Environmental Temperature and Cataract Progression in Experimental Rat Cataract Models

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Abstract

Purpose: To clarify whether or not ambient temperature relates to cataract development
or the progression of cataract formation.

Materials and Methods: 36 Brown Norway rats were divided into two groups, a high-
temperature ($35 \pm 2\,°C$, H = high) breeding group and a regular-temperature ($24 \pm 2\,°C$,
L = low) group. Each group was further divided into an experimentally induced diabetic
cataract subgroup (50 mg/kg streptozotocin, DM), an ultraviolet B exposure-induced cataract
subgroup ($200\,mJ/cm^2$, UV), and a normal control subgroup (C = control). Slit-lamp micro-
scopy and an anterior image analysis system (EAS-1000) were used to evaluate lens changes.

Results: Both the HC and HUV groups in the $35\,°C$ conditions showed higher light
scattering than that of the $24\,°C$ conditions (LC and LUV) 3 weeks after the start of the experi-
ment. Nine weeks after the start of the experiment, all the rats of the UV subgroups (HUV and
LUV) developed anterior subcapsular cataract. The temperature did not have much influence
on the progression of the UV-B-induced cataract. From 18 days after the start of the experi-
ment, the HC subgroup showed a wider light scattering area than the LC. An increase in abnor-
mal nuclear scattering light in the crystalline lens of group HC was found in 9 weeks after the
start of the experiment, and at the end of the experiment (78 weeks later), dense abnormal
nuclear light scattering was found including the prenuclear area. In contrast, the HDM group
in the $35\,°C$ conditions showed slower cataract progression than that of the LDM group at
$24\,°C$ room temperature.

Conclusions: Although further experiments are necessary before we can draw any con-
clusions about temperature and nuclear changes, paying attention to the effects of tempera-
ture on the lens is worthwhile.

Introduction

It is widely known that the mechanism of age-related cataract development, so-called 'senile cataract', is not caused by a single cataract risk factor, but has a multifactorial pathogenesis [1]. Exposure to solar ultraviolet radiation (UVR) is known as a risk factor for cataractogenesis. A large number of epidemiological studies [2–5] and animal experiments [6–8] that are concerned with the relationship between UVR exposure and cataract development are being carried out all over the world.

The authors' group has performed epidemiological cataract studies using the same study group with the same methodology in climatically different countries. The survey places were the main and southern island of Japan (Noto and Amami), Iceland and Singapore. The cataract prevalence, including early changes, was higher in Amami and Singapore. The main type of lens opacification was cortical in Noto and Iceland while that of Singapore was nuclear. A significant correlation was noticed between cortical opacification and the history of time spent outdoors [5]. These epidemiological results raised the question about the correlation between nuclear cataract and UVR exposure.

The UVR cataracts induced in the eyes of laboratory animals were cortical including anterior subcapsular cataract, and the opacity type remarkably differed from that of human age-related cataract. From the viewpoint that cataractogenesis can be multifactorial (diabetes, naphthalene, vitamin C-deficient guinea pigs, glutathione depression in rats), the authors have been carrying out a study which combines various cataract models with the UVR exposure. Up to now, however, we have experienced only cortical cataract, and no nuclear cataract [9]. The motivation behind this study came from the fact that the results of our epidemiological research did not agree with the observations of experimental research. The authors noticed that the tropical or subtropical zones, which had a high nuclear cataract prevalence and a high annual UVR exposure rate, also had a high ambient temperature and infrared exposure. The purpose of this study is to clarify whether or not ambient temperature relates to cataract development or the progression of cataract opacification.

Methods

All experimental animals were cared for and handled in accordance with the Guidelines for Animal Experiments in the Kanazawa Medical University and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Thirty-six 6-week-old male Brown Norway rats (BN/sea) were used in this experiment. The rats were divided into a high-temperature environment ($35 \pm 2\,°C$) breeding group (H = high) and a regular-temperature ($24 \pm 2\,°C$) breeding group (L = low). Each group

