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CARNITINE CONTENT OF RED BLOOD CELLS OF HUMAN SUBJECTS TREATED WITH PIVAMPICILLIN AND CARNITINE

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Total- and free carnitine content of washed pooled red blood cells collected from five children prior to and on the last day of combined pivampicillin and equal molar carnitine treatment were measured. On the last day of treatment (day 7) the level of total carnitine decreased from 47.5 ± 3.39 to 37.5 ± 2.48 nmol/ml, mean ± SEM ($p < 0.05$) with a concomitant decrease of free carnitine (from 19.2 ± 0.97 to 15.5 ± 0.99 nmol/ml, $p < 0.05$) as compared with the pretreatment control day (day 0). The calculated amount of acid soluble carnitine esters also fell (from 28.2 ± 3.38 to 21.9 ± 1.78 nmol/ml). The same effects were found when the carnitine levels were referred to haemoglobin or water content of samples.
These results demonstrate that in pivampicillin treatment the carnitine pool of erythrocytes also alters. In agreement with previous findings the data presented here suggest, that the administered carnitine was not sufficient to meet the enhanced needs of the organism caused by the pivalate load and that the organism utilized some of its stores for pivaloylcarnitine production. The decreased carnitine ester level of erythrocytes suggest, that the red blood cells do not participate in significant extent in pivaloylcarnitine transport or production.

INTRODUCTION

Pivampicillin (pivaloxymethyl ester of ampicillin) is widely used antibiotics in some countries. It was developed to reach better intestinal absorption of the drug /10/. The absorbed pivampicillin is hydrolysed to the active substrate (free ampicillin) and a part of the liberated pivalic acid conjugates

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with carnitine. The pivaloylcarnitine is eliminated probably predominantly via kidneys /5,11/. The urinary loss of carnitine as pivaloylcarnitine may ultimately produce carnitine deficiency/5/.

Although carnitine deficiency can be well tolerated, it is considered a potentially dangerous state because an additional metabolic stress like fasting, fever or acute illness may lead to metabolical changes with clinical symptoms, even life-threatening crises have been reported /3,9/.

In several types of carnitine deficiency administration of exogenous carnitine is recommended /1,2,7,9/. When pivampicillin was administered with equal molar carnitine in human subjects the magnitude of changes in urinary excreted carnitine esters showed that exogenous carnitine was a very good substrate for pivaloylcarnitine production /6/. However, the dose of carnitine was probably not sufficient to meet the enhanced needs implied by the plasma and urine carnitine values. The present work was undertaken to study the fate of erythrocyte's carnitine pool (as a different compartment) under the same conditions.

MATERIALS AND METHODS

Patients. Pooled erythrocyte samples were analyzed. The samples collected originally from five children (females, mean weight 36.4 kg, range 26.5 - 47.5 kg; mean age 9.4 years, range 8 - 12 years) who were participants of another study /6/. The clinical indications for the antibiotic treatment were ampicillin sensitive bacteriuria and upper respiratory tract infection. The daily dose of pivampicillin (Pondocillin, Leo Pharmaceuticals, Denmark) was 1.000 mg (n = 3) and 1.500 mg (n = 2) divided in 500 mg doses two- or three times daily. The dose of administered carnitine was equimolar to the pivampicillin: to each 500 mg tablet (containing 1.08 mmoles pivalic acid) 173.9 mg L-carnitine oral solution (100 mg/ml, Sigma-Tau Pharmaceuticals, Italy). Informed consent was obtained from the parents of participants.

Procedures. Prior to (day 0) and on the last day (day 7) of the treatment blood was taken between 7:30 and 8:30 AM after an overnight fasting into heparinized tubes. The samples were immediately centrifuged (600 g 5 min). After the removal of

plasma the sediment was washed twice in 0.9 % NaCl and centrifuged as previously. The sedimented red blood cells were stored at -20°C until analysis.

Chemical methods. Carnitine content of red blood cells (defined here as the carnitine within the cells and/or bound to cells) was measured by the DTNB method as described /8/ after partial purification of the samples. 500 µl erythrocyte mass was diluted with 100 µl distilled water and the protein was precipitated on ice with 100 µl concentrated perchloric acid. After centrifugation the pellet was washed with 500 µl 0.5 N perchloric acid. The combined supernatant was neutralized with 10 N KOH to pH 7.5 after addition of 100 µl 0.5 M phosphate buffer (pH 7.5). The perchlorate sediment was washed with 300 µl ethanol (40 %) and after centrifugation the supernatant was added to the above neutralized solution. The volume of the solution was measured and 20 µl DTNB (5 mM) was added to trap the free -SH groups. 250 µl mixture was applied to a small column (0.5 x 4.0 cm) containing Dowex 1X8 Cl- (200-400 mesh) resin and was washed with 2 x 250 µl water (the resin binds the free and sulphydril bound DTNB). The effluent was used for carnitine determination.

