11 | april 2015

MICE TIMES

Growing old with healthy eyes

Studies in animal models allow important advances in ophthalmic medicine

One in three people over the age of 70 suffers from degeneration of the macula. In this disease, for example, a pathological disease process leads to detachment of the macula lutea (a yellow spot at the center of the retina) and a worsening of eyesight. Only a few years after the diagnosis is established, patients with age-related macular degeneration can usually no longer read. Just ten years ago, there were hardly any ways of treating this widespread disease.

Macular degeneration is stabilized

But then researchers succeeded in mimicking the pathological detachment of the retina with the intravitreal injection of substances that inhibit what is known as vascular endothelial growth factor (VEGF). Today, medicines for the treatment of macular degeneration are in widespread use. In one clinic alone, the Ophthalmology Clinic at the University Clinic in Freiburg i. Br. (Germany), which played a leading role in the development of the new therapy, 10,000 elderly patients with macular degeneration are treated each year with antibodies to VEGF that are injected into the vitreous body of the eye. In most of these patients, the deterioration of their eyesight can be halted, and in many cases it is even possible to achieve a long-term improvement. Such a development would have been inconceivable without the initial use of these medicines in the mouse model.

"We need animal experiments in all areas of ophthalmology where we are looking to achieve further

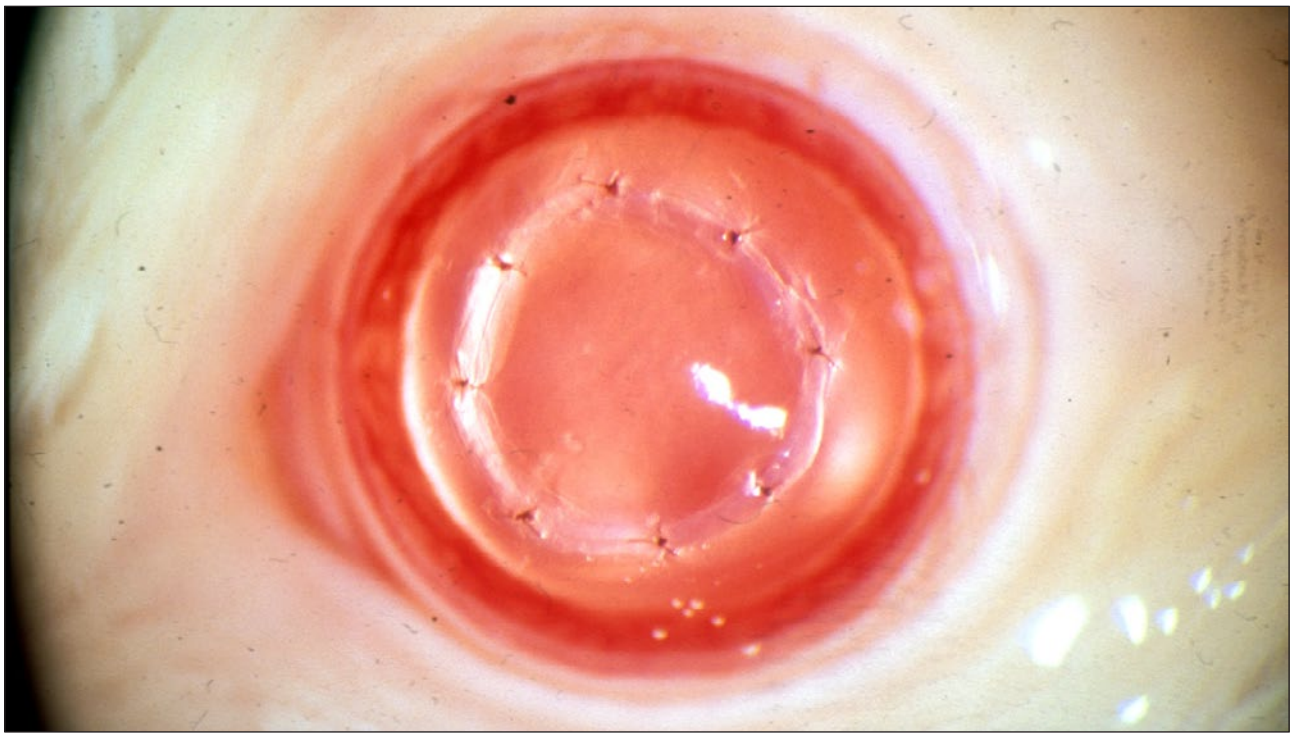

Fig. 1 Clear transplant after keratoplasty. Photo: Ophthalmology Clinic of the University Clinic Freiburg

advances in the future", says Prof. Thomas Reinhard, Director of the Ophthalmology Clinic at the University of Freiburg i.Br. A current field of research is in corneal transplantation. Thousands of patients a year receive a new cornea for the eye thanks to tissue donations. To avoid rejection reactions, ophthalmologists usually use cortisone eye drops for a limited period. The treatment is effective, but has substantial side effects: infections, dry eye, clouding of the transplanted cornea or cataract (= clouding of the lens). In 20 to 25 % of patients, the so-called steroid responders, longer-term use of cortisone can even destroy the optic nerve.