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was further divided into an experimentally induced diabetic cataract subgroup (DM), an ultraviolet B exposure-induced cataract subgroup (UV), and a normal control subgroup (C = control). Diabetes was induced by injecting 50 mg/kg streptozotocin into the tail vein. UV-B cataract was induced by exposure to a UV-B lamp (peak wavelength 310 nm, 40 μW/cm^2, 200 mJ/cm^2) every 2 days [10]. All rats were examined by slit-lamp microscopy under maximal mydriasis to see if there was any abnormality in the anterior segments, and then slit and retroillumination images were recorded by the anterior image analysis system (EAS-1000, Nidek) [11]. Lens image analysis was performed according to Kojima and Sasaki [11]. Changes in lens transparency were evaluated by applying a retroillumination image program, as previously described [12]. Scheimpflug slit images were evaluated by the program for retroillumination image analysis. The slit images were masked with a circle that did not overlap the images of the cornea or iris. The light scattering of the lens within the circle mask was measured as the total pixels after the corneal reflex had been eliminated. The light scattering of the lens was expressed by the pixels of the area that showed higher values than the surroundings.

The three subgroups of the high-temperature breeding group (DM, UV and C) were bred in a special breeding box. The high-temperature source was a ceramic heater, and the thermal management of the temperature of the breeding box was carried out by a thermostat which was maintained at 35 $\pm$ 2 °C from 9 a.m. to 4 p.m., and at 24 $\pm$ 2 °C for the rest of the time. The temperature and humidity in all the experimental terms were measured and recorded (Thermo Recorder TR-7S, T and D).

Body weight, blood glucose (Gluiest E, Sanwa) and urine glucose (Pretest 3a, Wako) were measured as an index of general health.


Results

General Health Conditions

The body weight of C subgroups (HC, LC) and UV-B subgroups (HUV, LUV) gradually increased. The highest body weight was in the LC group followed by HC > LUV > HUV > HDM > LDM groups (data not shown). In general, the high-temperature subgroups, such as HUV or HC showed a tendency towards lower body weight than the LUV or LC from 12 days after the start of the experiment. The tendency of the body weight difference between the H and L groups disappeared 2 months after the start of the experiment. The mean body weight in the HDM subgroup was always higher than that in the LDM subgroup 7 days after the start of the experiment, but we found that the statistical difference was only a few points. Again, the body weight difference disappeared 2 months after the start of the experiment.

DM Subgroup

All the DM rats showed a high blood glucose level ($>$200 mg/dl) 3 days after streptozotocin injection. Table 1 shows the blood and urine glucose in the DM rats after 2 weeks and 7 weeks of DM induction. There was no big

Table 1. Comparison of blood and glucose levels between the high-temperature and regular-temperature groups

|	Blood glucose		Urine glucose	
	2 weeks after DM	7 weeks after DM	2 weeks after DM	7 weeks after DM
Low	>500	482 ± 14	2,000	2,000
High	299 ± 118	430 ± 112	2,000	2,000
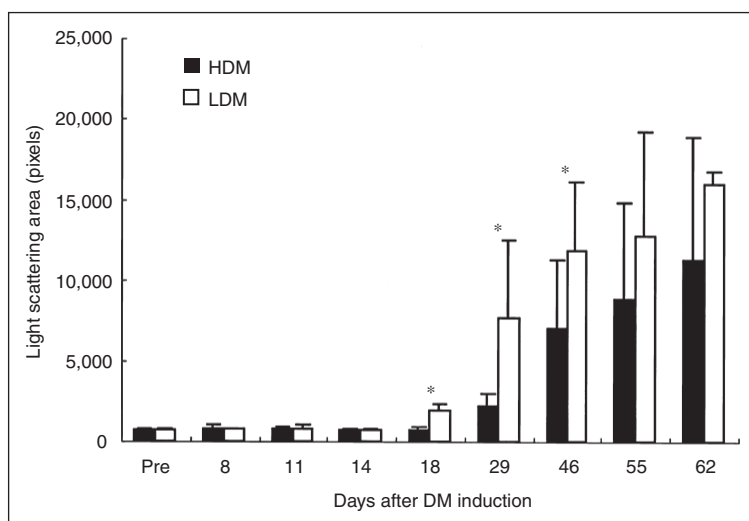
Fig. 1. Time course of light scattering in the lenses of the DM subgroups. Bars indicate mean ± SD. *p > 0.05 [reproduced by courtesy of Medical Aoi-shuppan, Ref. No. 14].

difference in urine glucose between HDM and LDM. In contrast, the blood glucose level of HDM showed a tendency towards a lower level compared to the LDM subgroup (table 1).

