THE EFFECT OF LASER ON THE RETINA IN EXPERIMENTAL AND PATHOLOGICAL CONDITIONS

Thesis

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INTRODUCTION

Laser treatment was first utilized for healing purposes in ophthalmology. At the first time the heat effect was utilized during the treatment. Afterwards, with the development of different laser types other histological effects was achieved. Nowadays a special laser surgery has developed where we utilize different effects of the laser: the coagulation, the disruption and the ablation. During coagulation the most important feature is the heat effect that we use in different retinal pathologies. Disruption in an intact eye globe means the break of continuity of different ocular tissues. We use this effect for example in the laser surgery of glaucoma, when we perform an iridotomy. The ablation is the key effect of the refractive surgery, the laser causes tissue vaporization on the surface of the cornea.

In ophthalmologic use the most often used laser base on the effect of heat production. Heat is absorbed by tissues that content pigments, this is why this kind of treatment is utilized in case of alterations of the retina, because of the melanin pigment of the retinal pigment epithelium (RPE).

When lasering the retina, there is direct cell damage at the site of the laser beam, due to the photocoagulation. Besides that there is a secondary effect, due to the heat produced around the laser spots. This effect is proportional to the heat generated, and it is also proportional to the power applied. The cellular response after laser treatment can be motitorized by direct histological and biochemical methods.

A special laser treatment is when we use a 698 nm „non-thermal” diode laser, which activates the verteporfin molecules administered earlier intravenously, and damages active neovascularisations without damaging the surrounding tissues. The effect is called photodynamic treatment (PDT). The selective effect the PDT treatment is due to the selective vasoocclusion. This laser is utilized not only in the treatment of age-related macular degeneration (AMD), but can be used in other subfoveal neovascular conditions, such as pathological myopia, angioid streaks, presumed ocular histoplasmosis, and tumors with important capillary network.

AIMS

After the facts revealed in the introduction it is obvious that the cicatrisation caused by laser treatment that rest on the coagulation, and the therapeutic effect is proportional to the conducted heat and its effect on the tissues. At the site of the coagulation there are different cellular damages that finally lead to cicatrisation.

In human practice there is only a very shallow difference between the therapeutic and damaging effects. Because the for mentioned effects are very difficult to test *in vivo* we performed *in vitro* experiments in cell cultures. We wanted to examine the following questions:

1. to measure the temperature change in cell cultures after laser treatment,
2. to monitorise the cell damage with GABA and glutamate uptake experiments.

With the special photodynamic laser we looked upon its effect on the retinal angiomatosis in vivo:

1. to examine the cicatrisation of the angiomatosis,
2. to examine its blood supply, with the help of examining its feeder and draining vessels,
3. can vessel constriction or closure be observed,
4. how much is the feeder and draining vessels are involved in the procedure,
5. is the change of the vessel diameters provisional or not?

METHODS

1. RPE – laser treatment – monitorising the biochemical changes

(This experiment was performed in the Cell Research Center, of the Tampere University, Finland)

RPE cell culture

The primary RPE cell cultures were prepared by the method described by Khatami. The isolated cells were resuspended in DMEM with 20% fetal bovine serum, penicillin G (100 IU/mL), streptomycin (100 g/mL) and amphotericin B (0.25 g/mL). The RPE cell suspension was diluted to the final volume and plated at cell densities of 4–6 X $10^4/cm^2$ in 2.0 ml cell culture dishes. The cells were cultured in a humidified atmosphere of air/CO2 95:5% at 37°C. The confluent RPE cultures were used for the laser treatment.

Laser Photocoagulation

Laser photocoagulation of the confluent primary RPE cells was accomplished using a semiconductor diode laser (678 nm) (Tampere University of Technology, Tampere, Finland). Laser irradiation of the RPE cells was delivered through an endolaser probe with an optical fiber, a handle, and a distance adjuster. The irradiation was carried out with power setting of 800, 1000, 1250 and 1600 mW with short pulse duration for 0.186 second and spot size 1000 μm.

Temperature Measurement

An NTC-thermistor (Negative Temperature Coefficient) was used to measure changes in temperature next to the laser spot. The measurements by the semiconductor resistor were based on the changes in resistance caused by temperature and the results were quantified by a digital oscilloscope.

WST-1 cell proliferation assay

The WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) assay is a colorimetric test that detects proliferating and viable cells. It is based on the cleavage of tetrazolium salt to colored formazan by mitochondrial dehydrogenases. A WST-1 assay was used for evaluation of the viability of RPE cells after laser treatment.

