

Human Age-Related Cataract: A Condition with No Appropriate Animal Model

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Abstract

A case is made in this article that research on animal models of human age-related cataract has contributed little to our understanding of this blinding disease. This surprising conclusion comes about not so much for the reason that humans are different from other animals, although important aspects of human lens biochemistry are not matched by experimental animals, but more so because of the very long periods of time that are required before human cataract become evident.

Insidious processes associated with aging are required to establish the conditions necessary for human cataract, and laboratory animals simply do not live long enough to act as useful models. In relation to human nuclear cataract, the large sums of money spent on animal models would have been spent more productively on investigating the processes that underpin human lens aging. Lessons derived from human cataract may apply more widely to other human age-related diseases.

Introduction

From an historical perspective, research on human cataract formation has been overwhelmingly dominated by the use of animal models. Vision research is not unusual in this regard, since it is commonplace to study the mechanism of most human diseases using one or more animal model systems. Indeed this approach is so typical, that the strategy is often considered as the first line of attack.

In relation to this, editors of some scientific journals devoted to ageing will not readily accept articles on aspects of human aging if they are not accompanied by experimental results derived from an animal model. Otherwise data derived solely human studies, no matter how comprehensive, can be dismissed as being merely “descriptive”. Is this a valid argument, or might there be aspects of human aging that are hominid-specific? One aspect of this will be addressed in this manuscript.

The advantages of employing animal models for human diseases are obvious and include; ease of access to experimental animals, simplified ethical approval, cost, the availability of inbred lines, replicates, the ability to control diet and environment, the relatively short time between experimentation and outcome, and the possibility of modifying genetic makeup or expression.

By comparison, the study of human tissues is problematic, is increasingly an ethical minefield, is often limited to a particular age range and is bedevilled by a multitude of issues that are not encountered by animal investigators. As just one example, time between tissue collection and bench experimentation is sometimes variable and beyond the control of the investigator.

With regard to animal models and their use in studies purported to be relevant to human aging, short-lived animals have an additional advantage that several generations can be studied within the period of grant funding.

Now the human lens may, at first glance, seem to be a tissue that may not suffer to the same extent from some of these disadvantages. Although fresh human lens tissue is not readily obtainable, intact lenses can be sourced from donors, although there are issues with regard to the time delay between collection and analysis and the availability of

lenses from younger donors. However, for the reasons listed above, historically animal tissues have been used for studies of the mechanism of human cataract.

Before assessing whether animal experiments have been instructive in informing the scientific community as to the cause of human cataract, it is worthwhile reviewing salient aspects of human cataract.

Multiple causes of lens opacification do not necessarily mean that human cataract is multi-factorial

The fact that many agents both endogenous (e.g. mutations) [1], and exogenous (e.g. chemicals, UV light) [2,3], can cause lens opacification lead some investigators to conclude that human age-related cataract must be multi-factorial. This is not necessarily the case.

Despite observations that numerous factors can interfere with lens transparency, it is quite possible that there may be just one, or very few, primary causes of age-related cataract. These could be responsible for the vast majority of human cataract. In this sense the use of the term “multi-factorial” may be counterproductive, since it could act to discourage researchers from trying to understand the predominant molecular basis of human age-related cataract.

Even though there may be just one primary agent, or potentially very few underlying causes of age-related cataract, the great genetic, as well as environmental, variability of the human population coupled with the length of time required for onset, will ensure that there will likely be a suite of clinical presentations.

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The molecular basis for age-related nuclear cataract

Although the basis for human cortical cataract remains obscure, recent data indicates that there may be one primary cause of human age-related nuclear (ARN) cataract. The underlying basis for the onset of ARN cataract appears to be the inexorable deterioration of life-long macromolecules. This slow deterioration explains why ARN cataract is age-related.

The molecular basis for this, and the reason for its dependence on age, has been elaborated elsewhere [4] but the key elements can be summarized briefly. Over decades, lens proteins unfold due mostly to the intrinsic instability of certain amino acid residues. This leads to extensive racemisation [5,6] deamidation [7,8] and truncation [9-11] of crystallins, as well as other proteins, in the fibre cells of the lens centre.

Proteins are the major constituents of lenses, and do not turnover [12]. They undergo denaturation continuously over time. Initially, in lenses of young people, unfolding proteins are intercepted and bound by another abundant lens crystallin; the chaperone, alpha crystallin. High molecular weight aggregates are produced. After age 40, the supply of alpha crystallin that we were allocated at birth is exhausted [13] and subsequent protein denaturation takes place in the absence of such a chaperone. The outcome in this altered environment appears to be quite different. After middle age, large-scale protein binding to cell membranes occurs [14-16].

One consequence of such extensive binding is that the pores of the membranes become blocked and the passage of small molecules such as water [17] and glutathione [18] from cell to cell, become impaired. Since glutathione is the major antioxidant of the lens, its restricted flow leaves the centre of human lenses older than middle age, susceptible to oxidative insult. Protein oxidation is the key factor that differentiates ARN cataract lenses from age-matched normal lenses [19].

