

INVITED EDITORIAL

Are We Ready to Try to Cure Alkaptonuria?

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It is very gratifying to see that alkaptonuria has now finally caught up with most of the other metabolic disorders formerly called "inborn errors of metabolism." Thanks to these recent advances we have a much better understanding of the molecular basis and mutational events that produce this well-known prototypic metabolic disorder. Alkaptonuria is characterized by the passing of urine that blackens with the addition of alkali, by a gradual development of an intensive connective-tissue melanin-like pigmentation (ochronosis), and, with advancing years, by a type of arthritis that resembles osteoarthritis in its distribution but that also includes acute inflammatory episodes (La Du 1995).

The Spanish investigators responsible for the very informative and landmark article appearing in this issue of the *Journal* (Beltran-Valero de Bernabe et al. 1998) previously reported the cloning of the AKU gene (Fernandez-Canon et al. 1996), and they also published a detailed description of the human gene coding for homogentisic acid oxidase (HGO) (Granadino et al. 1997). A complete deficiency of this enzyme is responsible for an accumulation of 2,5-dihydroxyphenyl acetic acid (HGA), an intermediary compound arising during the metabolism of the aromatic amino acids phenylalanine and tyrosine. Practically all of this was predicted by the English physician Sir Archibald Garrod, in the early 1900s, in his Croonian lectures (Garrod 1908). His conception of inherited metabolic diseases was far ahead of his time, and his correct hypothesis that defective genes are responsible for particular enzymatic deficiency diseases was a remarkable achievement, especially considering how little was known about enzymes, human genetics, and intermediary metabolism, at that time.

The article by Beltran-Valero de Bernabe et al. (1998) is a comprehensive study of the genetic defects in 14

unrelated representative alkaptonuric subjects from six European countries, Algeria, and Turkey. Point mutations believed to be responsible for the alkaptonuric phenotype were identified in 11 of the 14 exons, and several intronic defects also were found to be associated with this hereditary disorder. For three disease chromosomes from alkaptonuric individuals, Beltran-Valero de Bernabe et al. were unable to identify the nature of the defect. Nevertheless, it is obvious that a wide variety of molecular defects can lead to the common phenotype alkaptonuria. Presumably, most sporadic patients have a combination of two rare, different alleles and are not homozygous for one particular HGO mutation. Tracing of the different allelic variants associated with alkaptonuria will be aided by the careful characterization of haplotypes, on the basis of several new polymorphic markers and repetitive sequences in the gene that also are identified by Beltran-Valero de Bernabe et al.

Another laboratory, studying alkaptonuria in the Slovak regions, which have a relatively high incidence of this disorder, independently cloned the human HGO gene and found a frameshift mutation at position 454-457 and a missense mutation, G481A, in two of the alkaptonuric families of that area (Gehrig et al. 1996). Further experience will indicate whether a few mutations predominate among the extended alkaptonuric families in that rather isolated region. The reported higher incidence of alkaptonuria in the Dominican Republic, which at one time appeared to support the possibility that a dominant type of alkaptonuria existed, probably will be explained by the frequency of a single or, more likely, of a few abnormal alleles circulating within a limited population, owing to consanguinity and the intermarriage customs of that ethnic group.

Attempts to treat alkaptonuria by use of genetic engineering to replace the missing enzyme no doubt will be made soon. This disorder also has the attractive feature that any significant correction of the missing enzymatic function can be easily detected and monitored simply by following the degree of reduction in the amount of HGA excretion in the urine each day, provided that the patient has been on a fixed dietary-protein intake. However, genetic treatment of this disorder should proceed with due caution. Adult alkaptonurics

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on a regular diet produce ~5-7 g of HGA/d and even more than that on a diet higher in protein. Most of this product is efficiently eliminated in the urine, because HGA is actively secreted by the kidneys. Renal secretion of HGA remains the important line of defense for the alkaptonuric: it reduces the accumulation of HGA in alkaptonuric tissues and thereby slows the development of ochronosis and arthritis (La Du 1995).

