Morphological change in the corneal endothelium due to ultraviolet radiation in welders

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SUMMARY To clarify the relationship between morphological changes in the corneal endothelium and ultraviolet (UV) radiation, specular microscopic examinations were performed on both eyes of 118 welders and 85 controls. The results showed: a decrease in the hexagonal cells in welders (20-29 years) in comparison with the controls (20-29) ($p < 0.05$); an increase in the mean cell size of the endothelium and a decrease in the hexagonal cell population with increasing age in both groups; increases in standard deviation (SD) and the coefficient of variation (CV) of the mean cell size in both groups; increases in SD and CV of the mean number of cell sides in both groups; and no difference in the mean cell size between the two groups. These results show that UV radiation damages not only the corneal epithelium but also the endothelium, and suggest that it causes more pleomorphic change (a decrease in hexagonal cell population) than enlargement of the mean cell size.

Arc welding generates visible light and much ultraviolet (UV) light. Most of the UV radiation is absorbed by the superficial layers of the cornea. UV light of 265-275 nm wavelength causes photokeratitis (keratitis superficialis diffusa).¹ UV light of longer wavelength reaches the corneal endothelium, and that over 295 nm reaches the lens and can cause cataracts. However, there has been no report on the effect of UV radiation on the endothelial morphology of the human cornea.

UV radiation is now thought to be one of the factors promoting cataracts with aging.^{2,3} The endothelium is also affected by aging.⁴⁻¹⁰ The mean cell size of the endothelium increases⁴⁻⁸ and hexagonal cells of the endothelium decrease with age.^{9,10} The effects of aging on these changes are not clear. We assumed that UV radiation has an important role in the endothelial aging phenomenon and tried to study the relationship between the endothelial changes and UV radiation in welders, who are occupationally exposed to excess UV radiation.

Subjects and methods

A total of 118 Japanese male welders of mean age 44.1 years (range 24-66 years) in two welding factories in Osaka were studied. Their mean employment duration as welders was 17.4 years (range 1-39 years). The control group comprised 85 men of mean age of 41.8 years (range 20-73 years), employees in two chemical factories (42 males), and male outpatients (43 persons) without any corneal diseases from the Department of Ophthalmology in two hospitals. The welders were asked about their length of employment as a welder, the welding method used, and personal history of ocular disease. We excluded one welder with corneal disease, and one control who had once worked in welding. We also excluded persons with myopia gravis (over -6 dioptres) from this study.

Specular microscopes (Keeler-Konan camera, and Bio-Optics) were used to photograph the central corneal endothelium of both eyes of the subjects three times after treatment with ophthalmic surface anaesthetic (0.4% oxybuprocaine hydrochloride). Kodak film (Tri-X pan, ASA 400) was used for all

Fig. 1 Cell-free area of the endothelium of a 43-year-old male welder. After extensive cell loss, healing seems to be taking place with invasion by the surrounding cells.

photographs. The film contrast was increased twice by force-developing, followed by normal fixing and printing. Sharp focusing was essential, and we excluded data from 15 eyes of welders and 19 eyes of the controls because of poor photographic quality. The best focused film from three films for each eye was chosen for printing. A 390-fold cell magnification on printing paper was used, and 100–160 cells with

Fig. 2 Typical rosette formation of endothelial cells. Numerous small cells usually surround a large one, producing a rosette pattern, which is characteristic of pleomorphism with aging.

Fig. 3 Pattern of cumulative frequency of endothelium size. About 80% of normal endothelium is composed of 0–350 μm² cells. The arrow shows a curve indicating a tendency of enlarging cell size.

completely visible outlines in a cluster were traced on a printing paper by two trained technicians who were unaware of the purpose of the study.

A digitiser (Muto Industry, Japan) was used to digitise the cell boundaries of each tracing. The following morphological characteristics of 100–160 cells for each eye were recorded by computerised morphometry (Muto Industry—Cannon, Japan): (1) cell size; (2) number of sides (polygonality) of each cell; (3) mean cell size, standard deviation (SD), and coefficient of variation (CV); (4) mean number of cell sides, SD, and CV; (5) enlarging tendency of the cell; and (6) endothelium with cell-free area (Fig. 1). About 80% of normal endothelium is composed of 0–350 μm² cells (Fig. 3). We counted the number of cells where less than 50% of the endothelium was occupied by 0–350 μm² cells (e.g., Fig. 3, arrow).