Cornea transplants without cortisone

Researchers are therefore looking for alternatives to cortisone. Scientists around Thomas Reinhard are pinning their hope in particular on azithromycin. This antibiotic substance has been on the market in the form of drops for some time. It has been suspected for some years that azithromycin could also have an anti-inflammatory effect and may thus be suitable as a substitute for cortisone in cornea transplants. The Freiburg researcher Dr. Katrin Wacker recently showed in the rat that the substance actually inhibits the immune response by blocking pro-inflammatory cytokines (cell growth proteins that stimulate inflammation). "According to these animal experiments, the immunomodulatory action of azithromycin has an efficacy similar to that of cortisone", says Reinhard. "The substance prevents invasion of defense cells, which can trigger a rejection reaction, and avoids clouding of the cornea."

The Freiburg researchers achieved their results in the established cornea transplantation model (rat keratoplasty model). In this model, the cornea of Fischer rats was transplanted into Lewis rats. In syngeneic transplantation of the cornea – i. e. transplantation within a (genetically identical) inbred strain – the operation did not trigger a rejection or inflammatory reaction, because the immune system did not identify the transplant as foreign tissue. But the immunologically induced rejection effects did occur, on the other hand, in the case of an allogeneic cornea transplant (i.e. in a genetically different strain). It is on this basis that tests have been carried out in the past on the efficacy of potential medicines such as cyclosporin A or in this case now azithromycin. The studies in the rat model showed that, with the use of azithromycin, two-thirds of transplants remained clear and only a third were still rejected. This corresponds approximately to the therapeutic success achieved with cortisone. Clinical studies must now be carried out to show the extent to which azithromycin drops can reduce the use of cortisone in cornea transplants.

Eye research is counting on the mouse

"Animal experiments are mainly used in pharmacological research, whereas research in the surgical field usually makes use of other options for its procedures, such as the cadaver eye", says Prof. Thomas Kohnen, Director of Frankfurt University Eye Clinic. Kohnen speaks of a research project with primates on the accommodation process of the eye, which one of his senior physicians recently conducted in the USA. In the 1970s, the German ophthalmologist Rainer Sundmacher used monkeys in his research on keratitis caused by herpes simplex virus. But by far the most important animal model in ophthalmological research is the mouse, says Thomas Reinhard. The mouse is well defined and easy to maintain. In addition, rats and occasionally rabbits are also used. The latter are used, for example, in cataract research. This age-related disorder is associated with clouding of the lens. It is usually treated surgically with the implantation of an artificial lens. Since there is a relatively high proliferation rate in the capsular sac of rabbits, this animal model is an optimal solution for simulating human cataracts, says Prof. Kohnen.

The operation is safe and straightforward procedure. However, the operation does carry a risk of bacterial infection in the lens. This complication, the risk of which is 1 to 1000, can lead to loss of the eye. In animal experiments with rabbits, research is currently under way to establish whether infections can be prevented even more reliably than today by coating the lens. Scientists use the rabbit model for this, because the lens of the rabbit eye is more similar to that of the human eye than that of the mouse eye. Thanks to its size, artificial lenses lend themselves well to implantation.

Committed to the 3Rs

Ophthalmological research is making great efforts to replace animal experiments and to reduce the number of laboratory animals used and the stress to

which they are exposed in accordance with the principle of the 3Rs. One example is provided by the Aachen Center of Technology Transfer in Ophthalmology (ACTO). Thanks to the Ex Vivo Eye Irritation Test (EVEIT) developed here, experiments in live animals can be avoided. For EVEIT a rabbit cornea from the slaughterhouse is transferred to an artificial eye chamber suffused with fluid. Using this experimental arrangement ACTO is able, for example, to study the physical processes associated with the administration of a medicine (pharmacokinetics) outside the live eye.

"We are leading the world with this method", says Dr. Michael Dutescu, specialist in ophthalmology and scientific associate at ACTO. Using EVEIT, the researchers are investigating, for example, how medicines or care products can help to heal the cornea when it has been damaged by minor lesions on the surface. Another way of avoiding animal experiments has been taken by researchers at the Werner Reichardt Center for Integrative Neurosciences (CIN) at the University of Tübingen. They are testing methods of treating blindness and other forms of visual impairment directly in human tissue. This tissue comes from deceased cornea donors or from patients who had to have an eye removed.

Glaucoma research with and without animals

"For many diseases of the eye, such as normal-tension glaucoma, we do not have any ideal animal models", says Prof. Josef Flammer, senior physician at the Eye Clinic of University Hospital Basel and leading glaucoma researcher. Normal-tension glaucoma is the form of glaucoma that is not characterized by increased pressure in the eye. But this disease of the optic nerve, which occurs with advancing age, also has a form that is associated with an increase in intraocular pressure. Research again relies on animal experiments for this clinical condition, e.g. for measuring the intraocular pressure, which forms the basis for a better understanding of the disease and for effective treatments. The medical device company Implandata Ophthalmic Products has developed a pea-sized pressure sensor that can be implanted in the eye and used for telemetric readings of intraocular pressure. The sensor has been tested in laboratory animals. At present, an observational study is underway at six university eye clinics in Germany.

