Protective Effect of Catechin on Apoptosis of the Lens Epithelium in Rats with N-methyl-N-nitrosourea-induced Cataracts

Sung Min Lee¹, Il-Gyu Ko², Sung-Eun Kim², Dong Hee Kim³, Byung Nam Kang¹

¹Department of Ophthalmology, Myongji Hospital, Kwandong University College of Medicine, Goyang, Korea
²Department of physiology, Kyung-Hee University College of Medicine, Seoul, Korea
³Department of Ophthalmology, Chungju Hospital, Konkuk University School of Medicine, Chungju, Korea

Purpose: To investigate the effect of catechin on apoptotic cell death in the lens epithelium of rats with cataract.
Methods: Cataract was induced by intraperitoneal injection of 100 mg/kg N-methyl-N-nitrosourea (MNU) to ten day-old Sprague-Dawley rats. The neonatal rats were randomly divided into five groups (n=15 in each group): a control group, and four cataract-induction groups, treated with either 0, 50, 100, 200 mg/kg catechin. We performed slit-lamp biomicroscopic analysis, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, Western-blot for Bcl-2 and Bax, and immunohistochemistry for caspase-3.
Results: Apoptotic cell death in lens epithelial cells that increased following cataract formation in rats was suppressed by catechin.
Conclusions: Catechin inhibited cataract-induced apoptotic cell death in the lens epithelium and may prove useful for the prevention of cataract progression.

Key Words: Apoptosis, Cataract, Catechin, Crystalline lens, Epithelial cells, Rats

The lens is a unique tissue with long-lived proteins, called crystallins, which can be classified into three groups. The lens grows throughout the lifetime of an individual, and significant changes occur in the structure and function of the lens crystallins. Various modifications, such as deamidation, truncation, oxidation, glycation, and methylation, lead to structural changes in the crystallins. These mechanisms play a major role in converting the largely soluble pool of crystallins into a largely insoluble pool with aging. A cataract is an opacity that develops in the crystalline lens of the eye; it varies in degree from slight to completely opaque, obstructing the passage of light. The lens epithelium covers the anterior surface of the lens. Epithelial cells near the lens equator divide and differentiate into lens fibers. This process continues at a constant, slow rate throughout adult life, resulting in the steady growth of the lens fiber mass [1]. The mitotically quiescent central region of the epithelium is thought to protect the underlying fibers from various insults, to transport ions to and from the deeper layers of the lens, and perhaps to provide nutrients to the elongating lens fibers [2]. Damage to the lens epithelium has been a major focus in the identification of causes of cataract formation [3].

Apoptosis, also known as programmed cell death, is a form of cell death that serves to eliminate dying cells in proliferating or differentiating cell populations. Thus, apoptosis plays a crucial role in normal development and tissue homeostasis [4,5]. Previous studies have shown that apoptosis of lens epithelial cells plays an important role in the development of several types of cataracts [6-8]. These studies have suggested that apoptosis of lens epithelial cells appears as a common cellular mechanism mediating stress-induced noncongenital cataractogenesis [9,10].

Apoptosis can be detected using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, a measure of DNA fragmentation in tissue sections, and by observation of a DNA ladder, a measure of fragmentation in DNA extracted from cells or tissues [11,12]. In human cataract research, TUNEL-positive cells indicate apoptotic cell death in the lens epithelium [1,13]. Another important characteristic of apoptosis is caspase activation. Caspase-3 is one of the most widely studied caspases, and it is a key executor of apoptosis [14]. In addition to caspases, Bcl-2 family pro-
teins also play a pivotal role in the regulation of apoptosis. The Bcl-2 family is classified into anti-apoptotic and pro-apoptotic proteins according to function. The balance between pro-apoptotic and anti-apoptotic Bcl-2 family members determines the mitochondrial response to apoptotic stimuli [15].

Catechin is a naturally occurring polyphenolic compound found abundantly in green tea. Polyphenolic compounds include (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epicatechin (EC), the main constituents of catechin [16]. Previous studies have shown that catechin has diverse health benefits, including anti-oxidant, anti-hyperglycemic, anti-cancer, and anti-apoptotic effects [17-20]. Catechin has also been reported to exert a protective effect on UV radiation-induced epithelial cell damage of the retina [21] and lens [22].

The functional roles of catechin have been well documented, but its effects on the lens epithelium following cataract formation remain poorly understood. Although great advances have been made in surgical treatment, the incidence of cataract in developing countries is so high that it overwhelms the capacity of surgical intervention. Nonsurgical treatment alternatives are in high demand. Accordingly, we investigated the effect of catechin on apoptosis in the lens epithelium following cataract formation in rats using the TUNEL assay, Western-blot for Bcl-2 and Bax, and immunohistochemistry for caspase-3.

Materials and Methods

Animals and treatments

Neonatal Sprague-Dawley rats (seven days old) together with their maternal rats were obtained from a commercial breeder (Orient Co., Seoul, Korea). The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. Each animal was housed under controlled temperature (23±2°C) and lighting conditions at room temperature, with the exception of membrane transfer. Membrane transfer was performed at 4°C with a

| N   | -methyl-N-nitrosourea (MNU, Sigma Chemical Co.) intraperitoneally. Just before use, MNU was dissolved in physiological saline containing 0.05% acetic acid.

Morphological analysis of cataract

Slit-lamp biomicroscopic examination was performed on each eye to provide a morphological assessment of the degree of opacification at 15 days after cataract induction. Prior to the examination, mydriasis was achieved using a topical ophthalmic solution containing tropicamide with phenylephrine hydrochloride (Santen Pharmaceutical, Osaka, Japan). One drop of the solution was instilled in each eye every 30 minutes for 2 hours, while the animals were in a dark room. After 2 hours, the eyes were examined by slit-lamp microscopy at 12× magnification.

Tissue preparation

The rats were sacrificed immediately after determination of cataract formation with slit-lamp biomicroscopy (15 days after cataract induction). The animals were anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France). At necropsy, both lenses were quickly removed under a surgical microscope GL-99B-V7 (DAVIS, California, CA, USA); a complete necropsy was performed on all animals. The lenses were fixed in 4% paraformaldehyde, dehydrated in graded ethanol, treated in xylene, and infiltrated and embedded in paraffin. Coronal sections of 5 μm thickness were made using a paraffin microtome (Leica, Nussloch, Germany) and were mounted on coated slides, then dried at 37°C overnight on a hot plate. Six slice sections were collected on average for each lens.

Western blot analysis

The lenses were collected and immediately frozen at -70°C. The tissues were homogenized with lysis buffer containing 50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl2, 6H2O, 1 mM EGTA, 1 mM PMSF, 1 mM Na2VO4, and 100 mM NaF, and then ultracentrifuged at 50,000 rpm for 1 hours. Protein content were measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Forty micrograms of protein was separated on SDS-polyacrylamide gels and were transferred onto a nitrocellulose membrane. Mouse antibodies against actin (1:2000; Santa Cruz Biotech, Santa Cruz, CA, USA), Bax (1:1000; Santa Cruz Biotech), and Bcl-2 (1:1000; Santa Cruz Biotech) were used as primary antibodies. Horseradish peroxidase-conjugated anti-mouse antibodies for Bax and Bcl-2 (1:2000; Amersham Pharmacia Biotech GmbH, Freiburg, Germany) were used as secondary antibodies. The experiment was performed in normal lab conditions at room temperature, with the exception of membrane transfer. Membrane transfer was performed at 4°C with a