Nd : Yag Laser - Induced Lipid Peroxidation And Efficacy of Alfa - Tocoferol

AHMET ÇOLAK, MURAD BAVBEK, KAMER KILINÇ, BEKTAŞ AÇIKGÖZ, OSMAN EKİN ÖZCAN

Department of Neurosurgery (MB, BA, OEÖ) and Biochemistry (KK), Hacettepe University Medical Faculty
Turkish Organ Transplantation and Burn Foundation Hospital (AÇ), Ankara, Türkiye

Abstract : In this study, Nd:YAG laser induced lipid peroxidation in spinal cord and the efficacy of alfa-tocoferol on this process were investigated. The study was performed on 36 adult guinea pigs divided into three groups . The first group was the control group and only laminectomy was performed. In the second group, using a Nd:YAG laser, focal spinal cord trauma was induced and lipid peroxidation values were measured in the first and sixth hours after the laser application. In the third group, alfa-tocoferol was administered intraperitoneally, 30 minutes before the laser application and lipid peroxidation values were measured in the first and sixth hours after laser application. By comparing these results, statistical analysis revealed significant findings (For one hour $U=34$, $p<0.05$; and for six hours $U=36$ $p<0.05$). This study showed that the application of 30 watt Nd:YAG laser irradiation for one second on spinal cord induced a significant value of lipid peroxidation in guinea pigs and alfa-tocoferol significantly protected secondary spinal cord damage by reducing the laser-induced lipid peroxidation levels. This means that alfa-tocoferol may have a potential role not only in the treatment of laser-induced spinal trauma, but also in prophylaxis.

Key Words : Alfa-tocoferol, YAG-Laser, Lipid Peroxides

INTRODUCTION

Rosomoff and Carroll were the first to use lasers as a neurological instrument in 1965 (20). Since then different types of lasers including the Co2 and Nd:YAG laser have been used in neurosurgical practice (2,4,9,19,21,22,30,31,33,35). Most neurosurgeons prefer the Co2 laser because of its high absorption rate in water (17,27,31). It also has high vaporization and cutting effects on tissues. The Nd:YAG laser has a high scattered and absorptive effect in tissue (3,10,17,23,24,31,33,34,36). Although these qualities of the Nd:YAG laser produce deeper coagulation necrosis, these properties can be useful in shrinking tumors and coagulating the vessels. Experimentally, Beck et al. (3) first studied tissue changes following application of laser to rabbit brain, and the effects of Nd:YAG laser on cerebral microvasculature in normal rabbit brain, and the cerebrovascular and metabolic effects on rat brain after irradiation with Nd:YAG laser (16,17). The time course and spatial distribution of the Nd:YAG laser-induced lesions in rat brain were also investigated by Eggert et al. (10). Nd:YAG laser irradiation on brain and spinal cord was used as a model of a well-defined lesion for further investigation of brain edema and other changes of central nervous system injuries (7,10,11,16). For these reasons a standard dose, 30 Watt (20 joule), Nd:YAG laser was used in this experiment.

It is known that free radicals have an important role in tissue damage. A free radical is an atom or molecule with an unpaired electron in its outer orbital. It can react with a number of molecules and participate in chain reactions (lipoperoxidation) leading to denaturation of proteins, destabilization of cellular membrane and eventually tissue necrosis unless quenched by specific compounds (scavengers) like alfa-tocoferol ((1,5,6,8,12,13,15,16,26,28,29). Alfa-tocoferol is a well-known anti-oxidant which prevents lipoperoxidation by neutralizing free radical electron transfer (18,25). Review of the literature

revealed that there is a lack of information about changes caused by laser-induced free radicals. To our knowledge, the role of free radicals in laser-induced lesions, which mostly occur accidentally during coagulation of central nervous system tumors and vessels has not been studied previously.

