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Use of piracetam improves sickle cell deformability in vitro and in vivo

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SUMMARY Microsieving diluted suspensions of oxygenated sickle cell anaemia (HbSS) cells on polycarbonate filters shows that piracetam improves the red cell deformability in vitro. In vivo an oral intake of 160 mg/kg/day divided in four doses enhances the HbSS cell deformability as actively as it does in in vitro experiments. The drug is also able partially to restore the impaired deformability of physiologically deoxygenated HbSS cells. These findings are consistent with the results of clinical trials, which show that continuous treatment with piracetam reduces the incidence of vaso-occlusive crises in patients with sickle cell disease.

Piracetam (2-oxo-pyrrolidine acetamide) has been used over the past decade for the management of psychosenescent syndromes. The drug was tentatively suggested by Targino de Araujo and Nero[1] for the management of sickle cell disease, but their views remain unacknowledged.

Recent clinical trials conducted by Skondia[2] and ourselves[3] showed that piracetam taken orally (160 mg/kg/day, divided in four doses) was a prophylactic treatment for sickle cell vaso-occlusive crises. With maintenance treatment, the number of crises observed was reduced to about one fifth of what would be expected without piracetam.

In this paper we studied the effect of piracetam on sickle cell anaemia (HbSS) red cell deformability in vitro and in vivo by microsieving at pharmacologically obtainable serum concentrations.

Material and methods

All tests were carried out on fresh venous blood anticoagulated with 2 mg/ml of potassium ethylenediamine-tetraacetate.

Fifteen healthy black subjects with haemoglobin genotype HbAA were studied as controls. Of these, four were children under 10 years of age and 11 were adults aged from 25 to 36 years. The group of seven healthy blacks with haemoglobin genotype AS included four children (6 to 15 years) and three adults.

The group of 15 patients with sickle cell disease included 14 children from 1 to 15 years and one 23 year old woman. All were of the HbSS genotype, except one 10 year old child who had a sickle cell β thalassaemia. All the patients were in the steady state and studied as outpatients; they had been asymptomatic for at least three weeks before testing.

Haemoglobin genotype was determined by electrophoresis on cellulose acetate using Tris-buffer (pH 8·9). In each carrier of haemoglobin S the following tests were performed: demonstration of sickling by sodium dithionite; percentage of fetal haemoglobin by the "one minute residue" method of Singer; and percentage of A_2 haemoglobin by elution of an electrophoresis strip. The parents of each homozygous patient were similarly studied.

MEASUREMENT OF ERYTHROCYTE DEFORMABILITY

A sample of 10 ml of venous blood was collected in edetic acid. The filtration experiments were performed within two hours of sampling. After centrifugation at room temperature plasma and buffy coat were discarded and the red cell fraction was washed three times with two volumes of Ringer solution (sodium chloride 147 mmol/l, potassium chloride 2·5 mmol/l, calcium chloride 2·5 mmol/l, human albumin 0·5 g/dl, pH adjusted to 7·4 with 0·3 M Tris). After the final washing red cells were suspended again in the buffered Ringer solution and the haematocrit adjusted to 1%.

The number of red cells was checked by means of an electronic counter. It varied from 100 000 to 180 000 cells per μl. The number of white cells did not exceed 200 per μl.

Piracetam (concentrated 200 g/l: 1·4 mol/l buffered

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solution of Nootropil for clinical use) was added to 10 ml aliquots of the red cell suspension to obtain 0·05, 0·1, 10, and 100 mmol/l. All the cell samples, with or without piracetam, were incubated at 37°C for 30 minutes in open glass test tubes under air and with gentle shaking before starting the filtration experiments.

The condition of peripheral capillary blood was simulated as follows. An aliquot of 10 ml of red cell suspension (haematocrit 1%) was gently bubbled for 30 minutes using a gas mixture of nitrogen 96% and oxygen 4%. The procedure was expected to reduce the partial oxygen pressure to about 30 mm Hg, which is the partial oxygen pressure in the capillary. Immediately after bubbling the suspension of deoxygenated red cells was anaerobically transferred into the filtration device and filtered without delay.

APPARATUS AND FILTRATION PROCEDURE

The apparatus described by Reid *et al*[4] was used. All the experiments were performed with a 25 mm nuclepore filter of 5 μm mean pore diameter, pore density of $4 \times 10^5/cm^2$, the coefficient of variation in the pore diameter being 5·4%.[4]

After filling the dead space of the assembled filter holder with the cell suspension the holder was mounted on the reservoir and a 5 ml graduated plastic syringe without its plunger mounted on top of the filter. The graduated syringe was filled with the cell suspension up to the 5 ml mark. The reservoir under the filter was put on a negative pressure of 20 cm of water by opening a tap. A stopwatch was started at the same time. The time needed for filtering exactly 5 ml of fluid was used to measure the flow rate through the membrane filter. A new filter of the same batch was used for each measurement, and only filters with a flow rate of 27–30 ml/minute were used. For each determination of index of red cell deformability (IRCD) the respective flow rates of the Ringer solution and of the red cell suspension were successively measured on the same filter.

