Why do cataracts appear in cases of diabetes or galactosemia?

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ABSTRACT: The origins of cataracts can be very diverse. It is nevertheless quite clear that in many cases cataracts occur as a consequence of changes in the metabolism of carbohydrates. In this sense, diseases such as diabetes and galactosemia can initiate the process of cataractogenesis due to an excess of sugars in the blood and in the aqueous humour. The lens can use carbohydrates by means of three metabolic pathways: glycolysis, the pentose phosphate shunt and the polyol pathway. When the first two are saturated due to the excess of sugars, the polyol route starts to become relevant. This pathway mainly produces sorbitol and galactitol, which normally accumulates within the lens. The increase in these polyols generates an osmotic force that facilitates the entrance of water into the lens. Moreover, the high sugar levels glycosylate proteins, thus facilitating their self-aggregation. The mentioned protein aggregation plus the massive water influx produce changes in lens protein organization and visible aggregates can be observable. These protein clusters disperse light and impede the proper formation of images in the retina. There are ways to delay the development of this type of cataract, most of them interfering in the enzymes producing polyols. In particular, the development of RNAi for some lens proteins could help to halt the appearance of cataracts.

keep this structure perfectly transparent. Metabolic energy production depends almost entirely on glucose metabolism which is metabolized through three main routes: glycolysis, the pentose phosphate shunt, and the polyol pathway. Glucose uptake can take place through two types of glucose transporters in the lens; GLUT1 and GLUT3.

GLUCOSE

<table>
<thead>
<tr>
<th>Glycolysis</th>
<th>Pentose phosphate</th>
<th>Polyol</th>
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| To produce ATP (Cell Energy) | To produce NADPH (Cell Maintenance) | ???

Figure 1. Main glucose metabolic pathways used by the lens to produce different metabolites. Although they can all use glucose, the first pathway to be activated is glycolysis. When this is saturated the second to be stimulated is the pentose phosphate shunt and if this is also saturated then polyol pathway starts to produce metabolites. The polyol pathway generates metabolites with cataractogenic properties.

Three metabolic pathways and one single problem: cataracts

There are three metabolic pathways which convert glucose in energy (ATP) and other relevant metabolic molecules; glycolysis, the pentose phosphate shunt, and the polyol route (Figure 1).

The glycolytic pathway that occurs in the lens is no different from that existing in other tissues. Interestingly, one of the main regulatory enzymes of this pathway, hexokinase, is present at low levels and only two isoforms have been described: hexokinase I and II. The $K_m$ of type I is lower than that of type II, however, hexokinase type II is more abundant than I. It is likely that the latter is used when glucose levels rise.

Hexokinase concentration decreases as the individual ages. This may be one of the reasons why during aging the lens has more of a predisposition to pathologies such as cataracts. The drop in ATP levels prevents the normal functioning of the lens in processes such as active control of electrolyte balance. Poor control of electrolytes will produce an osmotic driving force that may produce a massive influx of water into the lens. This entrance would produce alterations in the metabolism as well as disorganization of the well-structured proteins present in the lens. The final result is the aggregation and precipitation of proteins which are generally known as cataracts.

Some of the glucose is metabolized by the pentose phosphate shunt. This pathway does not generate large amounts of ATP, but is essential because it allows the synthesis of significant amounts of NADPH + H+ by the first enzyme of this pathway, glucose 6-phosphate dehydrogenase. NADPH + H+ is essential for glutathione reductase and also for the function of the polyol pathway. It has been shown that about 1.4% of the glucose is metabolized by this pathway. The activation of this pathway is triggered under conditions of oxidative stress since glutathione (GSH) must be available.

The third metabolic pathway for glucose is the polyol route, also known as the sorbitol pathway. This route was described by van Heyningen in 1959, after verifying the accumulation of polyols in the lens. Those with diets including high quantities of glucose, galactose, or xylose developed cataracts in experimental models.

The route of sorbitol is formed by only two enzymes, firstly aldose reductase, using NADPH + H+ as a cofactor and secondly polyol dehydrogenase which uses NAD+ as coenzyme. It is considered that about 1/3 of glucose entering the lens is metabolized through the sorbitol pathway.

Human aldose reductase has a $K_m$ for glucose of 200 mM, and it is present mainly in the epithelia, where 70% of the activity of this enzyme has been located. This is important because it means that the concentration of sorbitol accumulates in a very small area of the lens, so that the final effect is an increase of 50 times the concentration that would be expected if the distribution of the enzyme was homogeneous throughout the lens.

Polyol dehydrogenase (sorbitol dehydrogenase) is present in the lens of many species but has not been studied in as much depth as aldose reductase. Sorbitol dehydrogenase is distributed more evenly than aldose reductase. 50% of polyol dehydrogenase is found in the lens epithelium and 50% in the cortex. Interestingly, unlike aldose reductase, polyol dehydrogenase cannot metabolize inositol, glycerol nor dulcitol (galactitol).