MATERIAL AND METHODS

Thirty-six adult guinea pigs, selected at random, each weighing between 360-490 grams with a median of 410 grams were anesthethized with intramuscular injection (60mg/kg) of ketamine (Ketalar, Parke-Davis, Eczacibaşı, İstanbul), and xylazine (9mg/kg) (Rompun, Bayer, İstanbul). Additional doses were occasionally administered during the surgical procedure. The temperature of the animals was maintained at 36-37 degrees with a heating pad and lamp. Direct arterial pressure and heart rate were measured intermittently. Animals were placed on a frame. In all animals, laminectomies were performed with the aid of a surgical microscope under magnification of X16 (OPMI-99, microscope, Carl Zeiss Inc., Germany).

The animals were divided into three groups of 12.

Group I: Control group

In this group, without disturbing the dura mater, midthoracic laminectomies (T6-T7) were done. After one hour, thoracotomy was performed and using the intracardiac route, total body perfusion was carried out with 200 ml 0.9% saline solution. The spinal cord was removed and the dura mater was excised totally. The cord was embedded in liquid nitrogen and transported for lipid peroxidation study.

Group II: Laser group

Laminectomies were carried out as described in group I. After the laminectomies 30 watt (20 joule) Nd:YAG laser energy delivered from a Nd:YAG source (Medilas, Fa., MBB, Munich, Germany), was applied for one second on the thoracic spinal cord at T6-7 level as described in previous studies (7,10,16). With a light guide (divergence 5.5) and a lens beam system having a 3cm focal length, the laser was focused on the spinal cord just at the midline. Pulse duration was chosen as one second. In six out of 12 animals, one hour after laser application, thoracotomy and intracardiac perfusion were performed as in group I. Spinal cord was removed and the dura was excised and then embedded in liquid nitrogen for lipid peroxidation study . The same procedure was performed on the remaining six animals 6 hours after the laser application.

Group III : Experimental group.

After the laminectomies, 30mg/kg alfa-tocoferol was given intraperitoneally (Ephynal Amp. 100mg. Hoffmann & LaRoche). Fifteen minutes later, Nd:YAG laser was applied as described in group II. Six of the animals in this group were perfused one hour and the other six were perfused six hours after the laser application. Their spinal cords were removed and after excision of the dura, cords were embedded in liquid nitrogen for transportation to the laboratory for measurement of lipid peroxidation. For lipid peroxidation measurement, the thiobarbituric acid method of Uchiyama and Mihara was used . Lipid hydroperoxide reacts with thiobarbituric acid and forms malondialdehyde. The molar absorbtivity of this metabolite was determined by spectrophotometry as nmol/gr wet tissue (32).

STATISTICAL ANALYSIS

The values of lipid peroxidation in the different groups were compared using the Mann Whitney U test. Results were significant at $p<0.05$.

RESULTS

The clinical status of the animals did not change during the surgical interventions. No perioperative death occured. In group I, the values of lipid peroxidation one hour after the laminectomies ranged from 77.84 to 81.79nmol/gr wet tissue with a median of 79.86+1.59. The values, six hours after laminectomy, ranged from 81.30 to 82.30 with a median of 82.15+0.42nmol/gr wet tissue. In group II, the values of lipid peroxidation one hour after laser application were between 97.51 and 98.76. The other values for six hours are shown in table I. Total amount of lipid peroxidation increased significantly according to group I, not only at the first, but also at the sixth hour postoperatively. (for one hour U=36; for six hours U=36, $p<0.05$) (Table II). All these results revealed that a standard dose of Nd:YAG laser application on spinal cord induced spinal cord injury which produced a significant increase in the lipid peroxidation level. In group III, alfa-tocoferol reduced the level

Table I : The mean values of lipid peroxidation (nmol/gr)

GROUP	1ST HOUR	6TH HOUR
Group I	79.86∓1.59	82.15∓0.42
Group II	96.00∓4.21	124.14∓3.78
Group III	83.32∓5.83	92.38∓2.63

