

# Intraocular irrigating solutions and barrier function of retinal pigment epithelium

Makoto Araie, Minoru Kimura

## Abstract

**Aim**—To study the effect of intraocular irrigating solutions on the barrier property of the retinal pigment epithelium (RPE).

**Methods**—The isolated rabbit RPE-choroid mounted on Ussing-type chambers under short circuit conditions was used. According to a previous study, the inward (from the choroid to the vitreous side) permeability of the tissue to carboxyfluorescein was adopted as a quantitative index of the barrier function of the RPE cells.

**Results**—Of the three solutions tested, Krebs-Ringer solution, a commercially available glucose glutathione bicarbonate solution (BSS plus), and glucose citrate-acetate bicarbonate solution (Opeguard), BSS plus gave a significantly lower permeability ( $1.1 \times 10^{-6}$  cm/s on average) than Krebs-Ringer solution or Opeguard ( $1.9$  or  $1.8 \times 10^{-6}$  cm/s on average, respectively) (unpaired *t* test with Bonferroni's correction,  $p < 0.05$ ). Since the major chemical difference between BSS plus and the other two solutions is the incorporation of oxidised glutathione (GSSG), the effects of GSSG were studied using solutions having an identical composition to BSS plus, but with various concentrations of GSSG. The solution containing 0.3 mM GSSG gave significantly lower permeability than that without GSSG ( $1.1 \times 10^{-6}$  cm/s *v*  $2.0 \times 10^{-6}$  cm/s) (unpaired *t* test with Bonferroni's correction,  $p < 0.05$ ).

**Conclusion**—It was suggested that BSS plus is less harmful to the barrier function of the RPE cells and that GSSG has a beneficial effect on its maintenance.

(*Br J Ophthalmol* 1997;81:150-153)

Vitrectomy allowed us to manage otherwise untreatable ocular pathologies, but this technique significantly involves the ocular tissues surrounding the vitreous cavity. Substitution of the vitreous by an artificial intraocular irrigating solution would probably disturb the physiology of the retina and choroid, and thus influences of intraocular irrigating solutions used in vitrectomy on the retinal function are of primary clinical importance.

Many of the previous studies which addressed this problem have examined the function of the sensory retina, using the electroretinogram (ERG),<sup>1-6</sup> fluorescein angiogram,<sup>7</sup> or rate of glycolysis and levels of some

metabolites<sup>8</sup> as factors to be compared. Aside from a study by Shirakawa and associates comparing phagocytotic activity of cultured RPE cells in various intraocular irrigating solutions,<sup>9</sup> there is a relative paucity of information on the effects to retinal pigment epithelium (RPE). RPE cells are directly exposed to the irrigating solution during vitrectomy when the eye is complicated with rhegmatogenous retinal detachment and these cells play an important role in maintaining the physiology of the sensory retina through their metabolic activity and barrier properties. In the present study, we focused attention on the RPE and compared the effects of various intraocular irrigating solutions on the barrier property of the isolated RPE-choroid preparation.

As irrigating solutions, Krebs-Ringer solution, a glucose glutathione bicarbonate solution, BSS plus (Alcon Laboratories, Fort Worth, TX, USA), and a glucose citrate-acetate bicarbonate solution, S-MA<sub>2</sub> (Opeguard, Senju Pharmaceutical Co, Osaka, Japan), were examined. BSS plus and S-MA<sub>2</sub> were chosen because both were commercially available intraocular irrigating solutions widely used for intraocular surgery including vitrectomy. Oxidised glutathione (GSSG), an ingredient of BSS plus reportedly has a beneficial effect on the barrier function of the corneal endothelium and the blood-aqueous barrier.<sup>10,11</sup> Therefore, the effects of GSSG on the barrier property of the isolated RPE-choroid was also studied.

## Materials and methods

### TISSUE PREPARATION

Details of tissue preparation have been described in a previous paper.<sup>12</sup> Briefly, the eye was enucleated immediately after sacrificing adult New Zealand albino rabbits with an overdose of pentobarbitone. The enucleated eye was bisected at the equator and the posterior half was placed in a Petri dish containing Krebs-Ringer solution maintained at 37°C and bubbled with 95% oxygen and 5% carbon dioxide. The eye cup was opened via three meridional incisions and the vitreous and sensory retina were carefully peeled off. The suprachoroidal tissue was separated from the sclera with meticulous care and a rectangular RPE-choroid complex was isolated. The isolated RPE-choroid was then placed between two halves of an Ussing-type chamber according to procedures described previously.<sup>12</sup> The exposed tissue surface area was 0.14 cm<sup>2</sup> and the volume of each chamber was 7.0 ml. Both chambers were filled with one of the test solutions bubbled with 95% oxygen and 5%

