Table. The results of 5 studies of different doses of alglucerase in Gaucher disease

Authors	Barton et al.	Barton et al.	Pastores et al.	Figueroa et al.	Zimran et al.
# of Patients	12	10	33	14	29
Dose (U/kg/4 wk)	120	20	60–120	27.6	27.6
Frequency/4 wk	2	2	2	12	12
Duration (mo)	6–9	6	6–9	6	6
Pt Age (yr ± SD)	20.3 ± 13.5		32 ± 17.1	30.2 ± 16	25.1 ± 14.6
Haemoglobin (Δg/dl)	+3.0 ± 1.35	+1.0	+1.6 ± 1.4	+1.3 ± 1.1	+1.0 ± 1.1
Platelets (1000s)	+31	+9	N/A	+78	+24.8
Liver Size (Δ%)	−17.8	−5.7	−22.1	−23.4 ± 3.7	−16.7 ± 13.4
Spleen Size (Δ%)	−33	−11.7	−32	−23.9 ± 7.3	−25.1 ± 15.9

Modified from Zimran et al., Am. J. Med. *97* (1994) 3.

glucocerebrosidase, which is needed for the enzymatic degradation of complex lipids, globosides and gangliosides. In the absence of sufficient glucocerebrosidase activity, the catabolic product glucocerebroside accumulates.

We have cloned the cDNA and the gene coding for glucocerebrosidase. Over 40 different mutations that decrease glucocerebrosidase activity have been documented. Among Ashkenazi Jews, a people in whom Gaucher disease is relatively common, one of these, *1226G(N370S)*, accounts for 75% of the mutations and a second mutation 84 GG for an additional 12%. Altogether five mutations account for over 97% of the deficient mutant alleles among Ashkenazi Jews. Haplotypic markers are produced in this gene complex by polymorphisms in the glucocerebrosidase gene and in the closely adjacent liver type pyruvate kinase gene. The common Jewish mutations are each always found in context of the same haplotype, a finding that suggests a single common origin for each of the mutations and implies an as-yet-unknown selective advantage conferred by glucocerebrosidase deficiency genes upon heterozygotes. The type of mutation inherited is correlated with the disease phenotype, but there is much variation within genotype, and much still needs to be learned about the factors that affect disease severity.

Because the Gaucher disease phenotype is a result of abnormal storage in macrophages, the concept of introducing exogenous enzyme into these cells as a treatment modality has long appeared attractive. Although varying degrees of success attended such therapeutic efforts in the 1970's, the implementation of enzyme replacement therapy had to await the large scale commercial production of the enzyme. This enzyme is modified by deglycosylation to expose inner mannose moieties with the idea of targeting it to macrophage. We have shown that in reality it is not targeted to macrophages, and that only a minute fraction of the large amount infused reaches these cells at all. However, for reasons that are not entirely clear, treatment with this enzyme is effective in reducing the liver and spleen size of patients with Gaucher disease and improving their peripheral blood counts. Moreover, the usually recommended doses of this very expensive enzyme (US$ 400'000 per year per 70 kg patient), are 5 to 10 times as large as those required to give an optimal therapeutic response. The table summarises the data on the effectiveness of different dose schedules in the treatment of this disease. Gaucher disease may be considered to be a disorder of hematopoietic stem cell; the macrophage, which is responsible for the disease phenotype, is a progeny of this cell. However, since there is no cell-to-cell correction in Gaucher disease, marrow ablation would be necessary for gene transfer to be clinically successful. From this point of view, systemic administration of genes, using a vector such as cationic liposomes may prove to be a more practical means for the ultimate correction of the disease.

The chloride effect in human hemoglobin: A new kind of allosteric mechanism

M. F. Perutz

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (Great Britain), Fax + 44(223)213556

Abstract

In human hemoglobin hydrogen ions, chloride, 2,3-diphosphoglycerate and CO_2 cooperate to shift the oxygen equilibrium curve to the right. Bovine hemoglobin, by contrast, has an intrinsically low oxygen affinity: when stripped, it is as low as that of human hemoglobin in the presence of 0.1 M NACl + 0.1 M DPG.