Figure 1 shows the light scattering area of HDM and LDM. The light scattering area of the LDM subgroup showed a rapid increase 18 days after DM induction. The HDM subgroup, however, showed a gradual increase in the light scattering area. The light scattering area of the LDM subgroup was wider than that of the HDM subgroup, and was statistically significant 18, 29 and 46 days after DM induction (fig. 1). All of the LDM rats had developed mature cataract by 9 weeks after DM induction while the LDM subgroups mainly showed cortical cataract and only a few rats had mature cataract (fig. 2).

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24°C (LDM)
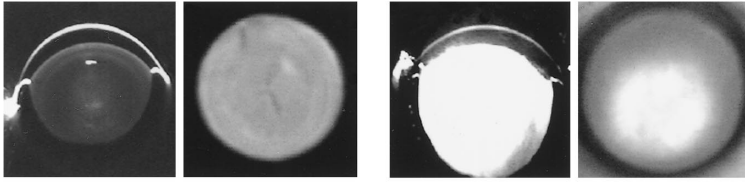
35°C (HDM)
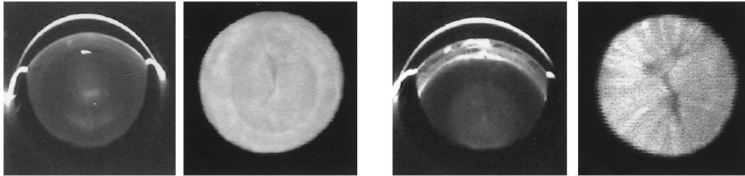
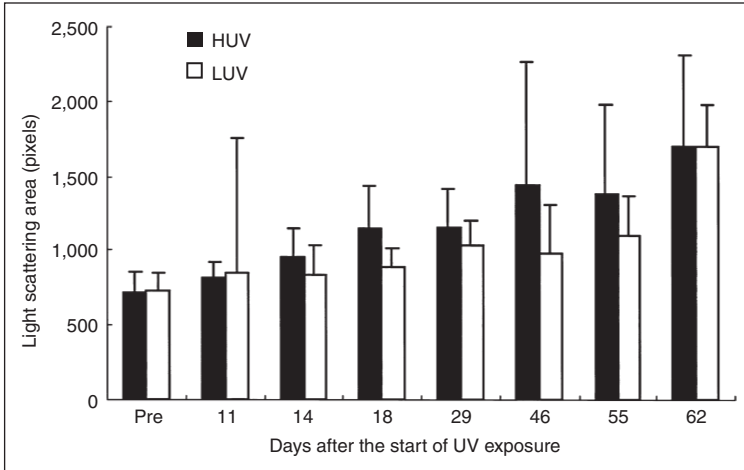Baseline   9 weeks after DM induction

Fig. 2. Representative lens conditions before the start of the experiment and 9 weeks after DM induction in both the regular-temperature (24°C) and high-temperature (35°C) subgroups [reproduced by courtesy of Medical Aoi-shuppan, Ref. No. 14].

UV-B Subgroup

All UV-B-exposed rats developed photokeratitis after UV-B irradiation. There was no significant time difference in the healing of corneal photokeratitis between HUV and LUV (data not shown). The light scattering area of all rat lenses showed a gradual increase. The HUV subgroup showed a tendency towards a wider light scattering area than the LUV. This tendency, however, disappeared in the late experimental phase (fig. 3). Nine weeks after the start of the experiment, all the rats of the UV subgroups had developed anterior subcapsular cataract (fig. 4). Figure 4 shows the condition of the lens 6 months after the start of the experiment. It seems that the temperature did not have much influence on the progression of the UV-B-induced cataract, aside from the increase in nuclear light scattering (fig. 4).