Glutamate and GABA Uptakes

The cultured cells were incubated at 37°C for 10 minutes with L-[3H]-glutamate or [3H]-GABA. The total concentration of glutamate or GABA was 10 μM in the final incubation volume of 2 ml. Aliquot of this medium was transferred to liquid scintillation fluid, and the radioactivity was determined with an LKB Wallace 1219 Rackbeta liquid scintillation counter (Turku, Finland).

Protein Determination

A Pierce BCA Protein Assay was used for the protein measurement. The determination is based on a bicinchoninic acid-based modification of the Lowry method.

Statistical Methods

All calculations and determinations were done in triplicate. The statistical significance of differences between control and treated cultures was calculated using the independent paired Student's *t*-test.

2. Retinal capillary haemangioma – Photodynamic treatment – Retinal vessel analyser (The experiment was carried out at the Semmelweis University, Budapest, Department of Ophthalmology)

Photodynamic treatment (PDT)

The benzoporphyrin-MA (Visudyne®, Novartis Ophthalmics AG, Hettlingen, Swiss) molecule iv. was used as the photosensyteser. The Zeiss laser apparatus was employed for

photosensitizing (Visulas II, Zeiss, Oberkochen, Germany). The treatment was performed with a 692 nm emitting light, and the power density was 600 mW/cm^2. In our case during the PDT treatment one single laser spot was transmitted onto the capillary hemangioma.

Monitoring of the diameters of the retinal vessels with the Retinal Vessel Analyser (RVA)

With this machine we monitorised the big vessels close to the optic disc head (Imedos, GmbH, Weimar, Germany). We compared these results of the vessel diameters. Vessel diameters were obtained before the PDT, 5, 12 days and 6 months after the treatment.

RESULTS

The effect of direct laser treatment on porcine primary retinal pigment epithel monolayer cell cultures (*in vitro* experiment)

RPE cell culture

Cells in the culture wells started to grow extensions at the first day. This procedure increased during the following days. Cells started to fuse after 1 week. The porcine primary pigment epithelial cells reached confluence after 7–10 days.

Temperature Measurement

Temperature during treatment in the near vicinity of the laser spots increased linearly as a function of the intensity of the laser. The temperature increase in the center-, and 0.5 mm from the center of the spot showed the same tendency. After laser treatment, acute necrosis of RPE cells was observed in the central part of the spots. At some places the cells were detached from the bottom, being completely stripped off.

WST-1 cell proliferation assay

In the WST-1 assay, the production of formazan was significantly reduced after the photocoagulation. When the laser was set to 800 mW the production of formazan was reduced to 79.1%; when it was set to 1600 mW it was reduced to 80.1%, compared to the controls

Glutamate and GABA Uptakes

The glutamate uptake after laser treatment decreased to 86.9% using 800 mW and even more to 59.4% using 1600 mW compared to controls. Changes were exactly the opposite in GABA uptake. The GABA uptake increased to 127% after 800 mW and increased furthermore to 280.8% after 1600 mW laser photocoagulation compared to controls

The control and follow up of the feeder and draining vessels of the retinal capillary haemangioma (RCH) with the Retinal Vessel Analyser (RVA) after photodynamic therapy (PDT) (Clinical human experiment)

A 26-year-old woman with a complaint of blurred vision in her left eye of 4 weeks' duration was referred to our clinic. Her best corrected visual acuity (BCVA) was 20/25 in the left eye. On the fundus, superior from the optic disc, a large (approximately two disc diameter) RCH associated with fine subretinal fluid accumulations at the foveola was found. The lesion was treated using PDT, according to the guidelines of the treatment of age-related macular degeneration with photodynamic therapy (TAP) Study. One day after PDT treatment, VA dropped to 20/200 with subretinal fluid accumulation of the macula that totally resorbed over the following 12 days. Over the next 6 months the size of the tumor regressed by one third. Increased fibrosis on its surface and minor irregularity of the foveolar pigment epithelium was observed, and the BCVA returned to normal. Before PDT and at 5 days, 12 days and 6 months after treatment the retinal vessel analyzer (RVA) was utilized to measure the diameters (μm) of the feeder and draining blood vessels of the tumor. Following PDT, the feeder and draining vessel diameter decreased by approximately 20% and 40%, respectively. This decrease was already present 5 days after treatment and 6 months later remained the same.

