Does Higher Red Blood Cell (RBC) Lactate Transporter Activity Explain Impaired RBC Deformability in Sick Cell Trait?

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Abstract: Lactate and H⁺ are suggested to promote the sickling process in red blood cells (RBCs) containing hemoglobin S. We demonstrated greater activity of the RBC monocarboxylate cotransporter *MCT-1*, lower RBC deformability,

and impaired hematological indices in sick cell trait (SCT) carriers compared to control subjects, suggesting an involvement of *MCT-1* in hemorheological disturbances in SCT carriers. [The Japanese Journal of Physiology 55: 385–387, 2005]

Key words: hemorheology, sickle cell trait, lactate.

Sickle cell trait (SCT) is the heterozygous form of sickle cell anemia (SCA), which is characterized by the presence of both hemoglobin A (HbA) and hemoglobin S (HbS). SCT is usually considered benign and asymptomatic [1], but several studies have introduced doubt about this assumption [2–4]. Two recent studies [5, 6] reported impaired red blood cell (RBC) deformability in SCT carriers even though the environmental conditions were normal. Low deformable RBCs may disturb blood rheological properties in microvessels and increase flow resistance [7, 8], which adversely affects capillary perfusion. Although never investigated, Smith *et al.* [9] hypothesized that lactate/H⁺ uptake could be involved in the sickling process of RBCs containing HbS because of subsequent dehydration triggered by the activation of the volume- and pH-dependent K⁺/Cl[−] cotransport channels. This may lead to impaired sickle cell rheology and altered blood flow through the microcirculation that could contribute to some of the unexplained cases of health complications in SCT carriers. The major pathway of lactate transport across the RBC membrane is the cotransporter lactate-proton *MCT-1*, which is responsible for 80%–90% of the total lactate uptake by RBCs [10], and we

recently found higher *MCT-1* activity in SCT carriers compared to a control group [11]. Thus on the basis that high *MCT-1* activity could lead to high RBC rigidity in SCT carriers, we investigated the relationships between *MCT-1* activity (results already published in [11]) and (i) RBC rigidity, (ii) hematological indices such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

Eight SCT carriers (SCT group, 23.5 ± 6.9 yrs, 182.6 ± 3.5 cm, 77.0 ± 10.3 kg) and six subjects with normal hemoglobin (control group, 19.8 ± 1.3 yrs, 178.5 ± 5.1 cm, 70.3 ± 7.3 kg) participated in the study after giving their informed consent. Subjects with hypertension, malaria, and hemoglobinopathy other than SCT were excluded.

After blood sampling, the hemoglobin type was screened by isoelectric focusing, and the results were confirmed by citrate agar electrophoresis. The various hemoglobins were isolated and quantified by high-performance liquid chromatography (HPLC, Hemoglobin Testing System Biorad, Biorad Laboratories, Germany). The presence of HbS was confirmed by a test of solubility. Positive test results for SCT were determined by the presence of HbS (less than 50%) and

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Table 1. Michaelis-Menten parameters for MCT-1 and hematological and hemorheological parameters in the SCT and control groups.

	SCT group	Control group
$V_{\max}$ (nmol·ml ⁻¹ ·cells ⁻¹ ·min ⁻¹)	1,364.9 ± 392.0 [†]	776.9 ± 61.6
K_m (mM)	7.2 ± 3.2	7.0 ± 6.1
[Hb] (g·dl ⁻¹)	14.7 ± 0.8	14.3 ± 0.8
MCV (fl)	84.6 ± 3.8 [†]	92.8 ± 3.0
MCHC (pg)	35.1 ± 0.7*	33.0 ± 1.9
% ret (%)	1.0 ± 0.2	1.3 ± 0.6
[HbA]	51.5 ± 1.3 [†]	87.9 ± 1.2
[HbF]	0.3 ± 0.3	0.3 ± 0.3
[HbA2]	3.4 ± 0.3	3.1 ± 0.3
[HbS]	38.7 ± 1.6 [†]	–
η_b (mPa·s ⁻¹)	5.94 ± 0.62*	5.51 ± 0.20
η_p (mPa·s ⁻¹)	1.69 ± 0.11	1.72 ± 0.06
Hct (%)	45.75 ± 1.96	46.79 ± 0.72
T_k	0.86 ± 0.03 [†]	0.80 ± 0.03

Values are means ± SD. $V_{\max}$, maximal lactate transport capacity for MCT-1; K_m , constant of Michaelis-Menten for MCT-1; [Hb], hemoglobin concentration; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; % ret, percentage of reticulocytes; [HbA], concentration of hemoglobin A; [HbF], concentration of hemoglobin F; [HbA2], concentration of hemoglobin A2; [HbS], concentration of hemoglobin S; η_b , blood viscosity; η_p , plasma viscosity; Hct, hematocrit; T_k , coefficient of red blood cell rigidity. Difference between the two groups, * P < 0.05; [†] P < 0.01.

