Treating genetic disease

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The treatment of genetic disease has developed over the past thirty years, and includes: the classical approach, which aims to prevent organ damage from excessive amounts of metabolites generated by the metabolic defect or which adds substances that are not synthesised; enzyme replacement therapy, which attempts to introduce a specific enzyme with a normal configuration into organs where the enzyme is missing or malfunctioning because of structural abnormalities; the transplantation of cells, especially bone marrow cells, and whole organs; and somatic gene therapy. The clinical outcomes of attempts to treat genetic disease have only been partially successful. More developmental work is required before the therapies can be said to be effective, especially in the prevention of brain damage.

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Introduction

A series about inherited metabolic disorders is not complete without addressing the options for treatment. The principles of such treatments "have followed logically from a number of factors, (i) a better understanding of the nature of the fundamental problem, (ii) the pleiotropic effects of the problem, which result in altered metabolism, (iii) the consequences for the pathogenesis of the clinical manifestations of disease," to quote L Nyhan.[1] This article summarises the substantial progress that has been made in treating genetic disease over the past four decades, beginning with the dietary treatment of classical phenylketonuria in the early 1950s.[2]

Classical forms of treatment

The classical approaches include,[1] (i) the restrictive intake of specific dietary components to diminish the metabolic load on defective metabolic steps, e.g. restricted phenylalanine intake in phenylketonuria and of the branched-chain amino acids isoleucine, leucine, and valine in maple syrup urine disease; (ii) the binding of metabolites "blocked" up-stream of the metabolic hindrance. For example, in urea cycle defects with diminished elimination of the immediate precursor to the cycle, ammonium, orally administered sodium benzoate can be used to react with glycine, forming hippuric acid, which can be excreted in the urine. By this mechanism, the urea cycle can be bypassed, preventing the generation of toxic amounts of ammonium; (iii) the oral replacement of enzymic cofactors in hereditary conditions, where the synthesis of the cofactor is affected, e.g. biopterin in tetrahydrobiopterine deficiency or biotine in biotinidase deficiency; (iv) modification of the activity of the malfunctioning enzyme, e.g. allopurinol to inhibit xanthine oxidase and prevent uric acid being formed in gout; (v) the use of chelating agents, classically of penicillamine for the elimination of copper in Wilson's disease, and cystine in cystinuria. These forms of treatment have been surprisingly successful, given the complexity of the biochemical situation. Good examples include phenylketonuria, where the carefully monitored restricted intake of dietary phenylalanine will ensure that the affected child will reach his/her intellectual potential, and the treatment of gout by allopurinol. The disadvantage is the difficulty in maintaining life-long treatment, again exemplified by phenylketonuria, when previously well-managed patients have reached adolescence and rebelled against the treatment.[3]

Enzyme replacement therapy

A second alternative treatment is enzyme replacement therapy,[4] which has been tried in lysosomal storage disorders including the mucopolysaccharidoses, Tay-

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Sachs disease (lack of hexosaminidase A), Gaucher's disease (lack of β-glucosidase), and lately, in severe combined immunodeficiency disease (lack of adenosine deaminase). Treatment rests on the principle that an enzyme, having been administered intramuscularly or intravenously, is taken up by a specific target cell and transported to its intracellular site, e.g. to the lysosomes. The efficiency of enzyme replacement therapy is not very high. The purified enzymes, after having been injected intravenously, are rapidly taken up by cells other than the target cells, or destroyed by the target cells themselves, and immunogenic reactions have been observed. As a result, research into this form of therapy was abandoned for almost a decade. It has recently had a renaissance, with the discovery that modified enzymes with specific carbohydrates attached to the enzyme protein (as for DNA vectors) could increase the uptake into specific target cells and inhibit uptake by other cells. Adenosine deaminase linked to polyethylene glycol (PEG) is eliminated at a much slower rate from the circulation than the native protein, and clinical effects of treatment with the modified enzyme for severe combined immunodeficiency are promising.[5] Modified enzymes may also prove to be less immunogenic.[5]

Transplantation of cells and organs

The third form of treatment involves transplantation of normal cells and organs.[6,7] Cells in bone marrow transplants can replace defective lymphocytes in severe combined immune deficiency (SCID), Wiskott-Aldrich syndrome (an X-linked, recessive condition with abnormal T lymphocytes, thrombocytopathy, and eczema) and thalassaemia—all defects in the haematopoeitic system. The success of treatment has been varied—excellent in SCID, and encouraging in the other conditions, where problems with previous blood transfusion and graft versus host disease (GVHD) have been encountered.