The IRCD, ranging from 0 to 1, was calculated as follows:

$$\text{IRCD: } \frac{\text{Flow rate of red cell suspension ml/minute}}{\text{Flow rate of Ringer solution ml/minute}}$$

Repeated measurements on the same sample showed that the method was reproducible with a coefficient of variation of about 5%. The results were statistically evaluated using Student's *t* test.

Results

In a first series of experiments the IRCD was measured without added piracetam. The IRCD and mean (SD) of normal controls (HbAA: 0·92 (0·097)) and heterozygous patients (HbAS: 0·92 (0·073)) were similar. The IRCD and mean (SD) of homozygous HbSS patients was 0·47 (0·13). The difference between the means of HbAA controls and patients with sickle cell anaemia was highly significant ($p < 0·001$).

In a second series of experiments (table 1) the influence of incubation with piracetam (0, 0·5, 1, 10 and 100 mmol/l) on the cells of both genotypes HbAA and HbSS was tested. The IRCD of normal HbAA subjects was not modified by piracetam, but the IRCD of HbSS patients progressed towards normal values after incubation with 0·5–1 mmol/l piracetam. Higher concentrations of piracetam did not improve the gain obtained with 0·5–1 mmol/l (table 1).

At this stage we investigated whether the drug improved the deformability of the sickle cells in vivo and to what extent. For this purpose, the IRCD of HbSS patients receiving the drug orally (160 mg/kg/day divided in four doses) was measured directly on samples of venous blood collected within six hours after the last dose had been given. Piracetam given orally actually improved the IRCD in HbSS patients ($p < 0·001$) and was effective both in vivo and in vitro (table 2).

For the sake of simplicity, all the in vitro experiments were done under air—that is, at about 150 mm Hg partial oxygen pressure, and it was therefore important to see how the drug influenced deoxygenated HbSS cells. Comparative assays were done on five individual samples, both at atmospheric oxy-

Table 1 *Effect of piracetam in vitro on IRCD of HbAA and HbSS subjects*

	Without piracetam	*With piracetam (mmol/l)*			
		0·5	*1*	*10*	*100*
HbAA					
No tested	15	12	15	10	15
Mean (SD) IRCD	0·92 (0·097)	0·95 (0·04)	0·93 (0·06)	0·95 (0·047)	0·93 (0·07)
HbSS					
No tested	15	9	15	15	8
Mean (SD) IRCD	0·47 (0·13)	0·83 (0·1)*	0·81 (0·17)*	0·84 (0·1)*	0·82 (0·1)*

*Student's *t* test $p < 0·001$.

Table 2 *Comparison of effect of piracetam on IRCD of HbSS cells in vitro and in vivo*

	IRCD		
	Control	*In vitro*	*In vivo*
No tested	15	15	12
Mean (SD) IRCD	0·47 (0·13)	0·81 (0·17)*	0·84 (0·09)*

*Student's *t* test $p < 0.001$.

gen pressure and at reduced partial oxygen pressure, either without or with piracetam (table 3).

At low oxygen pressure, the IRCD of HbSS cells was reduced, considerably. This phenomenon indicated that the flow through the pores of the filter almost completely stopped. The addition of piracetam to the incubation medium partially prevented the flow from slowing down. Nevertheless, the IRCD values of the deoxygenated sickle cells remained lower than the IRCD values of oxygenated sickle cells treated in a similar manner by piracetam.

In control experiments the red cells of healthy subjects of the HbAA genotype retained unchanged IRCD values when deoxygenated, as described above. In healthy subjects of the HbAS genotype the IRCD values of the deoxygenated cell suspension also remained almost completely unchanged.