For total carnitine determination 500 µl red blood cell mass was treated with 100 µl 5 N KOH (60 min, 55 oC). After this hydrolization of the esterified carnitines the same procedure was done as described above.

The haemoglobin was measured by routinely used KCN reaction. For calculation of water content of samples 200 µl erythrocyte mass was stored in desiccator until reaching a constant weight. Thus, the water content measured in the present work is not equal to the cellular water because small amount of extracellular water was also involved.

Statistics. The Student's t test for paired samples was used with the help of an IBM compatible computer package.

RESULTS

The carnitine content of the pooled ruptured red blood cell mass is shown in Table I. Both fractions (total and free, amount of carnitine esters was calculated by substraction: total minus free) of carnitines decreased on the last day of treatment (all patients responded) as compared with the initial pretreatment value (b versus a in the Table I). No differences were found in the haemoglobin and water content of the samples comparing the day 0 and day 7 values (not shown) indicating that the yield of the erythrocytes was the same in the samples obtained prior to and on the last day of treatment (no effect of the drugs, washing and pooling procedures on these

parameters). Thus, when the carnitine levels were expressed as µmol/mmol haemoglobin on nmol/ml water the same feature was found like when the carnitine values were expressed as nmol/ml erythrocyte mass.

TABLE I

Carnitine content of red blood cells of patients taking pivampicillin and carnitine (a: day 0; b: day 7).

		total	acyl	free
nmol/ml red blood cell	a	47.5 ± 3.39	28.2 ± 3.38	19.2 ± 0.97
	b	37.5 ± 2.48*	21.9 ± 1.78*	15.5 ± 0.99*
µmol/mmol haemoglobin	a	2.44 ± 0.20	1.45 ± 0.19	0.99 ± 0.06
	b	1.94 ± 0.14*	1.14 ± 0.09*	0.80 ± 0.06*
nmol/ml water content	a	75.2 ± 3.96	44.5 ± 4.41	30.7 ± 1.73
	b	60.6 ± 3.29*	35.4 ± 2.40*	25.1 ± 1.45*

*$p < 0.05$ or less

DISCUSSION

The data show proportionally decreased total, free and esterified carnitine content of red blood cells collected from patients on the last day of combined pivampicillin and equimolar carnitine treatment compared with the initial carnitine status.

As it was shown previously, pivampicillin treatment causes exceptional formation of pivaloylcarnitine in humans evidenced

by marked excretion of this xenobiotic acyl ester of carnitine in the urine /5/. The pivampicillin treatment was associated with changes of plasma carnitine levels: the plasma total carnitine decreased due primarily to a decrease of the free carnitine, whereas an expansion in the short-chain acylcarnitines was seen /5/. These effects of the drugs were recently confirmed by others /4/, who noticed that the resulting carnitine deficiency should be considered as a risk factor regarding that in many forms of carnitine deficiency serious complications have been observed. Indeed, the present knowledge is that carnitine deficiency (insufficiency) a multiple deterioration of intracellular processes may develop leading to a spectrum of pathological changes /3,9/.

Therefore, in a previous work the fate of supplemental carnitine was studied in pivampicillin treatment /6/. At the end of the study the ratio of acylcarnitine/free carnitine in plasma increased due to a decrease of circulating free carnitine and an increase of carnitine esters /6/. The increase of carnitine esters was probably caused by the presence of pivaloylcarnitine in the plasma as suggested by gas-chromatographic analysis of an exctract of pooled, combined and concentrated plasma samples /6/. Although the ester profile of red blood cell acylcarnitines was not determined in the present study, the decrease of carnitine esters in the erythrocytes (Table I) on the last day of treatment strongly suggests that this compartment does not participate significantly or at all in the pivaloylcarnitine transport and production. By contrast, the decrease of total, free and acylcarnitine on the last day of treatment shows that the carnitine pool of red blood cells contributed to the enhanced needs caused by the increased pivaloylcarnitine generation.

Based on the present data it is raised, on the other hand, that measurement of carnitine levels of red blood cells may be a useful additional parameter in the evaluation of carnitine status of human subjects. It should be considered particularly during surveys on efficiency of carnitine supplementation.

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