Brief Report

Effect of l-carnitine on erythroid colony formation in mouse bone marrow cells

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Abstract

Background. L-Carnitine can alleviate uraemic anaemia in haemodialysis patients by improving erythrocyte membrane functions or erythropoiesis, which are depressed under uraemic conditions. l-Carnitine and palmitoyl-l-carnitine were reported to increase the formation of colony-forming unit-erythroid (CFU-E) colonies in cultures of fetal mouse liver cells, an effect that depended on the concentration of palmitoyl-l-carnitine but not of l-carnitine. In this study, we investigated l-carnitine’s effect on CFU-E colony formation in cell cultures of mouse bone marrow cells.

Methods. Bone marrow from normal female mice was placed in 35 mm culture dishes containing a medium composed of methylcellulose and various nutrients. The dishes were incubated for 48 h, and the colonies of erythroblasts, which were differentiated from CFU-E, consisting of \( /C21 8 \) cells, were counted in each dish using an inverted microscope.

Results. The numbers of CFU-E colonies correlated well with both the initial numbers of bone marrow cells and concentrations of recombinant human erythropoietin (rhEPO) in the methylcellulose medium. In the presence of 0.5 or 1.0 IU/ml of rhEPO, l-carnitine at concentrations of 200 and 400 \( \mu \)mol/l significantly enhanced CFU-E colony formation \( (P < 0.001) \).

Conclusion. L-Carnitine significantly increased the number of CFU-E colonies in mouse bone marrow cell cultures. This finding suggests that l-carnitine stimulates erythropoiesis, partially accounting for its mitigating effect on renal anaemia.

Keywords: anaemia; l-carnitine; CFU-E; erythropoiesis; mouse bone marrow cell; rhEPO

Introduction

Anaemia is often present in patients with renal failure, especially in those undergoing dialysis, because most of these patients produce almost no erythropoietin (EPO) in their kidneys. Renal anaemia in patients with end-stage renal disease is caused mainly by (i) a reduction in erythropoiesis resulting from a substantial decrease in the renal production of EPO; and (ii) a shortened red blood cell (RBC) lifespan arising from a derangement in RBC membrane integrity as a result of renal insufficiency and haemodialysis (HD). Thus, possible treatment strategies for renal anaemia include: (i) stimulating erythropoiesis; and (ii) preserving RBC membrane function to prolong the lifespan of the RBCs.

Recombinant human EPO (rhEPO), which has a direct effect on erythropoiesis, is presently the treatment of choice for HD patients with renal anaemia. Meanwhile, l-carnitine is the only drug proven to prolong the lifespan of RBCs. Carnitine plays an important role in myocardial and skeletal muscle energy metabolism, acting as a transporter of long-chain fatty acids into mitochondria for the generation of energy. Long-term intermittent dialysis is associated with a significant reduction in plasma and tissue l-carnitine levels [1]. This secondary carnitine deficiency syndrome can be treated using l-carnitine. In fact, the efficacy of oral l-carnitine in improving diminished l-carnitine levels in HD patients has been confirmed by a number of studies. The patients’ haematological status, in particular, can be improved by increasing the amount of haemoglobin or allowing
a reduction in EPO dosage without changing the amount of haemoglobin, as suggested in reviews by Goral [2], Bommer [3] and Golper et al. [4].

The efficacy of l-carnitine for the treatment of anaemia in experimental animals was also reported by Azzadin et al. [5]. In their study of rats, a concomitant treatment with oral l-carnitine and rhEPO significantly restored the haematocrit depressed by partial nephrectomy. l-Carnitine may alleviate renal anaemia by both stimulating erythropoiesis and preserving RBC membrane integrity. Several lines of research have confirmed the latter mechanism as manifest in an improvement in RBC membrane stability [6], an increase in ouabain-sensitive Na⁺K⁺ ATPase activity on the RBC membrane [7], the activation of RBC membrane enzymes for lipid incorporation [8], and so on. Evidence of the former mechanism, however, has been provided only by a few groups [4,9]. Matsumura et al. demonstrated that l-carnitine and palmitoyl-l-carnitine increased the number of erythroid colonies in cultures of cell from fetal mouse liver, a major location of erythropoiesis during the embryonic period [9]. Some reports have indicated that the elevation in inflammatory cytokines and oxidative parameters correlated with rhEPO resistance, and that l-carnitine administration improved these abnormalities [4]. Nevertheless, that l-carnitine has a positive effect on erythropoiesis has not yet been clearly shown.

