The effect of cyclosporine on taurine transport in human cord blood cells

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Cyclosporine has been used in long-term organ transplant treatment and there are reports of an increased occurrence of intrauterine growth retardation (IUGR) in pregnancies in such patients (Pickrell et al. 1988; Cockburn & Krupp, 1989). Indirect evidence suggests that this might result from an inhibitory effect of cyclosporine on taurine uptake by the placenta (Ramamoorthy et al. 1992). Here we investigated taurine transport by peripheral blood mononuclear cells (PBMs) enriched from cord blood (CB), an alternative and easily obtainable fetal tissue to placental tissue. We determined whether cyclosporine inhibited taurine uptake by these cells.

CB was obtained from placentas from normal term pregnancies in accordance with local ethical approval. PBMs were enriched by dilution with Hanks' biological salt solution (HBSS, containing (mm): NaCl 137, KCl 5.4, KH2PO4 0.4, NaHPO4 0.34, NaHCO3 4.2 and glucose 5.6) and centrifuged at 400 g for 30 min (modified from Wan et al. 1999). [3H]taurine uptake was measured with or without 10 mM [3H]taurine by methanol, the vehicle used to dissolve cyclosporine. Student's paired t-test). There was no effect on the uptake of [3H]taurine by methanol, the vehicle used to dissolve cyclosporine.

In conclusion, the effect of cyclosporine on taurine transport by peripheral blood mononuclear cells (PBMs) enriched from cord blood (CB), an alternative and easily obtainable fetal tissue to placental tissue. We determined whether cyclosporine inhibited taurine uptake by these cells.


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All procedures accord with current local guidelines.

The effect of 1-chloro-2,4-dinitrobenzene on K⁺ transport in human red blood cells

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1-Chloro-2,4-dinitrobenzene (CDNB) lowers [reduced glutathione] and increases passive K⁺ transport in human red blood cells (RBCs) (Shartava et al. 2000; Muzyamba et al. 2001). The major passive K⁺ transport pathways are the K⁺–Cl⁻ cotransporter (KCC) and the Ca²⁺-activated K⁺ channel (Gardos channel, Iₖ). Here we investigated taurine transport by peripheral blood mononuclear cells (PBMs) enriched from cord blood (CB), an alternative and easily obtainable fetal tissue to placental tissue. We determined whether cyclosporine inhibited taurine uptake by these cells.

CB was obtained from placentas from normal term pregnancies in accordance with local ethical approval. PBMs were enriched by dilution with Hanks' biological salt solution (HBSS, containing (mm): NaCl 137, KCl 5.4, KH2PO4 0.4, NaHPO4 0.34, NaHCO3 4.2 and glucose 5.6) and centrifuged at 400 g for 30 min (modified from Wan et al. 1999). [3H]taurine uptake was measured with or without 10 mM β-alanine at 37°C with or without pre-incubation with cyclosporine (5 μM), 37°C for 10 min using methods similar to those previously described (Ayuk et al. 2000). Data are expressed as means ± s.e.m.; n = number of placentas from which CB samples were taken.

Uptake of [3H]taurine by CB PBMs was linear over 15 min (5.49 ± 0.92 fmol (10⁶ cells)⁻¹ min⁻¹, n = 6), inhibitable by β-alanine (0.22 ± 0.09 fmol (10⁶ cells)⁻¹ min⁻¹, n = 6 P < 0.05, Student’s paired t test). Pre-incubation with cyclosporine (5 μM) inhibited [3H]taurine uptake by 29.3 ± 5.3% (n = 8, P < 0.05, Student’s paired t test). There was no effect on the uptake of [3H]taurine by methanol, the vehicle used to dissolve cyclosporine.

In conclusion, the effect of β-alanine on taurine uptake into CB PBMs suggests that this was mediated by system β, although further characterisation is required. The inhibitory effect of cyclosporine on taurine transport in CBPBs was comparable to that seen in choriocarcinoma cells (Ramamoorthy et al. 1992). Thus the increased incidence of IUGR previously reported in mothers being treated with cyclosporine A might be due partially to effects on taurine uptake into fetal and placental tissues.


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All procedures accord with current local guidelines.
The effect of nitrite on passive K⁺ transport in human red blood cells

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Red blood cells (RBCs) from sickle cell patients, which are exposed to a greater oxidative challenge than normal, have elevated K⁺ transport. This characteristic is responsible for their dehydration. As part of a study to investigate the mechanisms responsible for this transport phenotype, we have examined the effects of oxidants on passive K⁺ transport in RBCs (Muzyamba et al. 2000, 2001). Stimulation of the K⁺–Cl⁻ cotransporter (KCC) correlated with both reduction of reduced glutathione (GSH) and accumulation of methaemoglobin (metHb), not GSH alone, whilst loss of O₂ dependence of KCC was independent of GSH but correlated with accumulation of metHb. Here we report the effects of nitrite (NO₂⁻), which stimulates KCC in RBCs from sheep and horse (Adragna & Lauf, 1998; Muzyamba et al. 2000), on human RBCs.

Normal human RBCs, taken with ethical permission from consenting volunteers, were pretreated for 60 min at 10% haematocrit (Hct) with or without NO₂⁻ (1, 3 or 5 mM). Cells were then equilibrated in air or N₂ after which transporter activity (80 mM K⁺, 2.5 mM Ca²⁺) was measured at 4% Hct using ⁸⁶Rb⁺ as a K⁺ congener, in the presence of ouabain (0.1 mM) and bumetanide (1 μM) to obviate transport through Na⁺/K⁺ pump and Na⁺–K⁺–Cl⁻ cotransporter. KCC activity was determined as the Cl⁻-dependent component of K⁺ influx (Cl⁻ replaced with nitrate). We also measured activity of the Ca²⁺-activated K⁺ channel (Gardos channel or I⁵) as the clotrimazole (5 μM)-sensitive component.

Increasing [NO₂⁻] produced progressive stimulation of KCC (3.5-fold at 1 mM; 6.5-fold at 5 mM) and loss of O₂ dependence (deoxygenation inhibited KCC in control cells by 90% but only about 50% in NO₂⁻-treated cells) (Table 1; P < 0.05, Student’s paired t test). As for other oxidants, treatment with NO₂⁻ caused accumulation of metHb (>60% at 5 mM) and depletion of GSH. I⁵, however, was not activated in either control cells or following treatment with NO₂⁻.

### Table 1. The effect of nitrite on K⁺–Cl⁻ cotransport in human red blood cells (RBCs)

<table>
<thead>
<tr>
<th></th>
<th>Oxygenated RBCs</th>
<th>Deoxygenated RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.27 ± 0.10</td>
<td>0.13 ± 0.13</td>
</tr>
<tr>
<td>1 mM nitrite</td>
<td>4.46 ± 0.65</td>
<td>2.14 ± 0.55</td>
</tr>
<tr>
<td>5 mM nitrite</td>
<td>8.23 ± 2.93</td>
<td>3.86 ± 0.31</td>
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Activity of the K⁺–Cl⁻ cotransporter was determined as Cl⁻-dependent K⁺ influx (Cl⁻ replaced by NO₃⁻), using ⁸⁶Rb⁺ as a K⁺ congener, in the presence of ouabain (0.1 mM) and bumetanide (1 μM). Influxes, in mmol (l cells h)⁻¹, are given as means ± S.D., n = 3.

In conclusion, NO₂⁻, like other oxidants, has profound effects on membrane transport in human RBCs altering the response to O₂ tension. Elucidating the target of oxidants will be invaluable for understanding the transport abnormalities observed in sickle cells.


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All procedures accord with current local guidelines and the Declaration of Helsinki.