Discussion

Piracetam (2-oxo-pyrrolidine acetamide, $C_6 H_{10} N_2 O_2$, molecular weight 142·15) is a substance which is quite soluble in water and easily diffusable in the tissues. It is rapidly absorbed after oral administration. Maximum plasma concentrations are achieved in about 30 minutes. In man piracetam is not metabolised and is rapidly excreted unchanged in the urine. The average plasma half life is four and a half hours.[5] In the adult an oral dose of 9·6 g/day divided in three doses (160 mg/kg/day) results in a peak of 121 (36) μg/ml after 30 minutes. With regular oral intake three times a day, the serum concentration fluctuates between 0·3 and 0·9 mmol/l; concentrations higher than 1–2 mmol/l are only occasionally reached by oral intake, but easily by parenteral route. The drug is very diffusible; the volume of distribution in the human body averages 0·64 l/kg.[6] It quickly enters the red cells (Leyssen MH, Grossens MF, Verwilghen RL, personal communication).[7]

Table 3 *Effect of piracetam (mmol/l) in vitro on IRCD of HbSS cells at high and low oxygen partial pressure*

Case No	*Piracetam (oxygen 155 mm Hg)*		*Piracetam (oxygen 25–30 mm Hg)*	
	0	*1*	*0*	*1*
1	0·58	1	0·27	0·36
2	0·58	0·7	0·01	0·02
3	0·38	0·9	0·09	0·66
4	0·45	0·9	0·15	0·59
5			0·36	0·67
Mean	0·43	0·87	0·18	0·44
SD	0·07	0·12	0·14	0·26

Piracetam has been used extensively over the past decade for the management of the psychosenescent syndrome. It seems to be devoid of severe reactions and toxic effects. Apart from its supposed intrinsic action on the nervous cells, the drug has effects on blood rheology,[6] which were discovered recently, Nalbandian *et al*[7–9] reported that piracetam had several previously unknown actions on the blood cells. Piracetam seems to be a multitargeted drug, capable of improving the microcirculation by suppressing platelet activity in vivo[10 11] by enhancing red cell deformability[12] and by reducing the adherence of damaged erythrocytes to endothelial cells.[13] The drug has been reported to be effective for the treatment of patients with sickle cell disease both during crises and as maintenance treatment[1–3 7–9]

In this report microsieving on polycarbonate membranes was the method chosen to study the influence of piracetam on sickle cell deformability. Microsieving is actually the most available and widely used technique for measuring the HbSS red cell deformability,[14] either in steady state or during a sickle cell crisis.[15] As recommended by Chien,[16] we filtrated the red cells properly. The cells were suspended in an isoosmotic buffered Ringer solution, enriched with serum albumin (0·5 g/dl) to preserve the cell hydration and to avoid crenation. Prior elimination of the buffy coat reduced possible obstruction of the pores by leucocytes. The red cell concentration was adjusted to a constant level. High dilution minimised cell-cell interactions and prevented early saturation of the microsieve.

Our findings in filtrating HbAA-AS-SS cells agree with those of Chien,[14] Usami *et al*,[17] and Lessin *et al*.[18] These authors, using more elaborated pressure flow filtration systems, showed that red cells of the genotypes HbAA and AS remain roughly identically deformable either when oxygenated or at physiologically reduced oxygen pressure. On the other hand, they observed that HbSS cells, with an already reduced deformability when oxygenated, behave like rigid cells when deoxygenated. Microsieving is also a valuable and sensitive technique for investigating the pathogenesis of sickle cell crisis. Kenny *et al*[15] showed that a primary loss of deformability occurs during the early stages of the sickle cell crisis. As the rheological properties of HbAS and HbSS cells assessed by microsieving match the clinical observations, microsieving seems to be an appropriate way of studying

drug action on sickle cells.

Our experiments have shown that piracetam (1 mmol/l), multiplies the IRCD values of oxygenated HbSS cells by a factor of 1·7. As the reduced deformability of oxygenated HbSS cells cannot be principally ascribed to their low content in viscous deoxy-HbS it is likely that piracetam is more effective in improving the membrane flexibility than in reducing the viscosity of the deoxygenated HbS solution within the cell. This view is consistent with the findings of Asakura *et al*,[19] who in their investigations into the chaotropic properties of the drug, showed that high piracetam concentrations (100 to 600 mmol/l) were needed for 50% sickling inhibition in vitro and for prolongation of the gelation time in deoxygenated HbS solutions. Similarly, from our preliminary experiments on filtration of sickled cells in the presence or absence of piracetam, it seems that the chaotropic desickling activity of the drug in the range of the pharmacologically obtainable serum concentrations, is rather poor.

The mechanism of action of piracetam on the viscoelastic properties of the red cell membrane is not known. When studying spectrin by gel filtration chromatography and measuring tetrameric (molecular weight 920 000) and dimeric (molecular weight 460 000) spectrin concentrations, Vincentelli *et al*[20] found that dimeric spectrin was enhanced by 25% when 1 mmol/l piracetam was present. A possible site of action of piracetam could therefore be the spectrin and actin network, which has a major role in the maintenance of red cell shape and plasticity.

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