Testing the Biocompatibility of a Glutathione-containing Intra-ocular Irrigation Solution by Using an Isolated Perfused Bovine Retina Organ Culture Model — an Alternative to Animal Testing

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Summary — The effects of a glutathione-containing intra-ocular irrigation solution, BSS Plus®, on retinal function and on the survival of ganglion cells in whole-mount retinal explants were studied. Evidence is provided that the perfused ex vivo bovine retina can serve as an alternative to in vivo animal testing. Isolated bovine retinas were prepared and perfused with an oxygen-saturated standard irrigation solution, and an electroretinogram was recorded to assess retinal function. After stable b-waves were detected, the isolated retinas were perfused with BSS Plus for 45 minutes. To investigate the effects of BSS Plus on photoreceptor function, 1mM aspartate was added to the irrigation solution in order to obtain a-waves, and the ERG trace was monitored for 75 minutes. For histological analysis, isolated whole retinal mounts were stored for 24 hours at 4°C, in the dark. The percentages of cell death in the retinal ganglion cell layer and in the outer and inner nuclear layers were estimated by using an ethidium homodimer-1 stain and the TUNEL assay. General swelling of the retina was examined with high-resolution optical coherence tomography. During perfusion with BSS Plus, no significant changes in a-wave and b-wave amplitudes were recorded. Retinas stored for 24 hours in BSS Plus showed a statistically significant smaller percentage (52.6%, standard deviation [SD] = 16.1%) of cell death in the retinal ganglion cell layer compared to the control group (69.6%, SD = 3.9, p = 0.0031). BSS Plus did not seem to affect short-term retinal function, and had a beneficial effect on the survival of retinal ganglion cells. This method for analysing the isolated perfused retina represents a valuable alternative for testing substances for their retinal biocompatibility and toxicity.

Key words: electrophysiology, experimental laboratory, pharmacology, retina, treatment surgery.

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Introduction

In this paper, we present the isolated perfused bovine retina as a sensitive tool for testing the retinal biocompatibility and toxicity of substances. Traditionally, such testing has been performed on rabbits, rats, mice and monkeys, and, occasionally, on other mammals (such as guinea-pigs, dogs and horses). However, testing in the animal that has the closest retinal structure to that of humans — the monkey — is hindered by moral and fiscal constraints. Smaller mammals, such as rats, mice and rabbits, are easier to use and less costly to maintain, but their retinal structures share less homology with the human eye.

In 2010, a total of 383,527 mammals were sacrificed for scientific purposes in Germany, of which 87% were mice and 10% were rats. A PubMed search for ‘electroretinogram AND toxicity’, retrieved 880 studies that involved the use of mice, 840 that used rats, and 1290 that employed rabbits. For about 30% of these studies, the actual number of animals used could not be found. For studies that disclosed such information, the average number of animals used ranged from 25 to 45.

The bovine retina is a good model of the human eye because, in contrast to the retinas of mice or rats, it has a photopic system, and a vitreous–lens ratio and vascular structure similar to those of the human eye (1). Thus, although small laboratory animals, mainly rats or mice, are widely used for retinal biocompatibility testing, their inherent physiological differences make toxicity studies performed on these animals less applicable to humans (2). For example, from phylogenetic analysis of the myocilin gene — a gene that in its mutated form causes autosomal dominant juvenile open-angle glaucoma — it is known that the bovine form is more closely related to the human form than to that of the rat or mouse (3). The myocilin protein...