G. Fermi and I tried to determine the chloride binding sites in bovine deoxy hemoglobin by collecting X-ray data of crystals in polyethylene glycol with 50 mM $Na_2HPO_4 \pm 0.1$ M NaCl or with HEPES ± 0.1 M NaBr. We found no halide binding sites in either medium. When told of this result, A. Arnone collected X-ray data from human deoxy hemoglobin crystals in polyethylene glycol ± 0.3 M NaF, NaCl or NaBr. He found a weak Br^- binding site near Val 1β, but no chloride binding site.

On the basis of earlier experiments by J. Manning, the Bonaventuras, J. Kilmartin and myself it was clear that chloride binds in the central, water filled cavity of hemoglobin which widens on loss and narrows on uptake of oxygen. The cavity contains 8 pairs of cationic and 3 pairs of anionic amino acids. The Bonaventuras proposed some years ago that electrostatic repulsion by the excess positive charges in the cavity destabilises the T-structure and therefore raises the oxygen affinity.

On the basis of all these results and ideas, I argued that widening of the cavity on deoxygenation allows additional chloride ions to diffuse in the cavity and to stabilise the T-structure by neutralising the excess positive charges. Earlier experiments had shown that neutralisation of only two pairs of charges, Val 1α and Lys 82β was sufficient to inhibit the chloride effect. I now argued that neutralisation of **any** one pair of positive charges in the cavity should halve the chloride effect, neutralisation of **any** two pairs should inhibit it and introduction of additional ones should enhance it.

D. Shih, D. Williamson and I found a series of abnormal human hemoglobins which confirmed that this is indeed the case. On the other hand, neutralisation of any pair of **external** positive charges did not diminish the chloride effect.

These findings suggested that abnormal human hemoglobins with fewer positive charges in the central cavity should have an intrinsically lower oxygen affinity than hemoglobin A and vice versa. A survey of all substitutions reported in the literature shows this to be true, with a few exceptions due to special stereochemical effects. In human hemoglobin half of the Bohr effect is inhibited in the absence of chloride. This chloride dependent Bohr effect is absent when two pairs of positive charges have been removed from the central cavity, which shows that the effect must be due to the raising of the pK_a of cationic groups in the cavity by chloride.

Regulation of the oxygen affinity by electrostatic interactions with diffusible anions not bound to any specific site represents a new kind of allosteric mechanism.

Scapharca dimeric hemoglobin: A new mechanism of information transfer between globin chains

E. Chiancone

CNR, Center of Molecular Biology, Dept. of Biochemical Sciences 'A. Rossi-Fanelli', University 'La Sapienza', I-00185 Rome (Italy), Fax +39(6)4940543

Abstract

The homodimeric hemoglobin component present in the red cells of the bivalve mollusc *Scapharca inaequivalvis*, HbI, is endowed with high cooperativity in ligand binding[1]. This behaviour is in contrast with that of vertebrate hemoglobins in which cooperativity is associated with a tetrameric assembly and the presence of two types of chain. Analysis of the aminoacid sequence[2] and immunological data[3] suggested that the assembly of HbI differed from that characteristic of vertebrate hemoglobins and hence that cooperativity had an unusual structural basis. Indeed the X-ray structures of the carbonmonoxy and deoxy derivatives at 2.4Å resolution showed that in HbI the heme carrying E and F helices are not exposed to solvent as in the vertebrate hemoglobin tetramer, but form the subunit interface and bring the two heme groups practically in direct contact through a network of hydrogen bonds[4]. Ligand binding brings about marked structural changes that are limited to the heme environment, whereas quaternary changes are only minor. The structural changes in the heme environment result in alterations in the network of interactions between the heme groups which lead to changes in ligand affinity[5,6]. In HbI therefore cooperativity in ligand binding is achieved through direct heme-heme communication as opposed to the long range information transfer operative in the vertebrate hemoglobin tetramer.

The direct communication between hemes is reflected in a number of properties with distinctive characteristics relative to vertebrate hemoglobins: information transfer between the hemes occurs in times no longer than a few nanoseconds, about 1000 times faster than in vertebrate hemoglobins[7]; the ligand-linked conformational changes take place with a half-time around 1 μs, a time regime characteristic of tertiary structural changes in mammalian hemoglobins[8,9]. In