Tablo II : Statistical analysis of the results

Compared Groups	1ST hour	6 TH hour
Group I-II	U=36, p<0.05	U=36, p<0.05
Group I-III	U=24, p>0.05	U=36, p<0.05
Group II-III	U=34, p<0.05	U=36, p<0.05

of lipid peroxidation in the injured spinal cord. The mean values of lipid peroxidation in this group were 83.32+5.83 and 92.38+2.63nmol/gr wet tissue in the first and sixth hours after laser application, respectively (Table I). When these results were compared with group II, the decrease in lipid peroxidation was statistically significant (for one hour U=34, p<0.05; for six hour U=34, p<0.05) (Table II). Comparing these results with group I, significant improvement was observed in the posst-laser first hour (U=24, p<0.05). In other words, a dose of 30mg/kg alfa-tocoferol provided almost complete improvement in the posst-laser first hour; significant but incomplete improvement was observed in the posst-laser sixth hour.

DISCUSSION

Today Nd: YAG and CO_2 lasers have become routine neurosurgical instruments in the operating theatre (2,4,9,19,21,22,27,30,31,35). Lesions induced by laser irradiation have been described as consisting of three or four identifiable zones (3,23,27,31,33,34,36). CO2 laser irradiation causes an area of charred tissue surrounded by a zone of vacuolized and disrupted tissue and an oedematous area delineated from normal nervous tissue (31). After radiation of the brain with Nd:YAG laser, a necrotic area surrounded by a vacuolized and oedematous zone was seen (10). With extensive use of laser, especially Nd:YAG laser, in neurosurgical practice, some problems such as deep tissue necrosis are encountered (14,31,34). The size and shape of laser induced lesions in central nervous system tissue are related to energy density, energy distribution and the optical properties of the tissue at the wavelength of the laser applied to the tissue (3,10,23,27,31,34,36). The size of the lesions corresponds in a semilogarithmic fashion to the applied energy density. The maximum size of lesion at the brain surface was obtained at an energy density of 2149 J/cm2. An increase of energy density up to 3070 J/cm2 did not result in any additional increase in size (10). In this study, the standard dose, 30 Watt (20 J) laser energy was used since this energy level produces a laser induced lesion in nervous tissue for experimental studies (7,10,11,16). Kiessling et al. (16) showed that at the irradiation site and in the perilesional area, cerebral blood flow was extensively affected, and protein synthesis was inhibited in the area distant to the coagulation necrosis. These factors lead to ischemia and oedema which probably induce free radical production. The ischemic region which became prominent in the first hour after irradiation was larger than the visible focus (16) and could cause postoperative neurological deficits. In our study, lipid peroxidation value averaged 96.00+4.21 in the first hour and postoperatively in sixth hour, it reached 124.14+3.78nmol/gr wet tissue. Our observation supported the suggestions above. It is well known that a laser beam is created by stimulation of active media such as CO2 or Nd:YAG. During this process, stimulated energy allows electrons to transfer to the upper circle. While receiving the electrons they emit photons which finally become the laser beam (31). Similar mechanisms are observed in the production of free radicals which are highly reactive chemical substances with an odd number of electrons. A compound becomes a free radical by either gaining or losing an electron. Addition of the first electron to molecular oxygen leads to the formation of the superoxide radical (O2-), hydroxyl radical and so on, as a chain of lipid peroxidation until they react with a detoxification system or a scavenger. Free-radical scavengers are grouped as enzymatic (superoxide dismutase, catalase), nonenzymatic (Deferoxamine, mannitol), hydrophilic (Ascorbic acid) or hydrophobic (Alfa-tocoferol) (1,5,6,12,13,28,29). Alfa-tocoferol stabilizes the reaction by giving an excess hydrogen atom to the free radical (8,18,25). Finally we believe that in the production of laser induced free radicals, laser-induced ischemia and energy conversion were the main causes. As an oxidant, alfa-tocoferol can be used as an alternative approach not only in the treatment of laser induced trauma which mostly occurs accidentally during surgical intervention, but also for perioperative protection of these injuries.

Correspondence : Bektaş Açıkgöz, MD
Department of Neurosurgery,
Hacettepe University,
Medical Faculty, 06100
Sıhhiye, Ankara, Türkiye

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