THE PENTOSE PHOSPHATE PATHWAY IN CYSTIC FIBROSIS ERYTHROCYTES

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SUMMARY

Glucose-6-phosphate dehydrogenase (G6PD), NADP and NADPH were compared in red blood cells (rbc's) of cystic fibrosis (CF) patients and sex and age matched controls. G6PD activity was higher in CF rbc's ($P = .03$). NADPH/NADP ratios were decreased in CF rbc's ($P= .003$). These findings are viewed in terms of an increased cellular need for NADPH generated by the pentose phosphate pathway (PPP). They indicate also that in terms of NADP CF rbc's are in a more oxidized state than are non CF rbc's.

Despite intensive study and recent observations of Spock et al.[1] Mangos et al.[2], Danes and Bearn[3], Johansen et al.[4], and Bowman et al.[5], the biochemical basis of cystic fibrosis (CF) is obscure. The only clinically secure phenomenon is failure of reabsorption of sodium in the duct of the sweat gland.[6] Recently, one of us described a cytological relationship between enzyme systems of the pentose phosphate pathway (PPP) and sites of active ion transport in rodent salivary glands.[7] Using histochemical methods, intracellular localization of the PPP was found in sites in which active ion transport occurs.[8,9] It was speculated that the reduced nicotinamide-adeinine dinucleotide phosphate (NADPH) generated by the PPP may be involved in active ion transport. Since salivary glands are classically involved in CF and because a defect in active ion transport has been implicated we chose to examine NADP and NADPH levels and glucose-6-phosphate dehydrogenase (G6PD) activity in cells of CF patients. Red blood cells (rbc's) were used because of their accessibility and because in CF they exhibit ion transport abnormalities.[10] We report what appears to be a clear difference in the ratio of reduced to oxidized NADP (NADPH/NADP) and in G6PD activity between rbc's from individuals with CF and their controls.

METHODS

We assayed for NADP, NADPH and G6PD in rbc's obtained from 10 pairs of CF patients and non CF controls. Diagnosis of CF was based on repeated elevated sweat chloride tests and clinical findings. All pairs were matched for sex. Matching for age was approximate (Table). Blood from pairs was obtained within the same hour, and prepared and assayed at the same time.

Approximately 5cc's of venous blood were collected into heparinized test tubes from each subject. Within one hour the blood was centrifuged at 4,000 rpm's (1935 g) for 10 minutes. The plasma and buffy coat were removed. The rbc's were washed twice with 3 volumes of cold saline and again centrifuged at 4,000 rpm for 10 minutes. The supernatant was removed and the rbc's mixed. 0.2ml were used for each of the assays. Cysteine was added to the 0.2ml used for the NADPH assays to a final concentration of 0.5mM. For NADP, rbc's were extracted with 0.3N $HClO_4$ and neutralized with 3N KOH to pH 7.8; for NADPH, rbc's were extracted with 0.25N alchoholic KOH, heated for one minute at 70^{0} C and then neutralized with 3N $HClO_4$ to pH 7.8.[11,12] Concentrations of oxidized and reduced NADP were estimated by an enzyme assay which couples specific dehydrogenases and non-specific diaphorase and measures the rate of reduction of 2,6-dichlorophenol-indophenol. The estimates were based on reduction of the dye by measuring the decrease in absorption at 600mμ in rbc's treated with G6PD, glucose-6-phosphate and diaphorase.[12] G6PD activity was estimated by measuring the increase in NADPH as determined by the change in optical density at 340mμ in rbc's treated with glucose-6-phosphate and NADP.[13] Each assay was run in duplicate and the mean value recorded.

RESULTS

The data for each CF patient and his control are given in the Table. No differences between the CF and control groups for absolute values of NADP or NADPH were noted. In 8 of 10 pairs G6PD activity was greater

NADP, NADPH and Glucose-6-Dehydrogenase in Erythrocytes

of Cystic Fibrosis Patients and Controls

	Control					Cystic Fibrosis				
Sex	Age	NADPH[1]	NADP[1]	NADPH/NADP	G6PD[2]	Age	NADPH[1]	NADP[1]	NADPH/NADP	G6PD[2]
M	10	26.88	9.38	2.86	140	14	29.12	11.47	2.50	180
F	24	25.63	6.56	3.90	166	24	25.80	9.37	2.74	174
M	12	27.5	7.5	3.67	174	7	26.30	10.3	2.55	166
F	19	35.75	10.2	3.50	140	17	32.13	8.6	3.72	203
F	17	23.75	6.8	3.48	155	7	20.80	8.15	2.55	145
M	25	26.65	8.98	2.97	126	11	30.2	10.25	2.93	243
F	18	28.80	9.4	3.07	141	7	24.3	8.8	2.76	145
M	28	20.25	6.77	3.0	126	18	18.2	7.38	2.47	135
M	33	29.8	10.37	2.92	120	20	20.63	9.57	2.16	126
M	22	18.1	7.53	2.4	141	14	25.0	13.0	1.92	188

[1] 10^{-3} μmoles/100 ml packed rbc's.

[2] units of enzyme activity/100 ml packed rbc's.

matched pair t (NADPH/NADP) = 3.828, P = .003

matched pair t (G6PD) = 2.196, P = .03

in the CF rbc's. In 9 of 10 pairs the NADPH/NADP ratio was lower in CF rbc's. These data were tested by matched pair t tests.[14] The probability of obtaining the results in the Table if in fact no difference between CF patients and their controls existed is P = .03 for G6PD and P = .003 for NADPH/NADP. In the Table it can be seen also that ages of controls were somewhat greater than their CF counterparts. Regressions of each of the assays on age were plotted. No correlation between age and assayed values was apparent in either group.

DISCUSSION

The intracellular localization of enzyme systems of the pentose phosphate pathway (PPP) coincides with sites of active ion transport in several tissues including salivary glands. These cytologic associations together with other data led us to speculate that NADPH generating systems are involved in active ion transport. If the PPP or one of its components is involved in metabolically dependent ion transport and if abnormal ion transport is a primary defect in CF then PPP enzyme systems may be abnormal in CF. Furthermore, rbc's of CF patients have been shown to possess decreased sodium pumping.[10] Accordingly, we compared NADP and NADPH levels and G6PD activity in rbc's of CF patients with age and sex matched non CF individuals. NADPH/NADP ratios were clearly decreased in CF rbc's. A relative decrease in NADPH might suggest its decreased generation by the PPP. However G6PD activity was higher in CF rbc's. Coexistent decreased NADPH/NADP ratios and increased G6PD activity in CF rbc's may be viewed in terms of an increased cellular need for NADPH generated by the PPP. This notion of increased NADPH-dependent reductions in CF rbc's is supported by significantly increased NADPH-dependent glutathione reductase activity in CF rbc's (unpublished findings). The role of NADPH as a source of hydrogen for synthesis of phospholipids and other membrane components speaks to the possible membrane defect in CF. Moreover transposition of hydrogen atoms between NADP and NADPH during oxidoreductions

represents a potential mechanism for energy transfer from its source to the site of the ion pump.[15,16] The differences from normal in PPP and NADP may thus be related to the ion transport defects in CF.

A further association between our findings and CF is the intracellular localization of PPP enzyme systems in olfactory nasal mucosa and the role attributed to them[17] and reports of enhanced olfactory perception in CF.[18]

Such speculations aside we conclude that: (1) G6PD activity is increased in CF rbc's; (2) in terms of NADP CF rbc's are in a more oxidized state than are control cells; and (3) these findings suggest an increased need for reductive power supplied by NADPH in CF rbc's.

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