Histopathology of Reversible Cataracts Produced by Hydroxyurea in vitro

Ikuo MIKUNI

Department of Ophthalmology, School of Medicine,
Tokai University
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A DNA synthesis inhibitor, hydroxyurea (HU) \(5 \times 10^{-2} \text{M}\), was added to the organ culture medium for a rat crystalline lens (male, white Wistar rats weighing 100g).

1. On the 3rd day, a macroscopic examination revealed diffuse opacity of the posterior capsule. Because of removal of HU from the culture medium on the 3rd day the crystalline lens had become lucid on the 6th day, approximately the same as that of the control of the 6th day. In a histopathological examination, the epithelial cells appeared to maintain a histo-architecture similar to that of the control. Lens fiber swelling was scarce at this time.

2. The crystalline lens cultured with HU showed diffuse opacity of the anterior capsule and localized opacity below the posterior capsule in a macroscopic examination on the 5th day. In histopathological examinations of the same culture, the lens epithelial cells and swelling of the lens fibers had disappeared.

(Key Words: Hydroxyurea, Epithel, Cataract, Rat)

INTRODUCTION

Hydroxyurea (hereafter abbreviated to HU) is an antineoplastic agent often used for the treatment of leukemia in man and mice (8).

HU is also known to cause fragmenting of DNA and chromosome aberrations in cultured mammalian cells (1). Probably the most interesting fact concerning this agent is that it inhibits synthesis of bacterial and animal cell DNA but, once it is removed, animal cells resume DNA synthesis (3). Furthermore, it is said that HU inhibits thymidine incorporation into HeLa cell DNA without affecting RNA or protein synthesis (9).

The author utilized these characteristic actions of HU — especially inhibition of DNA synthesis with the addition of the agent but resumption of synthesis after its removal. The author has proven in the past that experimental cataracts can be caused by DNA inhibitors in cultured rat crystalline lenses (5, 6). Therefore in the present study HU, a DNA inhibitor, was added to the culture medium of the lens to cause suppression of DNA synthesis in the lens epithelial cells and, after a fixed period, the agent was removed to induce resumption of DNA synthesis in the said epithelial cells. Subsequently, an investigation was made of the effects of HU on the lens epithelial cell DNA, histopathology of the epithelial cells and the degree of clarity of the opaque lens.

Furthermore, a histological study was performed on the reversible
change of the normal lens when the chemical was applied externally but
removed in the early stages of cataract formation.

EXPERIMENTAL MATERIALS AND METHODS

Male white Wistar rats weighing 100gms were used as the experimental
animals. After sacrificing them by a guillotine, the crystalline lenses were
removed by retrobulbar excision and Puck's solution. Four milliliters of Rat
Lens Medium (with TC 199 prepared in the Tokai University
Pharmacological Department) containing 5% calf serum (Gibco, U.S.A.)
was used as the medium and the lenses were cultured in an atmosphere of
5% CO₂ air at 37°C. Corning Petri dishes were used and the medium was
replaced every 3rd day. The right crystalline lens was used as the control
while the left was treated with HU (Sigma) of a final concentration of
5×10⁻² M HU. There were also lenses with no further addition of HU.
These specimens were cultured until the 5th and 6th days, respectively. The
cultured crystalline lenses on the 5th and 6th days were fixed in 10% formalin
dehydrated by alcohol, imbedded in paraffin, and subjected to
microtomy using a Jung-type microtome (Feather S 35 knife manufactured
by Daiwa Koki, Japan) to produce light microscopy specimens measuring
3µ in width. These were stained with hematoxylin-eosin and observed under
microscopy.

RESULTS

1) Macroscopic observation of the cultured rat crystalline lens

The control remained lucid on the 3rd, 5th and 6th days. On the 3rd
day the specimen cultured with HU showed diffuse opacity of the anterior
and posterior capsules. On the 5th day, the specimen cultured with HU
showed diffuse opacity in the entire anterior capsule and localized opacity
under the posterior capsule.

The crystalline lens which was cultured with HU for the first 3 days and
then cultured in the medium free of HU for an additional 3 days showed
that the diffuse opacity of the posterior capsule no longer existed and it
appeared lucid with slight and localized opacity of the anterior capsule.

2) Light microscopic observation of the cultured rat crystalline lenses

On the 5th and 6th days, the control lenses showed little stratification
of the epithelial cells in the bow area. On the 6th day, the same specimens
showed a slight swelling of the lens fibers (see Figs. 1 and 3).

In the observations of the crystalline lens epithelial cells on the 5th day
of cultivation with HU, only a few degenerated epithelial cells remained in
the bow area, but these cells had totally disappeared in the anterior section
of the lens. Closer observation gave the impression that a few of these cells
persisted at the center of the pupillary region. Mild swelling of lens fibers
was also noted (Fig. 2).