At least two other cell types, the Kupffer cells in the liver, and the microglial cells in the central nervous system, originate in the bone marrow and are continuously being replaced by cells from the bone marrow.[8] The Kupffer cells live in symbiosis with the hepatocytes and there is exchange of proteins including enzymes between the cells. Therefore, abnormally functioning Kupffer cells in many types of mucoplysaccharidosis will be replaced with normal cells after bone marrow transplantation. As a result, normal enzyme proteins are synthesised, transported into the hepatocytes, and taken up by the lysosomes, reversing the biochemical and clinical expressions of the gene defects. The clinical effect is fairly rapid, as the turnover and hence the replacement of Kupffer cells is rapid.[9]

Microglial cells in the central nervous system also exist in a symbiotic relationship with neurones and other brain cells. The turnover of microglial cells is much lower than that of Kupffer cells, and consequently abnormal glial cells in brain tissue are replaced by normal cells from transplanted bone marrow cells at a much lower rate than are abnormal Kupffer cells. The outcome has been used to attempt bone marrow transplantation in children with Krabbe's infantile globoid cell dystrophy, with encouraging results. This is vindicated by the outcome after bone marrow transplantation in a patient with Krabbe's disease, where no clinical improvement and normalisation of biochemical abnormalities was noticed until one year after the transplantation.[10]

Liver transplantation has been used successfully to treat patients with tyrosinosis and α_1-antitrypsin deficiency, as the genetic defect is specifically expressed in that organ.[6] This form of treatment is restricted by the nature of the surgical intervention involved and by a substantial shortage of donor organs. Immunosuppressive treatment is required both for the transplantation of cells and organs and long term success is dependent on close monitoring of the drug therapy used to avoid GVHD.

Gene therapy

The mapping of the entire human genome will soon be complete. Increasingly sophisticated methods have been introduced to investigate the genetic make-up of individuals and their families. These developments enable the diagnosis of specific mutations that lead to disease and for their repair, via gene therapy. The principle sounds simple enough. Mutant genes need to be replaced by normal genes that can code for functional proteins.

Germ line gene therapy

For philosophical and legal reasons,[11] germ line gene therapy has only been carried out in animal cells, where one-cell embryos have been injected with DNA. At subsequent cell divisions, the genetic material will be randomly inserted into the emerging cells. If the genetic material is incorporated into cells, eventually producing eggs or sperms, the proteins coded for by the inserted DNA will be inherited in a Mendelian fashion. Tissue specificity for the expression of the genetic material will also be expressed. In other instances,

chimeric animals (from chimera, imaginary product) may result—the DNA will be functional in cells where the genetic material is normally dormant. Insertion of DNA material may also render other genes inactive. Trials using animal models of inherited metabolic disorders with single gene defects including b-thalassaemia and ornithine transcarbamoylase deficiency, have been partly successful.[12]

Somatic cell gene therapy

Somatic cell gene therapy is based on the discovery that DNA attached to a retrovirus can be incorporated into the genome of cells that divide in vivo, mainly haematopoietic cells but also hepatocytes and endothelial cells.[13] Other vectors have been used for the transport of DNA into cells, which do not divide so rapidly. Asialoproteins linked to DNA bind to a specific receptor of the hepatocyte before being transported into the cytoplasm and incorporated into the nucleus.[14] Herpes simplex virus linked to DNA has been used as a vehicle to transport DNA into neurones.[15] DNA linked to an adenovirous and administered as an aerosol has been used to incorporate genetic material into alveolar cells in cystic fibrosis patients.[16] The clinical outcomes of these trials have so far not been outstanding, partly because of immunological reactions. A liposome complex as a carrier for the cystic fibrosis gene has shown more promise both with regard to clinical expression and reduced side effects.[17]