Photographs were taken with two specular microscopes using Keeler-Konan's camera, except in one hospital. Data on cell size obtained by a Bio-Optics camera were eliminated to exclude artefacts due to the difference in apparatus.

Welding methods used in the two welding factories studied were: shielded metal arc welding (SMAW), submerged arc welding (SAW), and metal active gas shielded arc welding (MAG). The welders did not use the same welding method for long periods and sometimes changed methods frequently. Therefore the effects of differences in methods were not considered.

Statistical significance was tested by Student's *t* test and the χ^2 test.

Results

Table 1 shows the mean cell size of the endothelium of both groups. The mean cell size increased with

Table 1 Mean cell size (± 1 SD) of the corneal endothelium

Age (years)	Welders		Controls	
	n	Cell size (μm^2)	n	Cell size (μm^2)
20-29	12	284 $\pm$ 17.2	9	290 $\pm$ 31.1
30-34	27	284 $\pm$ 33.4	22	296 $\pm$ 26.2
35-39	28	280 $\pm$ 27.8	16	282 $\pm$ 27.1
40-44	44	286 $\pm$ 30.6	22	300 $\pm$ 37.6
45-49	41	293 $\pm$ 26.4	14	302 $\pm$ 53.0
50-54	45	292 $\pm$ 34.9	15	302 $\pm$ 35.1
55-	24	302 $\pm$ 30.5	13	306 $\pm$ 31.9
Correlation coefficient	0.879**		0.919**	

Significant difference: ** $p < 0.01$.

increasing age (welders, $r = 0.879$, $p < 0.01$; controls, $r = 0.919$, $p < 0.01$). No significant difference was observed between the two groups.

Table 2 shows the mean SD and CV of cell size of both groups. Both values increased with increasing age in both groups (welders: SD, $r = 0.960$, $p < 0.01$;

CV, $r = 0.841$, $p < 0.05$; controls: SD, $r = 0.877$, $p < 0.01$; CV, $r = 0.695$, $p < 0.10$). No significant difference was observed between the two groups.

Table 3 shows the frequency of the occurrence of multisided cells. The value for hexagonal cells decreased with increasing age in both groups (welders, $r = -0.841$, $p < 0.05$; controls, $r = -0.846$, $p < 0.05$). The incidence of hexagonal cells in welders of 20-29-year-old-group was significantly higher than that in the controls ($p < 0.05$) (Fig. 4). But the mean number of cell sides was 6.0 for all ages and no difference was observed between the two groups.

Table 4 shows the mean SD and CV of the number of cell sides. Both values increased with increasing age in both groups (welders: SD, $r = 0.960$, $p < 0.01$; CV, $r = 0.841$, $p < 0.05$; controls: SD, $r = 0.817$, $p < 0.05$; CV, $r = 0.893$, $p < 0.01$).

Table 5 shows the incidences of large cells (over 700 μm^2), the enlarging tendency and the cell-free area of both groups. No difference in these values was observed between the two groups. But an increasing trend in these values was observed with increasing age in both groups.

Table 2 Standard deviation (± 1 SD) and coefficient of variation (± 1 SD) of mean cell size of the corneal endothelium

Age (years)	Welders			Controls		
	n	SD	CV	n	SD	CV
20-29	12	82.8 $\pm$ 11.3	28.9 $\pm$ 3.73	9	80.0 $\pm$ 13.4	28.5 $\pm$ 5.47
30-34	27	83.9 $\pm$ 15.2	29.9 $\pm$ 4.89	22	87.1 $\pm$ 13.6	29.5 $\pm$ 4.33
35-39	28	87.1 $\pm$ 13.2	33.0 $\pm$ 10.8	16	88.9 $\pm$ 16.2	31.5 $\pm$ 4.58
40-44	44	90.8 $\pm$ 13.4	32.3 $\pm$ 6.39	22	99.5 $\pm$ 32.8	33.4 $\pm$ 8.73
45-49	41	95.8 $\pm$ 17.3	32.6 $\pm$ 4.67	14	97.8 $\pm$ 29.6	31.8 $\pm$ 4.73
50-54	45	93.7 $\pm$ 14.6	33.0 $\pm$ 4.92	15	100.9 $\pm$ 20.2	32.5 $\pm$ 3.92
55-	24	103.7 $\pm$ 22.1	34.6 $\pm$ 5.97	13	97.5 $\pm$ 18.5	31.5 $\pm$ 5.38
Correlation coefficient	0.960**		0.841*	0.877**		0.695