CONCLUSIONS

The effect of direct laser treatment on porcine primary retinal pigment epithel monolayer cell cultures

When we perform focal photocoagulation it is very important to choose the optimal wavelength to avoid additional neuroretinal damage.

Our laser used *in vitro* conditions with its 678 nm, theoretically causes 35-65˚C temperature increase *in situ*, and this is mainly because of the RPE layer.

In our experiment the diode laser used with the same spot size caused temperature increase when increasing the laser power. We measured the temperature increase in the center and at the border of the laser spots. The measured temperature increase in the center of the beam was higher than at the border of the laser spot. The temperature increase at the two locations showed the same tendency. Our measurements met the results by other authors published earlier in the literature.

In our experiment the microscopical examination at the laser spot after laser treatment showed cell destruction. Cells were detached from the bottom of the cell culture wells and cell debris were observed.

Standard well visible laser treatment leads to 40-60°C temperature increase in the tissues. This temperature increase is much higher than the temperature caused by the ophthalmologically non visible laser treatment.

Laser spots become visible when there is damage to the neuroretina, and it looses its transparency.

Contrarily the short, micropulsed laser treatment causes effect only on pigmented tissues. When performing subthersold laser treatment, the possibility to cause effect onto the retina is 50%. This kind of laser reduces the harmful heat effect of the laser to the neuroretina and choroidea. The energy used for subthersold laser treatment is most of the time only the fraction of the energy utilized for periferial retinal photocoagulation.

The WST-1 cell proliferation assay bases on a color change reaction. It correlates with the viable and proliferating cells. Indirectly it can be used to detect cell damage.
Our in vitro experiment showed decrease of the viable cells after laser treatment. Laser treatment caused harmful effects to 20% of our cultured cells.

The glutamate uptake decreased after laser photocoagulation. The higher the power density used the higher the decrease of the glutamate uptake.
The GABA uptake changed contrarily after lasering the cells compared to the control cells. Our result has showed that the glutamate transporters of the RPE cells became less active compared to the GABA transporters after laser. This adverse event can be described by the possible disregulation of the different neurotransmitter receptors.
The cultured RPE cells after photocoagulation behave and regenerate differently from normal RPE cells *in situ*, and the production of cytokines and growth factors may be altered in quantity and quality. Although the effects of laser treatment are much more complex in the retina *in situ* than in cultured cells, our findings indicate that the primary RPE cell culture could be used for testing the effects of laser treatment.

The control and follow up of the feeder and draining vessels of the retinal capillary haemangioma (RCH) with the Retinal Vessel Analyser (RVA) after photodynamic therapy (PDT)

In 1878 Panas and Remy published fist its histopathological description. In 1882, Fusch published the first case report of the disease. The retinal capillary haemangioma (RCH) is the first sign of the von Hippel-Lindau (VHL) disease.

Most commonly the RCH is observed. When RCH causes complaints several treatment methods can be called upon: laser photocoagulation, cryotherapy, radiotherapy, hyperthermia, transpupillary thermotherapy, vitrectomy, photothombosis, and rarely for the blind painful eye enucleation

Photodynamic therapy (PDT) with vetreporfin is a useful method in the treatment of choroidal neovascularisation in age-related macular degeneration, or pathological myopia.

During PDT selective photothombosis of the pathological vessels occurs, leaving the overlying neuroretina unharmed. This is why the selective PDT might be a good treatment modality in treating vascular lesions in the retina.

Taking into consideration literature data PDT is a possible treatment for chorioideal heamangiomas. In vivo experiments demonstrated that RCH responded well to PDT: the size of the tumor and the subretinal exudation decreases after PDT. Unfortunately vascular complication might occur, for example vascular occlusion and the success is sometimes only provisional.

Until now we have only limited information of the PDT of RCHs. In the literature only a few case reports or small case series have been published. In most of the publications the follow up period is also quite short. This is the reason why we think that any additional information might be useful in the treatment of this rare disease.

In our case report we used successfully the PDT in a RCH diagnostized in a young female patient. We did not have any intraoperative complication. In the early postoperative period the BCVA decreased temporary because of a serous neuroretinal detachment.

Twelve days after PDT the serous fluid disappeared totally and her BVCA returned to normal. Six months after the laser only mild fibrotic changes could have been observed at the surface of the RCH, with a stable, normal visual acuity. The comparative color photos showed the decrease of the RCH. Five days after the treatment we was able to observe and measure a decrease of the feeder and draining vessel diameters of the capillary haemangioma. This decrease was also present 6 months after the PDT with no progression.

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