CONCISE REPORT

Antisickling Effect of Tellurite: A Potent Membrane-Acting Agent In Vitro

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Potassium tellurite (K₂TeO₃) was found to be a potent antisickling agent that inhibited red cell sickling at concentrations less than 10 μmol/L. The inhibitory effect depended on the incubation time, with the effect increasing with longer incubation periods. Because tellurite causes swelling of red cells, and because the antisickling effect of tellurite correlated with the degree of red cell swelling, the antisickling effect of tellurite is assumed to be due to the decreased mean cell hemoglobin concentration. Swelling of red cells by tellurite was accelerated by the addition of reduced glutathione. Tellurite appears to be a new type of antisickling agent that interacts with the red cell membrane.

ANTISICKLING AGENTS that interact with the red blood cell (RBC) membrane and decrease mean cell hemoglobin concentration (MCHC) are of particular interest because a small decrease in MCHC markedly delays the rate of polymerization of deoxy-HbS² and inhibits RBC sickling. Cetiedil, the first such chemical to be identified, induces RBC swelling at concentrations much lower than intracellular hemoglobin concentrations.³ ₄ Cetiedil increases RBC sodium and water influx.⁵ ⁷ Other agents that cause a decrease in MCHC are piracetam⁶ and monensin, a sodium-selective ionophore that produces sustained hydration of sickle cells.⁹

We report another membrane-acting antisickling agent, tellurite, which induces RBC swelling at micromolar concentrations and strongly inhibits sickling in vitro. Differences in the mechanism of swelling by tellurite from that of cetiedil will be discussed.

MATERIALS AND METHODS

Whole blood was freshly drawn from normal subjects or from patients with sickle cell disease, with sodium heparin or EDTA used as anticoagulants. The RBC were washed three times with 0.9% NaCl solution by centrifugation at 4,000 g for three minutes at 4°C. Potassium tellurite (K₂TeO₃) was obtained from Fisher Scientific Co, Pittsburgh, Pa. Other chemicals were obtained from Sigma, St Louis, Mo.

Degree of sickling was determined by direct microscopic examination of erythrocytes in rectangular glass microslides (Vitro Dynamics, Rockaway, NJ. No. 5005, path length 0.05 mm). Sample vessels contained 400 μL of buffer and 10 μL of washed HbSS cells. Incubation buffer was 150 mmol/L NaCl, 5 mmol/L KCl, 10 mmol/L TES [N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], and 5 mmol/L glucose (296 mosm/kg H₂O), pH 7.4, at 37°C. Deoxygenation of the RBC suspension was performed by flushing the sample vessel with humidified N₂ gas. The flow rate was adjusted to provide adequate deoxygenation without promoting significant evaporation of the sample (10–50 mL/min). For determining the percentage of sickled RBC, samples were collected through the gas outlet tube into the microslide and sealed with Vaseline. The microslide was then placed on a glass slide. RBC treated with tellurite exhibit similar morphological changes to those induced by cetiedil.³ Initially, cells increase in diameter and thickness, gradually becoming spherical. No morphological changes, such as crenation, are observed during incubation. Cell water content (Wₑ) was measured using the methods previously described.⁶ Mean cell volumes (MCV) were measured using a Coulter Counter model ZBI.

Oxygen equilibrium curves (OEC) of hemoglobin were determined in 0.1 mol/L potassium phosphate buffer, pH 7.0, at 20°C, as described elsewhere.¹⁰ Tellurite concentrations as high as 500 μmol/L had no effect on the OEC, suggesting that tellurite does not directly affect O₂-binding properties of hemoglobin.

RESULTS

To investigate the effect of different concentrations of tellurite on the size of RBC, normal RBC were incubated with 0.01–1.0 mmol/L K₂TeO₃. As indicated in Fig 1A, increasing the concentration of tellurite resulted in a faster increase in MCV. As expected, when the MCV reaches a maximum, RBC begin to hemolyze. As reported by DeMeio,¹² RBC swelling by tellurite is accelerated by the presence of reduced glutathione (GSH). No swelling or hemolysis takes place if RBC with decreased GSH levels are incubated with tellurite, but hemolysis does occur when GSH is added externally to these cells.