The polyol pathway is the main reason for the development of cataracts in diabetic and galactosemic patients

The polyol pathway is significant for individuals with elevated sugar levels in plasma and in the aqueous humour. In normal individuals, because glucose concentrations are between 0.7 and 2.2 mM, aldose reductase will not function. Nevertheless, under pathological conditions such as diabetes, where the glucose concentration is between 3 and 4.5 mM, aldose reductase has a chance to act. This pathway also operates in galactosemia, and high levels of galactitol are produced. The importance of this route is linked to the activity of another enzyme, hexokinase, whose activity decreases with age. Given that both enzymes have glucose as a substrate, the balance in the activity of both proteins is essential to determine the fate of glucose. The $K_m$ of hexokinase lens is 100 µM, so that
The polyol pathway generates metabolites with cataractogenic potential, as the second to be stimulated is the pentose phosphate shunt and if this pathway is not regulated, it can produce different metabolites.

Figure 1. Main glucose metabolic pathways used by the lens to generate energy from glucose. Some of the glucose is metabolized by the pentose phosphate pathway, while glucose is also used to generate sorbitol and dulcitol (galactitol) respectively. Although both sugars are similarly processed, sorbitol can be transformed to fructose via polyol dehydrogenase while dulcitol is not a substrate for the second enzyme as previously indicated.

It can be concluded that both substrates —glucose and galactose— induce the accumulation of polyols. This is faster in the case of galactose, which ultimately produces a hyperosmotic effect which results in water uptake via aquaporin 1 (AQ1) and aquaporin 0 (AQ0, aka MIP), which tends to counteract the osmotic gradient produced.

Is there a biochemical solution for glucose-induced cataracts?

In the last decade, RNA interference (RNAi), a new process of sequence specific post-transcriptional gene silencing, has emerged as a powerful tool for understanding gene function. This was initially studied in Caenorhabditis elegans. RNAi is mediated by small interfering RNA (siRNA) that is generated from long double-strand RNA (dsRNA) of exogenous or endogenous origin. These long dsRNA are cleaved by the ribonuclease II (RNase III) type protein Dicer. Dicer homologues can be found in Schizosaccharomyces pombe, C. elegans, Drosophila, plants and mammals, suggesting that small RNA-mediated regulation is evolutionary ancient, has been maintained, and may play critical biological roles. siRNA generated by Dicer is a short RNA duplex (21–23 nucleotides) with 2 nucleotides overhanging at each 3’ end. Each strand contains 5’ phosphate group and 3’ hydroxyl group.

siRNA is incorporated into a nuclease complex called RISC (RNA-induced silencing complex) that targets and cleaves mRNA complementary to siRNA. The initial RISC containing siRNA duplex is still inactive until it is transformed into an active form, which involves loss of one strand of the duplex by RNA helicase activity. RNAi can occur very quickly with proteins for many genes, decreasing within hours, and being completely absent within 24 h.

The idea of using RNAi for therapeutic purposes has been tested extensively in recent years. Where anti-sense directed therapeutics have failed, the enhanced delivery methods and potential gene therapy applications of RNAi are provoking excitement among investigators in multiple medical fields.

There are many targets that could be used to try to halt the development of cataracts. However, the silencing of certain proteins could, in theory, stop cataract progression. Some target proteins may be investigated. For instance, silencing aldose reductase could be of interest, since it leads to the generation of polyols that trigger the initial osmotic shock that initiates cataracts.

Another interesting protein could be the glucose transporter GLUT1. This protein has a $K_m$ greater than that of the aldose reductase.
WHY DO CATARACTS APPEAR IN CASES OF DIABETES OR GALACTOSEMIA?

than the other glucose transporter present in the lens, GLUT3. The latter is responsible for the massive influx of glucose in pathological situations such as diabetes.

A third possibility could be aquaporin AQ1, as it has a role in the entry of water from aqueous and vitreous humours and may be directly responsible for the entry of water when there is an over-concentration of polyols.

The reasons for choosing these three targets are, firstly that they play a dominant role in the development of cataracts. Secondly, silencing is not going to completely abolish processes such as sugar metabolism, transport or water traffic. Aldose reductase uses glucose, but this is not the sugar substrate. Glucose can enter the other transporter, GLUT3, which always is saturated under physiological conditions, ensuring the entry of glucose. Regarding the addition of aquaporin to AQ1 there is another, the AQ0 (also known as MIP), which ensures the flow of water into the lens.

It is the time to start the use of new biochemical approaches to reverse or halt pathological conditions such as cataracts. The possibilities revealed by siRNA technology may change the lack of interest by the pharmaceutical companies in the development of compounds for the treatment of lens opacification.

REFERENCES


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