Common to all trials using gene therapy, is the finding that the administered DNA has usually only been found in a few of the cells in the target organ.[18] This does not necessarily translate to a poor outcome. Most enzymes are present in excess of what is required for normal or near-normal physiological functioning, as is illustrated by early investigations of phenylketonuria. Intravenous loading tests clearly demonstrated that as little as a few per cent of the normal amount of phenylalanine dehydrogenase in the liver is sufficient to handle a normal intake of the amino acid in food.[18] A systems analysis of phenylalanine metabolism fully supports this conclusion.[19] These findings help to explain why the phenotypic expression of phenylketonuria is quite varied, and why some with the defect have not developed mental retardation without being on dietary treatment.[20] In general, small amounts of a normal enzyme protein in the right place could be sufficient to meet physiological needs.

Assuming that the technology for treating single gene disorders by gene therapy can be worked out, should such disorders be treated? The answer is not obvious, as several editorials in leading medical journals have highlighted.[21] The between-subject phenotypic expression of a single gene defect is varied and explained by compensating factors such as the use of dormant metabolic pathways, to eliminate a metabolite. One example is phenylketonuria, where

Table. Candidate human diseases for gene replacement therapy*

Disorder	Frequency
Atherosclerosis	Very high
Familial hypercholesterolemia	1 in 500 heterozygotes
Hemoglobinopathies	1 in 600 in ethnic groups
Lysosomal storage diseases	1 in 1500 for all types
Cystic fibrosis	1 in 2500 Caucasians
Duchenne's muscular dystrophy	1 in 3000 males
α_1-Antitrypsin deficiency	1 in 3500
Hemophilia A & B	1 in 10 000 males
Phenylketonuria	1 in 12 000
Huntington's disease	1 in 20 000
Urea cycle disorders	1 in 30 000 for all types
Glycogen storage disease 1a	1 in 100 000
Lesch-Nyhan syndrome	Rare
Adenosine deaminase and nucleoside phosphorylase deficiencies	Very rare
Leukocyte adhesion deficiency	Very rare

* Source: Beandet AL, et al. The metabolic and molecular bases of inherited disease. 1995.[18]

excess phenylalanine is metabolised to phenyllactate, a neurotoxic compound, but also to phenylacetylglutamine, which is non-toxic.[22] Brain damage would depend to a certain extent on the relative elimination rates through these two pathways.

Another important aspect is that some point mutations within a gene may cause serious disease whereas others are silent.[23] Extrapolated to multigene disorders, the situation becomes very complex with regard to the benefits of gene therapy. Counselling about gene therapy for a neonate with a prenatally diagnosed genetic condition in a family without an index case will be quite a complex issue.

A list of candidate human disorders for gene therapy is presented in the Table. It is evident that treatment with enzyme replacement therapy and bone marrow transplantation have been tried in many of these conditions. Combinations of treatments may well be the most effective approach in the long term.

Conclusion

Several alternative treatments for genetic disease have been developed over the past 30 years. Clinical outcome has often been substantially improved in the short term as judged by normalisation of biological functions, especially of cells in the liver, bone marrow, and bronchial epithelium. The enzymic or genetic treatment of the brain, when affected by genetic disease, has so far been surprisingly unsuccessful and awaits a major breakthrough.

In a famous statement from 1968, an international computer expert, when asked for his opinion of the newly-invented microprocessor, said: "What the hell is it good for?"![24] To those of us who have experienced the explosion of information technology and emergence of telemedicine since then, the statement seems to indicate that the human mind is not very good at realising the logarithmic development of a powerful concept. Gene therapy and enzyme replacement therapy are such concepts and no doubt, continuous progress in these areas will soon lead to the cure, rather than the care, of many hereditary conditions.

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