Figure 1B shows the effect of temperature on the swelling of RBC by 150 μmol/L tellurite. Incubation of RBC with tellurite at 0°C and 22°C produced no increase in MCV over time. However, the MCV values of RBC incubated with tellurite at 37°C increased nearly 70% as compared with control cells incubated at 37°C.

Because selenite also causes hemolysis of RBC,¹³ we examined the effect of different concentrations of selenite on MCV. As shown in Fig 1C, selenite
increases MCV of RBC in a fashion similar to that of tellurite, but with a smaller degree of effect.

To study the reversibility of the tellurite effect, RBC treated with tellurite were washed and incubation continued. Figure 2 shows that the basic course for cell swelling is not eliminated by washing, suggesting that cell volume increases are mediated by an irreversible interaction of tellurite with the membrane.

Tellurite increases Na influx many fold and produces a slight increase in K leak. To investigate if the increase in the Na and/or Cl influx is essential for RBC swelling by tellurite, RBC were incubated in solutions free of either Na and/or Cl. As shown in Fig 3, tellurite caused an increase in $W_c$ in each ionic solution. Incubation of RBC in a nonionic, isoosmolar sucrose solution displayed a smaller increase in $W_c$. Control samples showed no major change in $W_c$ values during this incubation period.

It has been suggested that an effective means of decreasing the rate of sickling would be to decrease the MCHC. The ability of tellurite to effect cell swelling and thus lower MCHC was investigated as a means of inhibiting sickling in vitro. Samples of HbSS RBC were incubated with various concentrations of tellurite and subjected to deoxygenation. Inhibition of sickling by tellurite was compared to sickling in controls under similar anaerobic conditions. Figure 4 demonstrates a significant inhibition of sickling at tellurite concentrations as low as 10 $\mu$mol/L.

**DISCUSSION**

Because of the strong dependence of the rate of polymerization of deoxy-HbS on its concentration, MCHC is assumed to play an important role in sickling of RBC in patients with sickle cell disease. The delay time prior to gelation of deoxy-HbS is inversely proportional to the 30th power of the deoxy-HbS concentration. In fact, osmotic shrinkage of HbSS erythrocytes markedly promotes RBC sickling. One way to decrease the MCHC is the infusion of hypotonic saline or distilled water. These attempts, however, cannot keep the MCHC reduced for a long period, as water is rapidly excreted from the kidney. Rosa et al used an antidiuretic hormone and a low sodium diet to reduce plasma osmolarity and thereby decreased MCHC. Although this method had some clinical success, it is difficult to control plasma sodium within a safe level.

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agent that induces RBC swelling under isotonic medium conditions. Incubation of RBC with higher than 100 μmol/L cetiedil causes a net influx of cations and increases Wc, thus swelling the RBC. The degree of swelling depended on the cetiedil concentration, and the swelling stopped after reaching certain levels. At higher concentrations of tellurite (>500 μmol/L), there is a large and rapid increase in MCV (Fig 1A). The increase in MCV was not as great at lower concentrations and began to level off, as shown in Fig 2. In addition, the tellurite effect was not eliminated by washing. The requirement for GSH and the loss of intracellular bases of therapeutic approaches. J Clin Invest 69:589, 1982

The degree of cell swelling induced by tellurite is much greater than that of cetiedil. The inhibition in shrink even in the absence of tellurite due to the net loss of Cl. This is related to some enzyme activity. Experiments in different media (Fig 3) showed that tellurite-induced swelling occurs even in an isoosmotic sucrose solution in which RBC shrink in the absence of tellurite due to the net loss of Cl. This is the major difference between the effects of cetiedil and tellurite, as RBC incubated in isoosmotic sucrose shrink even in the presence of cetiedil. In addition, the degree of cell swelling induced by tellurite is much greater than that of cetiedil. The inhibition of the tellurite effect by SITS (DeMeio, Shibutani, and Asakura, in preparation) suggests that tellurite may affect the anion transport system.

The net effect of tellurite, which is a decrease in MCHC, appears to be identical to that of the other membrane-acting agents, even though the way by which water influx is induced differs. These studies represent an investigation of the antisickling effect of tellurite in vitro. Extensive investigations, including animal experiments, of tellurite's effect in vivo are necessary before its clinical use could be considered. However, these studies on tellurite and other membrane-acting compounds are important in understanding the mechanism involved in antisickling agents that exert their effect by inducing a reduction in MCHC.

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