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Accepted for publication  
14 October 1996

Table 1 Chemical composition of solutions examined (mmol/l)

Constituent	BSS plus	S-MA <sub>2</sub>	Krebs-Ringer
Sodium chloride	122.2	112.9	118.05
Potassium chloride	5.1	4.8	4.69
Calcium chloride	1.0	1.2	2.15
Magnesium chloride	1.0	—	0.54
Magnesium sulphate	—	1.2	—
Sodium phosphate	3.0	—	1.01
Sodium bicarbonate	25.0	25.0	25.01
Glucose	5.1	8.3	11.1
Sodium acetate	—	4.4	—
Sodium citrate	—	3.4	—
Oxidised glutathione	0.3	—	—
pH	7.4	7.4	7.4
Osmolality (mOsm)	306	293	298

carbon dioxide and the system was maintained at 37°C. All experiments were carried out under short circuit conditions, wherein the short circuit current (SCC) was continuously monitored and the transepithelial potential difference was measured every 15 minutes throughout the experimental period of about 3 hours. Rabbits were treated according to the ARVO Resolution on the Use of Animals in Research.

#### MEASUREMENT OF BARRIER PROPERTY OF RPE

Since our previous study indicated that the inward (from the choroid to the vitreous side) movement of carboxyfluorescein across the isolated rabbit RPE-choroid occurs almost exclusively by passive diffusion through the paracellular spaces,<sup>13</sup> this dye was used as a tracer dye for evaluating the barrier property of the RPE. After an approximately 40 minutes of stabilisation of the SCC, reagent grade 5(6)-carboxyfluorescein (Eastman Kodak Co, Rochester, NY, USA) was added to the choroidal side chamber to give a final dye concentration of 300 µM in the chamber. Thereafter, the inward movement of the dye was determined for 2 hours and the inward tissue permeability to carboxyfluorescein (cm/s) was calculated as previously described.<sup>12</sup>

#### INTRAOCULAR IRRIGATING SOLUTIONS

In the first experimental series, Krebs-Ringer solution was freshly prepared in the laboratory just before use, freshly manufactured S-MA<sub>2</sub> (Opegard) was purchased from Senju Pharmaceutical Co Osaka, and BSS plus was kindly supplied by Santen Pharmaceutical Co, Osaka. The chemical compositions of these solutions are listed in Table 1. The inward permeability of the isolated rabbit RPE-choroid to carboxyfluorescein was determined using each of these three solutions and the results were compared.

Table 2 Comparison of BSS plus, S-MA<sub>2</sub>, and Krebs-Ringer solution

Solution	Transepithelial potential difference (mV)	Tissue resistance (Ωcm <sup>2</sup> )	Permeability* (×10 <sup>-6</sup> cm/s)
BSS plus	140 (47)	4.8 (1.2)	1.05 (0.16)†‡
S-MA <sub>2</sub>	114 (16)	5.6 (0.2)	1.79 (0.29)†
Krebs-Ringer solution	127 (25)	4.5 (1.6)	1.89 (0.69)‡

Values are mean (SD) in seven experiments.

\*Permeability of isolated RPE-choroid to carboxyfluorescein.

†Between group difference was significant at p<0.01 (unpaired *t* test with Bonferroni's correction for multiple comparison).

‡Between group difference was significant at p<0.05 (unpaired *t* test with Aspin-Welch's correction for unequal variance and Bonferroni's correction for multiple comparison).

In the second experimental series, solutions having the identical chemical composition to BSS plus, except that oxidised glutathione (GSSG) was omitted (BSS plus without GSSG) and a GSSG concentration of 0.1 mM (BSS plus with 0.1 mM GSSG) was added were also generously prepared and supplied by Santen Pharmaceutical Co. The pH and osmolality of BSS plus without GSSG or BSS plus with 0.1 mM GSSG was 7.4 and 303 mOsm, respectively. The inward tissue permeability was determined using BSS plus, BSS plus with 0.1 mM GSSG, or BSS plus without GSSG and the result was compared to evaluate the effect of the GSSG concentration added.