Normal Rat Group

The light scattering area of HC and LC gradually increased. From 18 days after the start of the experiment, the HC subgroup showed a wider light scattering area than the LC. A significant difference ($p < 0.05$) between the light scattering areas of HC and LC was noticed 62 days after the start of the experiment (fig. 5). An increase in abnormal light scattering in the crystalline lens was found in the lens nucleus 9 weeks after the start of the experiment, and at the end of the experiment (78 weeks later), dense abnormal light scattering was found in the nucleus including the prenuclear area (fig. 6).
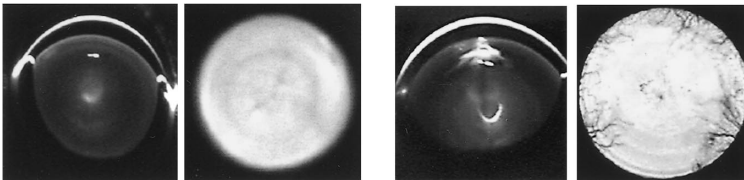
Fig. 3. Time course of light scattering in the lenses of the UV exposure subgroups. Bars indicate mean ± SD.
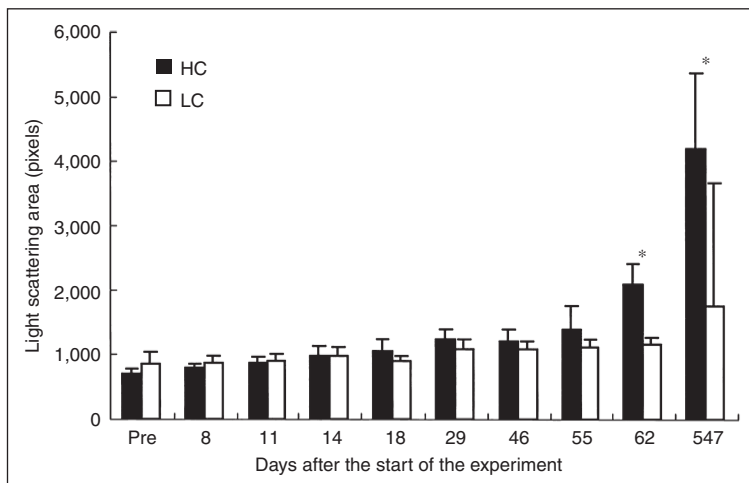

24°C (LUV)
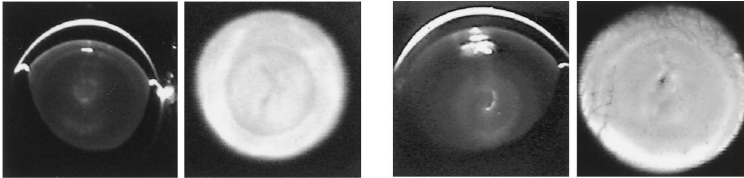
35°C (HUV)

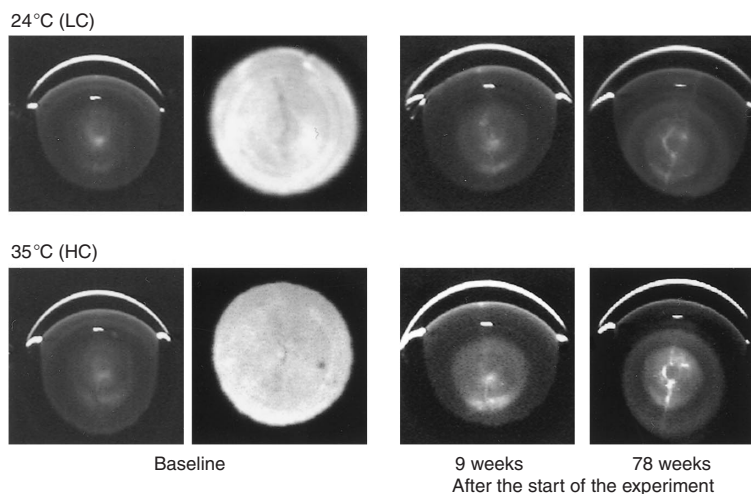Baseline   9 weeks after the start of UV exposure

Fig. 4. Representative lens conditions before the start of the experiment and 9 weeks after the start of UV exposure in both the regular-temperature (24 °C) and high-temperature (35 °C) groups.

Discussion

The high-temperature setting in this experiment was determined to correspond to the temperature fluctuation of the tropical zone [the maximum

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Fig. 5. Time course of light scattering in the lenses of the normal groups. Bars indicate mean $\pm$ SD. *p > 0.05.