Significant difference: * $p < 0.05$; ** $p < 0.01$.

Table 3 Frequency in polygonality in endothelial cells (± 1 SD)

Age (years)	Welders				Controls			
	n	4-5 sides (%)	6 sides (%)	7-10 sides (%)	n	4-5 sides (%)	6 sides (%)	7-10 sides (%)
20-29	12	19.4 $\pm$ 3.48	61.5 $\pm$ 7.42*	19.1 $\pm$ 4.15	20	16.5 $\pm$ 2.87	67.7 $\pm$ 5.38	15.7 $\pm$ 3.05
30-34	27	19.8 $\pm$ 4.26	60.6 $\pm$ 6.97	19.7 $\pm$ 2.76	26	18.2 $\pm$ 4.25	63.9 $\pm$ 8.56	17.9 $\pm$ 4.47
35-39	28	21.2 $\pm$ 4.24	59.0 $\pm$ 6.32	19.7 $\pm$ 3.45	18	19.5 $\pm$ 3.84	62.0 $\pm$ 7.61	18.5 $\pm$ 4.32
40-44	44	20.9 $\pm$ 3.72	58.1 $\pm$ 6.88	21.0 $\pm$ 3.93	24	21.0 $\pm$ 5.05	59.9 $\pm$ 8.72	19.1 $\pm$ 4.27
45-49	41	21.4 $\pm$ 4.04	57.9 $\pm$ 7.83	20.7 $\pm$ 4.21	22	21.4 $\pm$ 3.33	57.6 $\pm$ 6.55	20.9 $\pm$ 3.69
50-54	45	21.7 $\pm$ 3.52	57.7 $\pm$ 6.77	20.6 $\pm$ 3.84	19	20.5 $\pm$ 3.95	59.8 $\pm$ 7.22	19.6 $\pm$ 3.93
55-	24	22.1 $\pm$ 5.21	58.5 $\pm$ 8.55	19.3 $\pm$ 3.71	22	20.7 $\pm$ 5.31	59.5 $\pm$ 9.64	19.8 $\pm$ 4.97
Correlation coefficient	0.943**		-0.841*	0.352	0.834*		-0.846*	0.838*

Significant difference: * $p < 0.05$; ** $p < 0.01$.

Fig. 4 Frequency of the occurrence of multisided cells in 20-29-year-old subjects. The numbers of hexagonal cells in welders are significantly lower than in the controls due to pleomorphism ($p < 0.05$). Symmetrical frequency patterns were observed in both groups.

Discussion

UV radiation inhibits the synthesis of DNA, RNA, and proteins, inhibits cellular division, causes changes in cellular permeability and motility, and damages

corneal cells.¹ The corneal epithelium has a peak sensitivity to UV radiation at 270 nm wavelength^{11 12} (some say 288 nm¹³), and UV light below 295 nm is absorbed by the five corneal layers, including the stroma and the endothelium. UV light longer than 295 nm reaches the ocular lens and can cause cataracts.

Normal human corneal endothelium is a monolayer of polygonal cells covering the posterior surface of the cornea and regulates stromal hydration to maintain corneal thickness within a narrow range. Many findings from histological and specular microscopic examinations have shown that endothelial cells of normal cornea enlarge and are increasingly pleomorphic with age.¹⁴⁻¹⁶ The enlarged cells and increased pleomorphism in the endothelium found in older subjects implies that a gradual loss of cells occurs and the endothelium cannot adequately replace them because of the poor ability of endothelial cell division in humans. However, the effect of such morphological alteration on corneal function is not clear, since no direct correlation has been observed between the degree of cell loss and corneal function.

