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Brief report

The effect of anakinra on retinal function in isolated perfused vertebrate retina

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Abstract

Purpose: Blockage of the interleukin 1 (IL-1) signaling pathway has been proposed for treatment of inflammatory disorders like those affecting the retina and its adjacent tissue. Herein, we evaluated one of those inhibitory drugs, anakinra (Kineret®), based on its safety profile with emphasis on retinal function from an electrophysiological point of view.
Methods: Bovine retina preparations were perfused with two different concentrations of anakinra (1 mg/ml and 2 mg/ml). An electroretinogram (ERG) was recorded and b-wave recovery assessed.
Results: Exposure to anakinra at a concentration of 1 mg/ml did not decrease the b-wave amplitude, whereas 2 mg/ml resulted in a significant reduction.
Conclusions: Based on these preliminary results, anakinra at a dose as low as 1 mg/ml could be regarded as safe for retinal function. However, dosages of 2 mg/ml and more do have toxic electrophysiological effects, at least for the short-term.

Keywords: Anakinra; Interleukin 1; Toxicity; Retina; Electroretinogram

Introduction

Local and systemic inflammation is a hallmark of ocular diseases like uveitis, diabetic retinopathy, and age-related macular degeneration.[1] Various cytokines are up-regulated during the complex interaction of different cells and maintain the inflammatory milieu.[2] More and more cells become activated and through triggering of even more signaling cascades further proinflammatory cytokines and proteases are released, resulting in damage to surrounding tissue. Without timely and proper treatment, severe vision loss, which might be irreversible, is the consequence.

One of the major cytokines involved in the pathogenesis of intraocular inflammation is interleukin 1 (IL-1).[3] Its downstream via the IL-1 receptor activates the nuclear factor NF-κB, which enhances the expression of proinflammatory genes including cytokines, chemokines, and adhesion molecules.

Understanding these molecular mechanisms led to the invention of drugs like anakinra (Kineret®). Anakinra, which is an IL-1 receptor antagonist, is already approved for rheumatic arthritis and considered for even more inflammatory diseases.[4] While some studies exist in which anakinra is administered

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systemically to patients suffering from uveitis associated with Still's or Behcet's disease, local application to the eye has not been explored to a satisfactory extent, and research emphasizing ocular safety is still needed.[5,6]

As we have previously shown the isolated superfused bovine retina is a good, sensitive tool for pharmacological testing.[7,8] We were able to demonstrate the effects of various drugs on retinal function, and our results could also be transferred to the human retina. Since many types of retinal cells and synapses are involved in the generation of the b-wave, it might be obvious that a reduction of this parameter indicates substantial dysfunction of retinal neurons. Therefore, the b-wave amplitude of an electroretinogram (ERG) reflects a very sensitive indicator for overall retinal integrity and is a useful tool to investigate drug biocompatibility.

The aim of the present study was to assess anakinra based on its safety profile with regard to effects on retinal function from an electrophysiological point of view.

Methods

Preparations were performed as previously described.[7–10] In brief, freshly enucleated bovine eyes were opened equatorially. The vitreous was removed, and a circular piece of the most central posterior segment was obtained using a 7 mm trephine. The retina was separated from underlying pigment epithelium and mounted on a mesh occupying the center of a perfusion chamber.

ERG was recorded in the surrounding nutrient via two silver/silver-chloride electrodes on either side of the retina. The chamber was installed in an electrically and optically isolated air thermostat. The perfusion velocity was controlled by a roller pump (1 ml/min), and the temperature was kept constant at 30 °C.

The perfusion medium (NaCl 120, KCl 2, $MgCl_2$ 0.1, $CaCl_2$ 0.15, Na_2HPO_4 13.5, and glucose 5 mmol/l) was pre-equilibrated with oxygen, and the oxygen level was monitored by a Clarke electrode. Retinas were dark-adapted, and ERGs were elicited at 5-min intervals using a single white flash for stimulation. The flash intensity was set to 6.3 mlx at the retinal surface using calibrated neutral density filters. The duration of light stimulation (500 ms) was controlled by a timer. ERGs were filtered and amplified (100-Hz high-pass filter, 50-Hz notch filter, 100.000× amplification) using a Grass CP 511 amplifier. Data were processed and converted using an analog-to-digital (AD) data acquisition board (NI USB-6221; National Instruments, Austin, TX, USA) and a personal computer (PC). The ERGs were recorded and analyzed by DASYLab Professional Version 10.0.0 (National Instruments, Austin, TX, USA).

The retina was stimulated repeatedly until stable amplitudes were reached. Then the retinal preparations (n = 3 for each dosage) were superfused with agent medium for 30 min (retinal exposure time). Therefore, 50 mg or 100 mg anakinra (Kineret®) were dissolved in 50 ml standard medium. Afterwards the preparation was reperfused with drug free standard medium for at least further 30 min.