Fig. 6. Representative lens conditions before the start of the experiment, 9 weeks after and 78 weeks after the start of the experiment in both the regular-temperature (24 °C) and high-temperature (35 °C) groups.

temperature of Singapore: 33 °C, the lowest temperature: 23 °C, the maximum temperature of Bangkok (Thailand): 36 °C, the lowest temperature: 17 °C; Ala-Blance, weather information, http://www.jah.ne.jp/~abflt1/climate.html] where cataract epidemiology surveys are being carried out. The temperature of

the H group was set at 35 ± 2 °C from 9 a.m. to 4 p.m., and at 24 ± 2 °C during the rest of the time.

The cataract progression of the DM subgroup in the high-temperature environment (35 °C) was slower than that of those kept at the regular temperature (24 °C). The average weight of HDM was higher and the average glucose level was lower than those of the LDM subgroup. Although the above-mentioned results give the impression that the diabetic condition of the HDM was less severe than that of the LDM, it is too early to conclude that a high-temperature environment improves diabetes mellitus.

Regarding the relationship between diabetes mellitus and environmental temperature, an epidemiology survey compared the incidence of infant type I diabetes mellitus (IDDM) worldwide, and it was reported that the number of registered IDDM patients was smaller in regions where the annual mean temperature was high. The authors mentioned that the extraordinary geographic differences in IDDM distribution were caused by a host (genetic) factor, an environmental agent (virus, diet), or both, but the definitive cause remains unknown [13]. MacDonald et al. [14] compared the glycosylated hemoglobin concentrations of normal persons according to season, and reported that there is a seasonal variation in the glycosylated hemoglobin concentration: it is lowest in summer and highest in winter. As for the reason why the generation of diabetes mellitus increases at a low temperature, it is guessed that the secretion of insulin decreases in winter. There has been a report which examined the effect of temperature on aldose reductase (AR) which is closely related to true diabetic cataract. Ohtsuka et al. [15] measured erythrocyte AR enzyme activity of normal and type II DM patients after immersion in water at three different temperatures (25, 39, 42 °C). They found that the AR activity increased by 37.6% after the patients had taken a bath at a high temperature (42 °C, 10 min), in contrast to the lowered AR activity at 39 °C (–52.2%, $p < 0.01$) and 2 5 °C (–47.0%, $p < 0.05$). From the above-mentioned results, the authors hypothesized about the possibility of an adverse effect on DM complications in a high-temperature environment. Further examination, including of general conditions, is indispensable in order to clarify the relationship between environmental temperature and diabetes mellitus.

In the UV subgroups, a difference in the opacity area (HUV > LUV) was observed in the early phase of the experiment, and gradually disappeared in the late experimental phase. The two following causes were considered for this phenomenon. First, lens opacification could not be exactly determined by image analysis methods due to corneal photokeratitis. The other explanation for this phenomenon could be the acclimation of the rats to the environment. There appeared to be no interaction between UV and environmental temperature in ultraviolet-induced cataract.

The area of anomalous scattering light of the HC subgroup was significantly wider than that of LC, and the nuclear light scattering had increased (fig. 5, 6). On the correlation between cataract and temperature, Miranda [16] reports that an analysis of the data reported in the literature suggests that the onset and prevalence of senile cataract follow the same trend as that of presbyopia. That is, 'senile cataract' (age-related cataract) develops earlier and is more prevalent in warmer regions. Miranda [16] also speculates that lifelong exposure of the lens to regular small increases in temperature may constitute an accelerating cofactor in the aging process by accelerating the metabolic rate of the lenticular epithelium [16]. It is reported that Brown Norway rats often show an increase of nuclear light scatter with aging [17]. For most biological systems, a temperature increase of 10 °F doubles the reaction rate [18, 19]. The increase of the scattered light in the lens nuclear regions also points to the possibility that high-temperature breeding promotes aging alternations in the rat. As for the relationship between cataractogenesis and temperature, aside from special research such as that concerning the glassblower cataract, very little attention has been paid to the significance of the ambient temperature of living environments. The relationship between cataract and environmental temperature should be the focus of more research in the future.

Acknowledgment

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