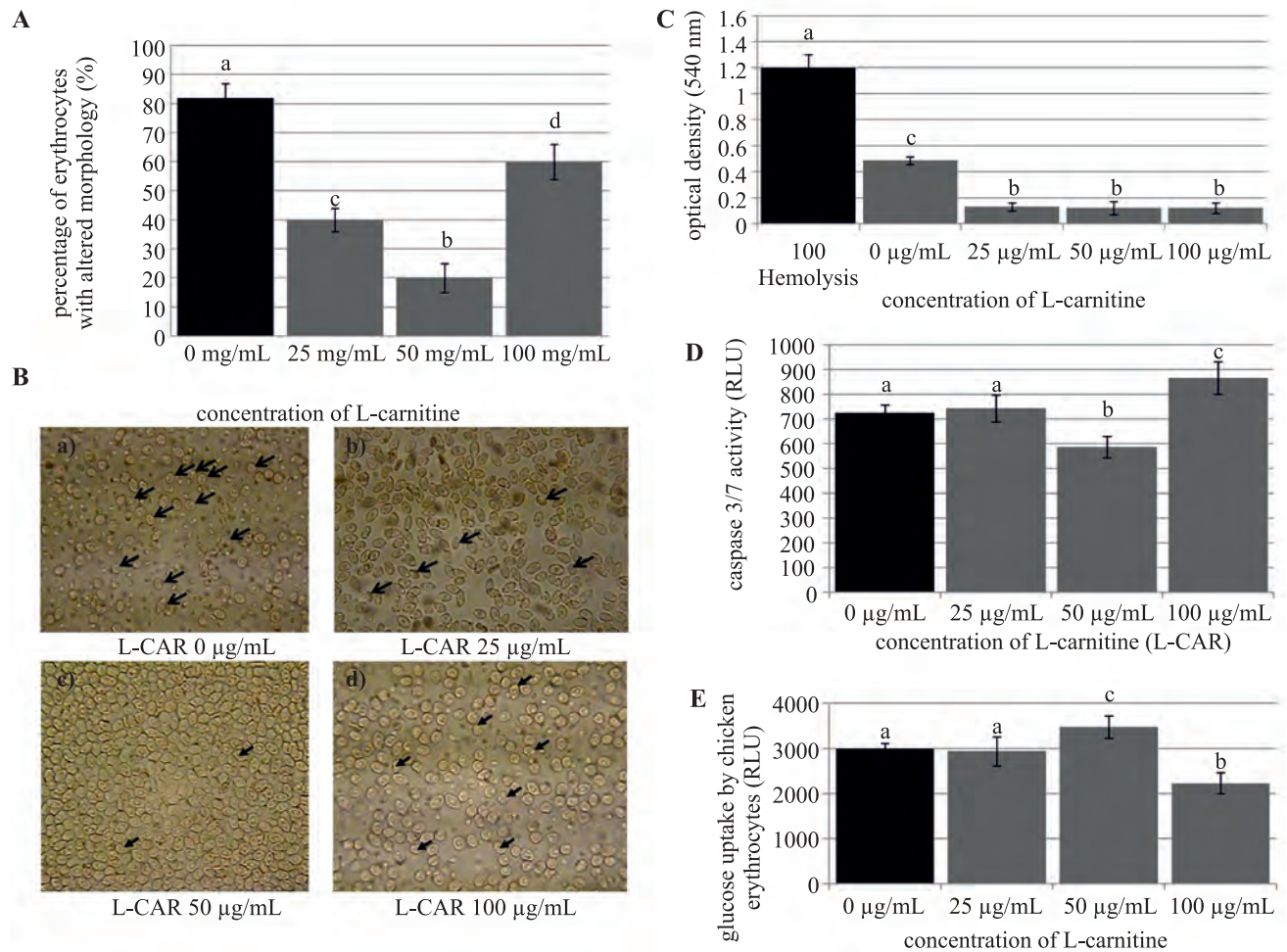


Fig. 1. Effects of L-carnitine on hen erythrocytes: **A**) percentage of cells with affected morphology; **B**) microscopic view of non-stained hen erythrocytes treated with different concentrations of L-CAR (400x magnification). Arrows indicate erythrocytes with abnormal morphology (swelling and irregular shape); **C**) percentage of haemolytic cells; **D**) activity of caspase 3/7; **E**) glucose uptake. The results are presented as means \pm SD, $n=10$, values not sharing a common superscript letter (a-d) differ significantly.

examined by light microscopy (magnification 400x). Caspase 3/7 activity and GU were determined using the Caspase-Glo® 3/7 and Glucose Uptake-Glo® (Promega Corp.) commercial kits respectively, according to the manufacturer's protocols with a Synergy HTX (Bio-tech, USA) reader. The results are presented as means \pm standard deviation (SD). Data were assessed for homogeneity of variance for ANOVA assumptions using Levene's test. Experimental data were analyzed using ANOVA followed by Tukey's test to detect differences among means. All analyses were performed using Statistica 13.1® software. Values were statistically significant at $p \leq 0.05$.

Results and Discussion

Most erythrocytes ($81 \pm 5\%$) incubated for 48 h in nutrient-deficient medium, showed abnormalities such

as loss of oval shape and swelling (Fig. 1a,b). In contrast, L-CAR treated erythrocytes showed fewer abnormalities. The lowest number of deformed cells ($20 \pm 5\%$) was found at 50 mg/mL of L-CAR. Partial haemolysis (about 42%) was noted in the non-treated cells (0 mg/mL) (OD [Optical Density]= 0.486 ± 0.03) after 48 h incubation, when compared to the positive control (100% haemolysis), (OD = 1.2 ± 0.03) (Fig. 1c). In contrast, haemoglobin release was reduced in cells exposed to all concentrations of L-CAR with OD values of 0.13 ± 0.03 , 0.12 ± 0.05 and 0.12 ± 0.04 at 25, 50 and 100 mg/mL, respectively. Caspase 3/7 activity was decreased in erythrocytes exposed to L-CAR at a concentration of 50 mg/mL ($RLU=587 \pm 44$) in comparison to the control ($RLU=725 \pm 32$) (Fig. 1d). In contrast, its activity was augmented in cells treated with 100 mg/mL of L-CAR ($RLU=867 \pm 65$). The amino acid, at a concentration of 50 mg/mL, induced a significant increase in GU ($RLU=3476 \pm 243$) when com-

pared to the non-treated cells (RLU=2994±120) (Fig. 1e). However, no significant alteration of this parameter was found at 25 mg/mL (RLU=2939±321) and 100 mg/mL (2227±234) of L-CAR, suggesting no significant differences in GU. Increase in GU was associated with decreased activity of apoptotic caspase 3/7, suggesting that a higher glucose intracellular level may reduce apoptosis of hen erythrocytes. Consequently, higher activity of caspase 3/7 contributed to the decreased GU. Our results are consistent with those obtained by other authors (Swenney et al. 2004, Caro-Maldonado et al. 2010). Taken together, L-CAR seems to improve the parameters of hen erythrocytes and reduce the activity of proapoptotic caspase and therefore it may be considered as a cellular protective agent, particularly during nutritional deficiencies.

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