The b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave, and the percentage of b-wave reduction after exposition was calculated. Changes of the b-wave amplitude were carefully monitored. Furthermore, the reversibility of the drug impact on the b-wave after reperfusion with standard medium was considered.

For statistical analysis of the b-wave amplitude, the software "Origin 6.0" (Microcal Inc, Northampton, MA, USA) was used. Experiments were replicated three times. Values are expressed as the mean ± standard deviation. Significance was calculated with a Student t test. Levels of $P \leq 0.05$ were regarded as statistically significant.

Results

Stable ERG amplitudes were reached within 30 min of perfusion. Environmental parameters such as pH, osmotic pressure, temperature, and pO_2 remained unchanged during the whole experiment.

In this model, exposure to 1 mg/ml anakinra did not decrease the b-wave amplitude significantly (−3.88% ± 5.38%; $P = 0.7855$), whereas 2 mg/ml applied for 30 min led to a statistically significant reduction (−51.24% ± 31.71%; $P = 0.0247$) of the amplitude (Fig. 1). Yet after reperfusion with standard nutrient solution, the b-wave amplitude convalesced, and at the end of the washout, a partial recovery was seen, in which the b-wave amplitude was not significantly lower compared to the phase before exposure to anakinra ($P = 0.8831$).

Discussion

The purpose of this study was to evaluate the effect of anakinra (Kineret®) in different dosages on retinal function.

Anakinra is a recombinant human IL-1 receptor antagonist that is identical to the naturally occurring nonglycosylated form with the exception of one N-terminal methionine.[3,4] It is systemically used to treat rheumatoid arthritis and patients

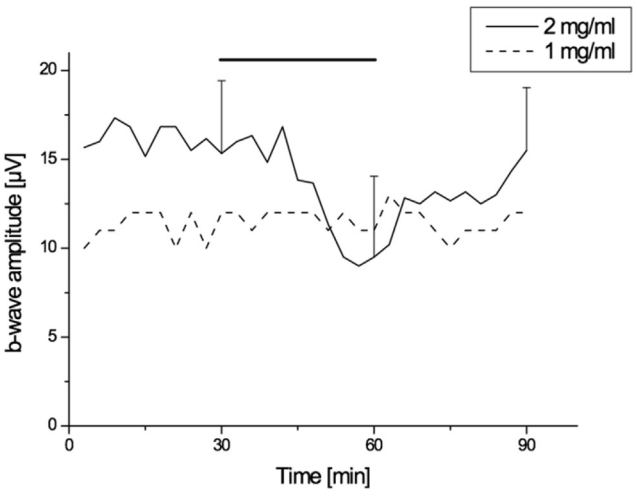

Fig. 1. Effects on anakinra on b-wave amplitude: Exposure to 1 mg/ml did not decrease the b-wave amplitude, whereas 2 mg/ml applied for 30 min led to a significant reduction ($P = 0.0247$, Student t test).

with neonatal-onset multisystem inflammatory disease (NOMID). Moreover, several trials are underway for various conditions underlying chronic inflammatory states such as cardiovascular disease, diabetes, and uveitis.[11–13]

In all previous studies, anakinra was administered systemically.[5,6] However, data based on local intraocular application, which would be favorable in terms of uveitis because of the blood-retina barrier, are limited. It has been shown that intravitreal injected anakinra suppresses autoimmune uveitis in rats through decreased levels of IL-1 and even TNF-alpha.[14] In another animal study, 0.75 mg anakinra injected into the vitreous (0.05 ml > 15 mg/ml) of rats was able to successfully inhibit the growth of choroidal neovascular membranes in an experimental model of exudative age-related macular degeneration.[15]

There are no published studies regarding ocular safety of anakinra. Our results suggest 2 mg/ml anakinra affect retinal function from an electrophysiological point of view at least temporary, while a lower dosage of 1 mg/ml might not. Considering that significant higher doses of anakinra were needed to have a significant anti-inflammatory effect in the animal studies mentioned before, a safe concentration with sufficient therapeutic outcome might not exist. Nevertheless, these results should be interpreted with caution. Despite the fact that our model mimics the retinal response to toxic agents quite properly, it remains an ex vivo model. Also, the safety of the drug in an ex vivo situation (cadaver eyes) may be different from an in vivo one (living eyes).

In addition, these animal studies have shown that the beneficial effect of anakinra is only temporary and repeated injections may be needed to achieve the best therapeutic result. Though, repeated injections might lead to more side effects and long-term dysfunction, which is not covered by our experimental setup. Moreover, inflammatory responses are not captured by our system, but should be regarded utterly important in real life since they might affect the threshold of susceptibility to drug-mediated toxicity.[16] Also, this study is limited by the small sample size.

In conclusion, the current data does not support the routine use of intravitreally administered anakinra in any retinal disease outside controlled trials. Further investigations, particularly dose-finding studies, will shed light on the potential therapeutic benefits for vascular and neovascular diseases of the choroid and retina.

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