The effects of aging on human endothelium can be divided into two categories: increasing cell size and decreasing hexagonal cell population. UV radiation seems to play an important role in endothelial aging. Therefore the aim of this study was to clarify the

Table 4 Standard deviation and correlation coefficient of hexagonal cell population of corneal endothelium (± 1 SD)

Age (years)	Welders			Controls		
	n	SD	CV	n	SD	CV
20-29	12	0.567 $\pm$ 0.088	10.5 $\pm$ 1.18	20	0.533 $\pm$ 0.071	9.4 $\pm$ 1.18
30-34	27	0.601 $\pm$ 0.085	10.9 $\pm$ 1.25	26	0.586 $\pm$ 0.077	10.6 $\pm$ 1.31
35-39	28	0.628 $\pm$ 0.066	11.4 $\pm$ 1.15	18	0.606 $\pm$ 0.100	10.9 $\pm$ 1.37
40-44	44	0.638 $\pm$ 0.094	11.5 $\pm$ 1.40	24	0.655 $\pm$ 0.130	11.8 $\pm$ 2.15
45-49	41	0.645 $\pm$ 0.089	11.5 $\pm$ 1.53	22	0.657 $\pm$ 0.076	11.8 $\pm$ 1.24
50-54	44	0.630 $\pm$ 0.090	11.6 $\pm$ 1.24	19	0.633 $\pm$ 0.098	11.4 $\pm$ 1.50
55-	24	0.642 $\pm$ 0.118	11.6 $\pm$ 1.90	22	0.654 $\pm$ 0.097	11.6 $\pm$ 1.71
Correlation coefficient		0.960**	0.841*		0.817*	0.893**

Significant difference: * $p < 0.05$; ** $p < 0.01$.

Table 5 Tendency of enlargement of the endothelium

Age (years)	Incidence of large cells (over 700- μ m ²)		Incidence of 0-350- μ m ² cells under 50%		Incidence of cell-free are	
	Welders	Controls	Welders	Controls	Welders	Controls
20-29	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
30-34	3.7	4.5	7.4	0.0	0.0	2.9
35-39	7.1	0.0	0.0	0.0	6.7	0.0
40-44	9.1	4.5	2.3	4.6	4.6	0.0
45-49	9.8	21.4	2.4	14.3	0.0	7.1
50-54	22.2	13.3	4.2	0.0	4.4	0.0
55-	33.3	7.7	4.2	7.7	20.9	21.4

relationship between these alterations and UV exposure.

This study showed no difference in cell size between the two groups but a tendency toward a decreased hexagonal cell population in welders compared with the controls. The results indicated that: (1) UV radiation causes loss of endothelial cells, which may be followed by chemical and physical changes in DNA, RNA, and protein; (2) cell loss without adequate replacement may result in cell-free areas, which may be covered eventually by migration and enlargement of the surrounding cells; and (3) a large cell occupying a cell-free area is surrounded by numerous small cells, producing a rosette pattern which is characteristic of pleomorphism.

Fig. 1 shows a typical cell-free area in the endothelium of welders, and Fig. 2 is an example of pleomorphism with a rosette pattern in the endothelium of welders. Small cells of tetragonal, pentagonal, and heptagonal shapes instead of hexagonal cells surrounding a large cell were seen (Fig. 2). A decrease in hexagonal cells resulted in symmetrical increases in pentagonal and heptagonal cells (Fig. 4). The question remains of why there was a significant difference in the pleomorphism but not in the cell size. For example, Fig. 2 shows a case in which loss of one hexagonal cell in 100 cells caused a 1% increase in a mean cell size but a 6% decrease in the hexagonal cell population. Therefore the mean number of cell sides changes more sensitively than the mean cell size. These changes also result in increased variation in cell size and polygonality. These increases in the variation with age were not significantly different between the two groups in older subjects.

This study clarified the effect of UV radiation on the corneal endothelium and indicated that UV radiation is an important factor in the gradual loss of the endothelium with aging. Another noteworthy

finding was that pleomorphic change is affected more than enlargement of cell size.

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