If the scenario outlined above is correct, then why should the lenses of some elderly individuals remain clear whilst those of others develop ARN cataract? We do not yet know the reason for this, but there are some interesting indicators, which suggest that the rate of protein denaturation may be a key factor. For example, the spontaneous cyclisation of one asparagine residue in gamma S crystallin (Asn 76) occurs to a greater extent in ARN cataract lenses than those of comparable age-matched controls [7]. If this is the basis for cataractogenesis, we clearly need to determine the factors within the lens that are responsible for determining the rate of such modifications.

Insidious macromolecular deterioration and animal models

If the process of gradual degeneration of macromolecules outlined in the previous section is indeed fundamental to the etiology of human ARN cataract, it becomes clear why no short-lived experimental animal can be used as an appropriate model. Only an animal that lives for many decades can mirror the gradual chemical processes that are required to establish all the conditions necessary for subsequent ARN cataract.

Naturally there are many issues associated with the housing and maintenance of long-lived animals. If one is contemplating establishing such a facility for the purposes of understanding processes that are responsible for human aging, it would probably be wise to focus on our closest cousins; the primates. Despite the fact that there are important ethical considerations surrounding the use of primates in medical

research, such a restriction would probably be necessary in order to properly understand the condition.

This is so, because the human lens differs from those of non-primate experimental animals, in some important ways. For example, primates have a UV filter pathway based on kynurenine metabolites and these compounds play a role in lens protein colouration and reactivity [20,21]. In addition, the phospholipid composition of lens membranes of humans – shown above to be a vital component of cataract – is quite different from that of commonly used laboratory animals [22].

Animal models can be misleading

The paradigm of investigating human diseases using animal models is so pervasive, that innate differences can sometimes be overlooked until late in the screening program. This can be very costly when the intention is to develop drugs for human use.

Two pertinent examples from the cataract literature serve to illustrate this.

Aldose reductase inhibitors: Following the discovery of polyols in the lenses of rats with sugar cataracts by van Heyningen it was widely believed that diabetic cataract resulted from the accumulation of a sugar alcohol (e.g. sorbitol from glucose) within the lens, which drew water into the lens via an osmotic effect [23]. The sugar alcohol that was formed by the action of the enzyme aldose reductase (AR) did not readily diffuse from the lens and disturbance of the ionic and fluid balance lead ultimately to lens opacification. In an effort to prevent such an outcome, and thereby delay osmotic cataract, a large research thrust over many years was devoted to the discovery of AR inhibitors. Rats were the main animal model used and rodents proved to be ideal experimental animals in this regard because their lenses were found to contain high levels of AR. Many drug candidates were developed during the course of an extensive drug discovery program. Alas, human lenses display very much lower levels of AR. Aspects of the search for AR inhibitors have been summarised [24].

Calpain inhibitors: Studies of calcium influx into lenses and subsequent proteolysis [25,26] lead to an investigation of calcium-activated proteases: calpains. In rodent lenses, calpain activation lead to specific cleavage of crystallins and the truncated crystallins tended to be insoluble. A pharmacological scenario based on these discoveries was therefore formulated. This strategy involved administration of calpain inhibitors to prevent calpain-induced proteolysis and thereby such compounds may act as anti-cataractogenic agents in man. Unfortunately human lenses respond quite differently from rat lenses to calcium, and it is thought that at least part of the reason for this rodent-primate difference is that human lenses contain a huge excess (300-1400 fold) of calpastatin over calpain, whereas the reverse is true for rats. In one of very few comprehensive comparisons of human and rat lenses it was found that humans contain just 3% of the calpain activity of rodents [27].

Other age-related diseases. Lessons from the lens?

Lens fibre cells are post-mitotic. The human body contains other cells that are post-mitotic, and one of the most important with regard to age-related conditions, are neurons. Many neurological conditions are strongly age-related and dementias, in particular, have become more prevalent in developed countries with ageing populations. The most common of the dementias, is Alzheimer's disease [28]. It will be interesting to discover whether the same, or similar, biochemical

processes that have been outlined above for human ARN cataract are also observed in the neurons of elderly people.

Epidemiology; ARN cataract versus age-matched normals

Epidemiology has contributed to an understanding of a number of human diseases, however, in the case of cataract; its impact has been marginal. Although studies have clearly demonstrated a pronounced age-dependence of cataract [29], other findings have been largely inconclusive. For example, despite numerous studies, at many sites on the globe, the role of UV light in cataract etiology remains unclear. On this basis, one may conclude that UV light is unlikely to be a principal causative agent for human cataract. This rather negative overall assessment of the impact of epidemiology to the present time should not downplay its potential role in prompting productive areas of scientific investigation. For example, the reasons behind the early onset of cataract in India [30] remain unknown, and understanding this would almost certainly provide valuable insights into the mode of formation of cataract in other populations.

Even if human lenses, rather than those of animals, had been utilised more widely in research studies, it is quite likely that a conventional experimental design of comparing cataract and age-matched normal lenses would have been employed. This is certainly a valid approach and it has allowed scientists to discover that oxidation and protein insolubility is a defining element of human ARN cataract [31,32]. It did not however permit an elucidation of the reasons why cataract lens proteins become so extensively oxidised. It has only been through a careful study of age-related changes to normal lenses [15,16,33] that it has been possible to understand the key elements that are responsible for ensuing cataract. This was a lesson that the author of this article was slow to learn, but in retrospect, this approach has been crucial to understanding the molecular basis for human ARN cataract.

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