In the lens cultured in the medium containing HU until the 3rd day but
cultured up to the 6th day without HU (HU was removed on the 3rd day),
the epithelial cells assumed an almost normal arrangement at the anterior
section and their number approximated that of the control. The lens fibers
also appeared almost normal (Fig. 4).

Fig. 1 Rat crystalline lens in culture on the 5th day. Stratification of the epithelial cells is almost absent. Hematoxylin-eosin, × 99.

Fig. 2 Rat crystalline lens in culture containing HU \((5 \times 10^{-5}\text{M})\) on the 5th day. The epithelial cells have all but disappeared on the frontal section of the lens. Light swelling of lens fibers is seen. Hematoxylin-eosin stain, × 99.
Fig. 3 The rat crystalline lens in culture on the 6th day. Stratification of the epithelial cells is almost absent but mild swelling of the lens fibers is noted. Hematoxylin-eosin stain, ×99.

Fig. 4 Crystalline lens which had been cultured in a medium containing HU (5 × 10^{-7}M) up to the 3rd day (at which time HU was removed) and then cultured for 3 more days. The number of the epithelial cells at the frontal section of the crystalline lens approximates that of the control. The lens fibers appear almost normal. Hematoxylin-eosin, ×99.
DISCUSSION

The author has indicated that the addition of DNA and microtubule inhibitors induces nuclear and posterior subcapsular cataracts in rat crystalline lens cultures (5, 6). The author considers that clarification of the following two issues will provide the key to the possibility of chemotherapy of cataracts when the activities of the epithelial cells are sufficiently retained and opacity is limited only to the anterior capsular or posterior subcapsular region: (1) the possibility of repair of the pathologically affected tissue and (2) the probability of arrest of the opacifying process.

One of the pharmacological properties of HU is that its DNA inhibitory action is reversed after short term treatment and the cells are returned to an ordinary medium where they resume DNA synthesis (3).

Based on this fact, the author assumed that the addition and subsequent removal of HU and observations of the recovery process of the once-opacified lens are highly significant in parallel with the following projects: (1) a study on the changes of the DNA synthesizing capacity of the lens culture system associated with the addition and removal of HU; (2) observations on the morphological changes of the lens epithelial cells which are believed to be furnished with DNA synthesizing capacity; and finally (3) substantiation of our hypothesis that an aberration of nucleic acid metabolism of the lens epithelial cells plays an important role in the pathogenesis of cataracts.

In the present study, the author used cultured crystalline lenses of Wistar rats weighing 100 grams. According to Jose et al. (4), DNA replication by rat crystalline lens epithelial cells continues with only a slight reduction in the rate even at an HU concentration of $5 \times 10^{-2}$M. Therefore, this high concentration ($5 \times 10^{-2}$M) of HU was applied in the present study considering the specificities of the organ — such as the membrane permeability of the lens capsule.

On the 5th day, the control lens appeared clear in a macroscopic observation. As seen in Fig. 1, the same organ showed normal epithelial cells and lens fibers in the histopathological examination. With the same time lapse, however, the HU-treated specimen showed macroscopically evident, diffuse opacity affecting the entire anterior capsule and localized opacity of the region below the posterior capsule. Microscopic examination revealed disappearance of the epithelial cells near the equator, debris of partially degenerated and atrophied epithelial cells, and swelling of the lens fibers (Fig. 2). These histopathological findings approximated those of cataracts induced by typical nucleic acid inhibitors (mitomycin®, daunomycin®, and 1-beta-D-arabinofuranocyl cytosine).

However, diffuse opacity of the anterior and posterior capsules which developed in the lens treated with HU up to the 3rd day of culture was dissipated when HU was removed on the 3rd day and the tissue was cultured up to the 6th day. As shown in Fig. 4, a histopathological examination revealed that the epithelial cells near the equator were preserved in a near perfect state and the lens fibers were approximately normal. These findings were interpreted to be an indication that the lens epithelial cells with their
DNA synthetic capacity suppressed initially by HU recovered the same capacity after HU removal.

According to Fournier et al. (2), de novo formation of the lens fibers is the dominant feature of reversible histological changes in cataracts induced by galactose *in vivo* in rats. Furthermore, Reddy et al. (7) projected that, in *in vivo* galactose-induced cataracts in rats, disappearance of cortical opacity in reversible changes is due to the recovery of the lens fibers from prior damage or formation of new lens fibers.

The disappearance of crystalline epithelial cells seen in HU-induced cataract and the reappearance of these cells at a reversible change in the present study are features much different from the reversible changes observed in *in vivo* galactose-induced cataracts.

In consideration of the developmental process of the crystalline lens in forming lens fibers by differentiation of the lens epithelial cells, it is probable that in HU-induced cataracts both the changes on the epithelial cells, and also the repair and/or formation of the lens fibers, are important factors in the reversible changes.

Accordingly, changes of microtubules which play an important role in the mechanisms of disappearance and development of the epithelial cells and their differentiation may be the major themes in future investigations.

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REFERENCES