In this study, we investigated the erythropoietic effect of l-carnitine, using cells from mouse bone marrow, where erythropoiesis takes place exclusively after birth.

Subjects and methods

We used female BDF1 mice (5 weeks old). Bone marrow was flushed from the femurs (excised under terminal anaesthesia with ether) and was added to Iscove’s modified Dulbecco’s medium (IMDM) containing 10% fetal bovine serum (FBS). The bone marrow cells were cultured according to the method described by Iscove et al. [10], with minor modifications. Briefly, the suspension of bone marrow cells from the femurs was centrifuged, and the resulting cell pellet, one for each mouse, was re-suspended with IMDM containing 10% FBS and was incubated at 37°C to obtain living non-adherent nucleated cells. These cells were plated at concentrations of 1.5×10⁵ in dishes, in a 1 ml volume of IMDM containing FBS and methylcellulose at final concentrations of 24 or 0.8%. Additionally, various nutrients were added to the culture medium. Then, 2-mercaptoethanol was added to a final concentration of 10⁻⁴ mol/l. RhEPO was dissolved and diluted to final concentrations of either 0.5 or 1.0 IU/ml. l-Carnitine was prepared and added to final concentrations of either 0, 200 or 400 µmol/l to the culture medium. When we examined the effect of l-carnitine on colony-forming unit-erythroid (CFU-E) colony formation, we prepared the following seven different compositions; 0 IU/ml rhEPO and 0 µmol/l l-carnitine as negative control and six different compositions of solution combining two concentrations of rhEPO and three concentrations of l-carnitine. The bone marrow cells were plated in four dishes per each composition of solution and were incubated for 48 h. The CFU-E colonies in each dish were counted using an inverted microscope [11].

Results

The number of CFU-E colonies from the bone marrow cells incubated in the methylcellulose culture media increased in parallel with the number of cells plated. In the preliminary experiments, no difference in CFU-E colony count was observed between cultures containing 1.0 or 3.0 IU/ml of rhEPO. We therefore used the lower concentration of 1.0 IU/ml of rhEPO to stimulate CFU-E colony formation. Between 1×10⁵ and 2×10⁵ plated cells per dish appeared to be appropriate for evaluating the erythropoietic effects of drugs, so we used 1.5×10⁵ plated cells/dish for subsequent experiments (data not shown).

The numbers of CFU-E colonies that the bone marrow cells formed (1.5×10⁵ cells/dish) in the presence of rhEPO at concentrations of 0, 0.05, 0.1, 0.5 and 1.0 IU/ml were 0, 56.4±17.2, 78.4±14.8, 122.1±3.2 and 131.1±5.8, respectively. The addition of l-carnitine to the culture medium at concentrations of 200 and 400 µmol/l in the presence of 0.5 or 1.0 IU/ml of rhEPO significantly (P<0.001; paired t-test) increased the numbers of CFU-E colonies (Table 1).

Discussion

This study points to the possible erythropoietic activity of l-carnitine in the presence of rhEPO, because we observed the enhancement of erythroid colony formation in mouse bone marrow cell cultures. Matsumura et al. reported that l-carnitine at concentrations up to 200 µmol/l also in the presence of rhEPO did not increase the number of CFU-E colonies in fetal mouse liver cell cultures, whereas l-carnitine at a concentration of 400 µmol/l or palmitoyl-l-carnitine, an acylated metabolite of l-carnitine, at a concentration of just 12.5 µmol/l did [9]. They concluded that long-chain acyl carnitine, but not free carnitine, was responsible for stimulating erythropoiesis. Wanner et al. [12] demonstrated that administering l-carnitine increased serum free carnitine levels from 31.2±4.7 µmol/l to 34.2±3.0 or 201.3±14.3 µmol/l at doses of 1 mg/kg (low-dose regimen) or 15 mg/kg (high-dose regimen), respectively, while serum long-chain acyl carnitine levels rose from 2.6±0.2 µmol/l to 3.6±0.48 or 10.9±1.1 µmol/l for low- and high-dose regimens, respectively. Thus, serum
Table 1. The effect of L-carnitine on the formation of colony-forming unit-erythroid (CFU-E) colonies in bone marrow cell cultures