a normal percentage of HbA2. The following hematological parameters were automatically determined (Max M-Retic, Coulter, USA): hemoglobin concentration ([Hb]), MCV, MCHC, and percentage of reticulocytes (%ret). The MCT-1 activity of SCT carriers and the control group was determined in a previous study (see [11]). In brief, MCT-1 activity was deduced from the difference between total lactate transport activity and the lactate transport activity determined with the use of 1 mM of p-chloromercuribenzenesulfonic acid (PCMBs) to block the MCT-1. The influx of lactate into washed RBCs was measured by a radioactive tracer technique using [¹⁴C]lactate (sodium salt, specific activity of 50 μ Ci/mM) at four lactate concentrations (1.6, 8.1, 41, and 81.1 mM). The Michaelis-Menten coefficients for MCT-1, i.e., the constant of Michaelis-Menten (K_m) and the maximal lactate transport capacity ($V_{\max}$), were determined by curve fitting using GraphPad prism 4 (USA). Blood viscosity (η_b) and plasma viscosity (η_p) were determined by using a cone plane viscometer (Brookfield DVII+, with CPE40 spindle) at a shear rate of 225 s⁻¹ and at 25°C.

Hematocrit (Hct) was measured by a micromethod after blood microcentrifugation (HEMA-C, Jouan, France). The index of RBC rigidity (T_k) from Dintenfass [5, 12, 13] was calculated at high shear rate (225 s⁻¹) according to the following equation: $T_k = (\eta_r^{0.4} - 1)/(\eta_r^{0.4} \cdot \text{Hct})$ where η_r is relative blood viscosity (η_b/η_p) measured at 225 s⁻¹.

We used the T_k index as a deformability index, such as high T_k value indicates low RBC deformability (i.e., high RBC rigidity).

All results are expressed as means ± SD. Hematological, hemorheological, and Michaelis-Menten coefficient parameters were compared, using the non-parametric Mann-Whitney test. A two-way analysis of variance (group × concentrations) for repeated measures was used to compare the MCT-1 activity at the four lactate concentrations between the two groups. Pairwise contrasts were used when necessary to determine where significant differences occurred. A Spearman correlation was performed between the $V_{\max}$ and T_k , MCV, and MCHC. Statistical significance was established at $P = 0.05$.

As already reported in a recent study [11], MCT-1 activity was higher in the SCT group than in the control group at 1.6 mM ($P < 0.05$), 41 mM ($P < 0.05$), and 81.1 mM ($P < 0.01$) (see [11] for more details). No difference was observed between the two groups at 8.1 mM. Although we found no difference for the K_m between them, we found higher $V_{\max}$ in the SCT carriers (Table 1). These results suggest overexpressed MCT-1 in the RBC membranes from SCT carriers, but confirmation by Western blot is needed. The reason for the greater MCT-1 activity in the SCT group is unknown, but it may be related to the occurrence of repeated episodes of hypoxia/hypoxemia in SCT carriers because hypoxemia is known to influence MCT-1 activity and/or expression in humans [11, 14–16]. The higher MCT-1 activity in the SCT group was accompanied by higher RBC rigidity (Table 1). Although hematological values were in the normal range for both groups (no hypochromic microcytosis), MCV was lower and MCHC greater in the SCT carriers. This could be a reflection of the dehydration of RBCs, which might in turn affect RBC deformability [17]. The poor RBC deformability observed in the SCT group led to higher η_b , even though η_p and Hct did not differ between the two groups (Table 1). Impaired RBC deformability and high η_b are able to cause blood flow structuring disorders in both the microcirculation and the macrocirculation [7, 8], and this might explain the reported health complications in SCT carriers [3, 4]. The significant correlation between $V_{\max}$ and T_k (Fig. 1, $\rho = 0.87$; P

Fig. 1. Relationship between red blood cell rigidity (T_k) and the $V_{\max}$ of MCT-1 ($\rho = 0.87$; $P < 0.01$).

< 0.01) suggests an influence of MCT-1 activity upon RBC deformability. Several studies observed that lactate impairs RBC deformability in healthy subjects [9, 13]. Indeed, the greater the MCT-1 activity, the greater the amount of lactate accumulates into RBCs, leading to impaired RBC deformability. The $V_{\max}$ was also correlated with MCV ($\rho = -0.73$; $P < 0.01$) and MCHC ($\rho = 0.59$; $P < 0.05$). Altogether, these results suggest that MCT-1 might regulate the deformability of RBCs, maybe via an effect on the dehydration process, as initially hypothesized by Smith *et al.* [9]. Recent results published by Pattillo and Gladden [16] suggest the same kind of mechanism in sickle cell anemia (HbSS genotype) because they observed high RBC' MCT-1 activity in these patients, and it is well known that RBC deformability from them is altered [18]. In conclusion, our results suggest a role of the MCT-1 protein in the sickling process and in the regulation of RBC deformability in SCT carriers. These alterations in blood rheology might have detrimental effects on blood microcirculation that will increase the risk for cardiovascular complications or vaso-occlusive events in this population.

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