## Results

### COMPARISON AMONG KREBS-RINGER SOLUTION, S-MA<sub>2</sub>, AND BSS PLUS

The addition of carboxyfluorescein at 300 µM caused no significant effects on osmolality or pH of the solutions. The SCC reached a steady state in 30 minutes and the addition of carboxyfluorescein showed no significant effect on the SCC. The transepithelial potential difference and the tissue resistance showed no significant change during the experiment and no significant difference was seen in the transepithelial potential difference or the tissue resistance among the three solutions (Table 2). The obtained inward permeabilities are summarised in Table 2. The inward permeability obtained with BSS plus was significantly lower than that obtained with Krebs-Ringer solution or S-MA<sub>2</sub> (unpaired *t* test with Aspin-Welch correction for unequal variance and Bonferroni's correction for multiple comparison, p<0.05). There was no significant difference between the inward permeability obtained with Krebs-Ringer solution and that obtained with S-MA<sub>2</sub>.

### EFFECT OF GSSG CONCENTRATION ADDED

The transepithelial potential difference and the tissue resistance showed no significant change during the experiment and no significant difference was seen in the transepithelial potential difference or the tissue resistance among the three solutions (Table 3). The inward permeabilities obtained are summarised in Table 3. The inward permeability obtained with BSS plus was significantly lower than that obtained with BSS plus without GSSG (unpaired *t* test with Aspin-Welch correction for unequal variance and Bonferroni's correction for multiple comparison, p<0.05), while it showed no significant difference from that obtained with BSS plus with 0.1 mM GSSG.

## Discussion

According to our previous study,<sup>13</sup> unlike the isolated dog RPE-choroid,<sup>14</sup> determination of the inward permeability to carboxyfluorescein in the isolated rabbit RPE-choroid is little affected by the outward (from the vitreous to the choroid side) active transport of carboxyfluorescein, and the inward permeability obtained using the present preparation is thought to almost exclusively reflect the diffusion of the

dye through paracellular spaces. The rabbit is cheaper to obtain and better suited for extensive studies. Further, since the effects of intraocular irrigating solutions on the barrier function of the corneal endothelium and the blood-aqueous barrier (BAB) have also been studied in rabbit eyes, the results obtained for the RPE can be directly compared with those obtained for the corneal endothelium and the BAB in the same species. In the present study, the transepithelial potential difference presently recorded was 4–5 mV (retinal side positive) and lower than those recorded in the RPE-choroid sclera preparation from the rabbit,<sup>15</sup> while the transepithelial electrical resistance was similar to that reported for the RPE-choroid preparations from dog<sup>16</sup> or sheep.<sup>17</sup> These results suggest that the present experimental condition may not be a perfect replication of the *in vivo* state of functioning of the RPE cells but the damage, if it existed, would not be serious, and that the present preparation can be used for studying effects of various intraocular irrigating solutions, as long as comparisons are carried out under the same conditions.

BSS plus or another glucose glutathione bicarbonate solution, glutathione bicarbonate Ringer (GBR), has been consistently found to be less harmful than S-MA<sub>2</sub> for the corneal endothelial cells<sup>18–21</sup> or the BAB.<sup>11</sup> However, the results obtained for these solutions in the retina or RPE were somewhat different; S-MA<sub>2</sub> was found to be better in maintaining ERG than GBR in rabbits<sup>2</sup> or than BSS plus in patients.<sup>6</sup> Phagocytotic activity of the cultured RPE cells was also reported to be better maintained in S-MA<sub>2</sub> than in BSS plus.<sup>9</sup> In contrast with these previous studies, the present result suggested that BSS plus is safer for the barrier function of the RPE cells than S-MA<sub>2</sub> or Krebs-Ringer solution, giving a lower permeability of the isolated RPE-choroid to carboxyfluorescein. In the previous studies, where the effects of irrigation with BSS plus, S-MA<sub>2</sub>, or other solutions on the corneal endothelial permeability<sup>18</sup> or the BAB permeability<sup>11</sup> were compared in rabbits, BSS plus was found to have the least damage on the barrier properties of these ocular tissues. The result obtained here on the isolated rabbit RPE-choroid agrees well with those of the above studies, suggesting that BSS plus is a safe commercially available irrigating solution for the intercellular junctions of ocular tissues.

Chemically, BSS plus, S-MA<sub>2</sub>, and Krebs-Ringer solution differ in several aspects: (1) S-MA<sub>2</sub> or Krebs-Ringer solution lacks oxidised

glutathione (GSSG), while BSS plus contains it at a concentration of 0.3 mM; (2) S-MA<sub>2</sub> contains about 4 mM of citrate and acetate, but these are absent in BSS plus and Krebs-Ringer solution, and (3) S-MA<sub>2</sub> lacks phosphate, while the other two solutions contain it. The second and third differences do not seem to be responsible for the presently observed discrepancy among the three tested solutions, since the isolated RPE-choroid showed a similar permeability to carboxyfluorescein in S-MA<sub>2</sub> and Krebs-Ringer solution.