Intraocular pressure, which is essential for eye function, is regulated by the aqueous humor. This is formed by the ciliary body, then flows in healthy subjects from the posterior to the anterior chamber and from here through the trabecular meshwork and Schlemm's canal into the bloodstream. If the outflow of the aqueous humor is disturbed, the intraocular pressure increases and leads to the optic nerve damage that is typical of glaucoma. To treat glaucoma, doctors today prescribe medication to try lowering intraocular pressure. Another approach consists in creating an artificial drainage channel through the sclera and the conjunctiva. A current research project at the University of Freiburg is using the mouse model to study how "long-

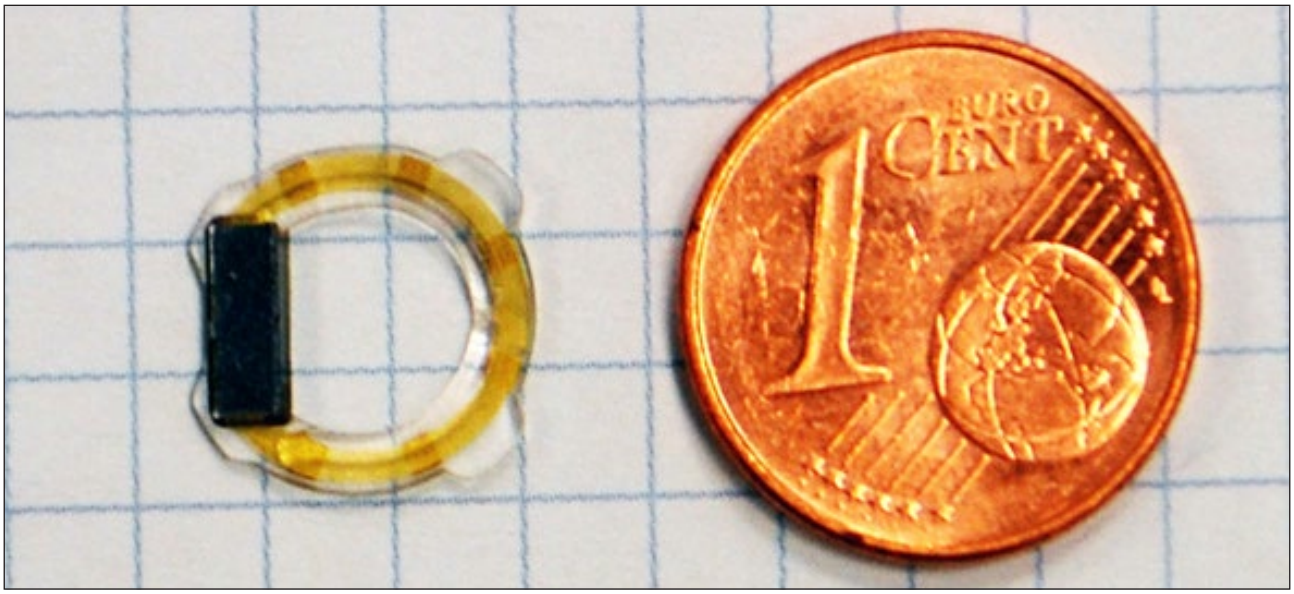

Fig. 2 The ring-shaped eye implant of medical device company Implandata Ophthalmic Products.
Photo: Uwe Seidenfaden/University Clinic Magdeburg

life" artificial drainage channels could be created using special coatings.

Mouse model for dry eye

A further research project of the Freiburg Eye Clinic is concerned with dry eye. "We know astonishingly little about the pathophysiology of dry eye", says Prof. Thomas Reinhard. To gain a better understanding of the disorder, the researchers are devising a suitable mouse model in collaboration with institute for animal experiments. As a first step, the scientists are seeking to establish the basic immunological mechanisms that lead to dry eye. In a second step they want to find out with which immunomodulatory substances dry eye can be more effectively treated than is possible today. "Thanks to animal experiments, we can reliably assess the benefits and risks of new treatments for patients", says Prof. Reinhard. "In most cases I would not be confident of trying new treatment approaches directly in humans."

It would be ideal if we could understand the complicated mechanisms of a body without stressful animal experiment. Unfortunately that is not yet possible today. But the dilemma will remain for a long time to come: basic research without experiments in animals would mean abandoning any medical progress. Mice Times aims to explain why and therefore reports on medical success stories that were only possible thanks to animal experiments.

IMPRESSUM

Editors:

Basel Declaration Society, www.basel-declaration.org

Forschung für Leben

www.forschung-leben.ch I www.recherche-vie.ch

Author: Dr. Benedikt Vogel
Editorial staff: Astrid Kugler, Managing Director