<table>
<thead>
<tr>
<th>rhEPO concentrations (IU/ml)</th>
<th>L-Carnitine concentrations (μmol/l)</th>
<th>No. of CFU-E colonies (per 35 mm dish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>106 ± 8.8</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>128.1 ± 11.0**</td>
</tr>
<tr>
<td>0.5</td>
<td>200</td>
<td>140.1 ± 10.9**</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>132.4 ± 7.0</td>
</tr>
<tr>
<td>1.0</td>
<td>200</td>
<td>148.5 ± 8.8**</td>
</tr>
<tr>
<td>1.0</td>
<td>400</td>
<td>163.3 ± 10.3</td>
</tr>
</tbody>
</table>

L-Carnitine was added to a methylcellulose medium to produce final concentrations of 0, 200 or 400 μmol/l. Each value represents the mean ± SE of the CFU-E colony count (n=12).

**Significantly different from control (0.5 IU/ml of rhEPO and 0 μmol/l of L-carnitine), P < 0.001.

†Significantly different from control (1.0 IU/ml of rhEPO and 0 μmol/l of L-carnitine) P < 0.001.

concentrations of long-chain acyl carnitine sufficient to cause erythropoiesis were obtained by the high-dose L-carnitine regimen in the HD patients, but serum free carnitine levels sufficient to cause erythropoiesis were not, according to the study of Matsumura et al., because 400 μmol/l L-carnitine was required to cause erythropoiesis [9]. However, an increase in CFU-E colony formation was induced by L-carnitine at a dose of 200 μmol/l in our study. In this case, a serum free carnitine level sufficient to cause erythropoiesis was probably obtained by the high-dose L-carnitine regimen. The exact concentration of carnitine in the bone marrow following L-carnitine administration, however, was not reported. Nevertheless, since in several animal species free carnitine accumulates in some tissues such as the epididymis at concentrations >1 mmol/l as reviewed by Jeulin et al. [13], carnitine may accumulate in bone marrow following L-carnitine treatment. L-Carnitine at a dose of 200 μmol/l markedly augmented CFU-E colony formation in our study but not in that of Matsumura et al. The source of the CFU-E may be responsible for this inconsistency; the CFU-E used in the present study, and obtained from bone marrow, may be more sensitive to L-carnitine than CFU-E from fetal liver.

Generally, carnitine plays an important role in mitochondria as a transporter of long-chain fatty acids for β-oxidation. During erythropoiesis, mitochondria persist in the CFU-E and generate the energy required for RBC maturation; and L-carnitine probably influences energy metabolism by oxidizing fatty acids. Alternatively, L-carnitine may affect differentiation during RBC maturation. Programmed cell death, or apoptosis, takes place during the differentiation of progenitor cells. Erythropoietin contributes to RBC maturation by retarding apoptosis, thus allowing erythroid progenitors to complete their differentiation programmes. Mutomba et al. [14] reported that L-carnitine inhibits the activation of caspase (which induces the apoptosis of progenitor cells) at various point in the Fas ligation pathway in Jurkat cells. Ishii et al. [15] also demonstrated that acetyl-L-carnitine and L-carnitine prevented neuronal cell death through an anti-apoptotic action in primary cultured neurons from rat embryos. These reports and our present study suggest that L-carnitine influences erythropoiesis, inhibits the apoptosis of progenitor cells, or both.

L-Carnitine deficiency may necessitate the escalation of the rhEPO dose in patients with ESRD. Since L-carnitine prolongs RBC lifespan [3,4], its administration is expected to reduce rhEPO requirements and improve hyporesponsiveness in patients with ESRD and rhEPO-dependent anaemia. Direct evidence supporting L-carnitine’s erythropoietic effect has not been obtained in clinical studies. In our study, however, the number of erythroblasts produced by a lower concentration of rhEPO in combination with L-carnitine was the same as that produced by a higher concentration of rhEPO without L-carnitine. Thus, the erythropoietic effect of L-carnitine may, in part, contribute to the amelioration of the rhEPO resistance seen in HD patients.

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Conflict of interest statement. None declared.

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