To examine the possibility that the observed difference was mainly attributable to the presence or absence of GSSG in the solution, the permeability was determined using three solutions containing various concentrations of BSS plus: commercially available BSS plus, BSS plus without GSSG, or BSS plus with 0.1 mM GSSG. The permeability obtained using BSS plus without GSSG averaged  $2.0 \times 10^{-6}$  cm/s. This value was similar to that obtained using S-MA<sub>2</sub> or Krebs-Ringer solution, 1.8 and  $1.9 \times 10^{-6}$  cm/s, respectively, but significantly greater than that obtained using BSS plus. On the other hand, the value obtained using BSS plus with 0.1 mM GSSG was not significantly different from that obtained using BSS plus. These findings suggest that as far as the permeability of the isolated RPE-choroid to carboxyfluorescein is concerned, absence of GSSG was mainly responsible for the higher value obtained using S-MA<sub>2</sub> or Krebs-Ringer solution.

Although the basis for the currently observed effect of GSSG remains conjectural, it may be related to the glutathione redox system which is involved in the protection of -SH groups in enzymes and membranes. In ocular tissues, it was reported that the redox state of glutathione in the corneal endothelial cells played a role in the maintenance of its barrier function.<sup>22</sup> Riley reported that a glutathione reductase inhibitor, BCNU, in the medium damaged the barrier function of the corneal endothelium rather than its active transporting function and that the addition of reduced glutathione, GSH, but not GSSG, protected the observed damage.<sup>23,24</sup> Anderson *et al* reported that exogenous GSSG rather than GSH is responsible for the increased corneal deturgescence and the effect of BCNU was inhibited by perfusion with a solution containing 0.1 mM GSSG.<sup>25,26</sup> Cultured human RPE cells contain GSH,<sup>27</sup> and glutathione peroxidase activity is present in the rabbit RPE cells.<sup>28</sup> Exogenous GSH of 0.01 mM or higher is reported to provide protection for cultured human RPE cells against oxidative injury.<sup>29</sup> Although its exact mechanism is unclear at the present time, it may be possible that GSSG in the intraocular irrigating solution exerted a beneficial effect on the intracellular redox state of glutathione and consequently a beneficial effect on the maintenance of the barrier property of the RPE cells.

Surgical outcome of vitrectomy is determined by many factors and the effect of the barrier function of the RPE cells on it is certainly only minimal, if any. The corneal endothelium irrigated with S-MA<sub>2</sub> showed

Table 3 Effect of oxidised glutathione (GSSG) concentration in solution

Solution	Transepithelial potential difference (mV)	Tissue resistance ( $\Omega\text{cm}^2$ )	Permeability* ( $\times 10^{-6}$ cm/s)
BSS plus (0.3 mM GSSG)	142 (39)	4.4 (1.1)	1.08 (0.17)†
BSS plus (0.1 mM GSSG)	119 (33)	5.1 (0.6)	1.22 (0.20)
BSS plus (without GSSG)	123 (35)	4.2 (1.4)	2.00 (0.70)†

Values are mean (SD) in seven experiments.

\*Permeability of isolated RPE-choroid to carboxyfluorescein.

†Between group difference was significant at  $p < 0.05$  (unpaired *t* test with Aspin-Welch's correction for unequal variance and Bonferroni's correction).

20% higher permeability to carboxyfluorescein than that irrigated with BSS plus.<sup>18</sup> In a randomised clinical trial, BSS plus was found to cause significantly less corneal swelling on the first postoperative day than did S-MA<sub>2</sub> in eyes that had undergone extracapsular cataract extraction with posterior chamber lens implantation.<sup>21</sup> Further, the eyes operated using S-MA<sub>2</sub> showed significantly more postoperative loss and deterioration of morphological characteristics in the corneal endothelial cells.<sup>21</sup> These findings in the above cited clinical study suggest that 20% deterioration of the barrier property may imply not only change in the intercellular structures, but also considerable damage in the cell function itself, as far as the corneal endothelium is concerned. In the present experiment, the RPE cells irrigated with S-MA<sub>2</sub> or BSS plus without GSSG showed 70% higher permeability to carboxyfluorescein than those irrigated with BSS plus with GSSG. The above results obtained in the corneal endothelium suggest that irrigation with these GSSG-free solutions may cause considerable damage not only to the blood-ocular barrier, but also to other physiological functions of the RPE cells. The use of BSS plus may be preferable and safer, especially in eyes where subretinal procedures are needed during vitrectomy.

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*Br J Ophthalmol* 1997 81: 150-153

doi: 10.1136/bjo.81.2.150

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