Providing the missing HGO to alkaptonurics should produce large amounts of the expected product maleylacetoacetic acid (MAA). This acid normally is then converted, by the next enzyme, an isomerase, to fumarylacetoacetic acid (FAA) (fig. 1), and the latter is split, by a hydrolase, into fumarate and acetoacetate (fig. 1). In normal liver, these sequential steps proceed very smoothly and efficiently, without the accumulation of MAA or FAA. Also, in normal liver, most of the HGA metabolism takes place in the soluble fraction of the hepatocytes, the fraction which also contains the subsequent enzymes. Interestingly, alkaptonuric liver also has normal levels of the later enzymes in the pathway, even though these enzymes presumably have never been called on to metabolize any substrate (La Du et al. 1958). From what we know today, localizing the recombinant HGO to its normal location in liver cells would seem to be the best strategy.

But what might be the consequences if recombinant HGO were localized in liver or other tissues, at some distance from the isomerase and the hydrolase? MAA and/or FAA then could accumulate in appreciable amounts. The hydrolase is rather widely distributed in tissues, but little is known about the tissue distribution of the isomerase. Maleic acid has been employed experimentally in animals, to deplete the liver of glutathione and other sulfhydryl compounds. The severe liver cirrhosis, renal Fanconi syndrome, and peripheral neuropathy that rapidly develop in young babies with the hepatorenal type of hereditary tyrosinemia, owing to deficiencies of the FAA hydrolase, are life threatening features of that deficiency (Mitchell et al. 1995). Mice deficient in FAA hydrolase also show extensive liver pathology, presumably as a result of the accumulation of these later tyrosine metabolites. In any case, model animal systems (either those representing known spontaneous hereditary deficiencies of HGO) (Montagutelli et al. 1994) or appropriate "knockout" animals with created deficiencies of this enzyme need to be tested before human trials are undertaken. The consequences of alkaptonuria are well known and established. We all hope that some, perhaps all, of these adverse effects can be prevented by new molecular therapeutic approaches. However, trading alkaptonuric problems for, quite possibly, even-more-serious metabolic disturbances, because

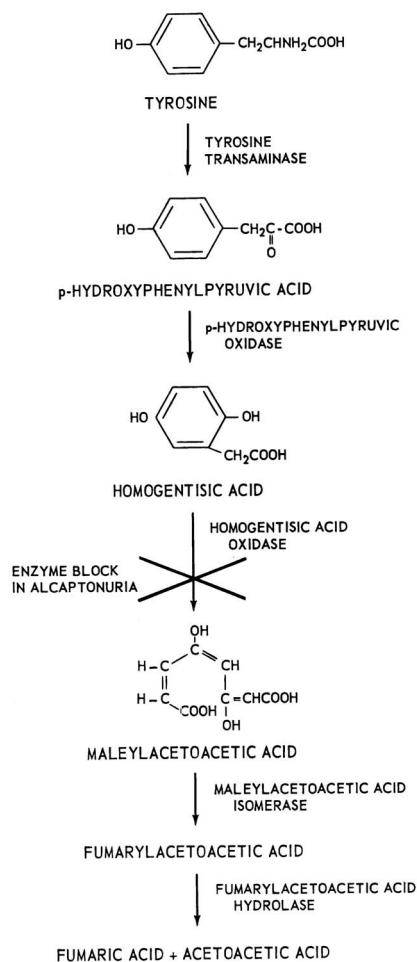


Figure 1 Scheme of tyrosine metabolism. A hereditary defect in HGO is responsible for alkaptonuria, a disease that is readily diagnosed through elevated levels of homogentisic acid in the urine. Awareness of this entire biochemical pathway will be needed in order to design gene therapies for alkaptonuria, to avoid creation of novel metabolic defects in the subjects.

of the pronounced toxicity of the later tyrosine metabolites, is not an acceptable alternative.

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