Basic research on intravascular low intensity laser therapy

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Intravascular low intensity laser therapy (ILILT) was popular in Russia in 1980s and in China in 1990s, respectively. There is photobiomodulation on blood cells and blood *in vitro* if the radiation intensity is so low that the photodynamic effects of endogenous photosensitizer can not damage membrane or cell compartments and the cells are not in health or normal states. The present *in vivo* research is of problem as the tip intensity of the fiber-optic used in ILILT might induce the apoptosis or necrosis of the blood cells near to the tip. Obviously, the *in vivo* research should be further done.

OCIS codes: 170.1420, 170.1610, 170.5380.

Intravascular low intensity laser therapy (ILILT) was put forward by Lee *et al.*[1]in USA in 1982, was popular in Russia in 1980s, and then was popular in China in 1990s, respectively. As Chinese therapeutic applications of ILILT were widely used in the world, its basic researches, such as intracellular signal transduction research[2], blood research *in vitro*[3], animal blood research *in vivo*[4], human blood research *in vivo*[5] and traditional Chinese medicine research[6], were also very progressive in China. Its basic researches will be reviewed in this paper.

According to quantum mechanism[7], the transition rate of a molecule irradiated with monochromatic light with angular frequency ω, intensity I and radiation time t from the ground state $|k\rangle$ with energy E_k to the excited state $|n\rangle$ with energy E_n can be written as

$$r = \frac{1}{2\hbar^2}\left|D_{kn}\right|^2 I\frac{\sin\left(\omega_{kn}-\omega\right)t}{\omega_{kn}-\omega} \overset{\text{resonance}}{\longrightarrow} \frac{1}{2\hbar^2}\left|D_{kn}\right|^2 It, \tag{1}$$

where $\hbar$ is the Plank constant, D_{kn} is the matrix element, and $\omega_{kn} = (E_n - E_k)/\hbar$. For the resonant transition, $\omega_{kn} = \omega$, we then have the reciprocity rule (Bunsen-Roscoe law)[8] according to the second equation of Eq. (1), the photochemical response is independent of the intensity of light I and the radiation time t when the dose It is kept constant.

There are two kinds of pathways mediating cellular photobiomodulation[7]. The specific one is mediated by the resonant interaction of light with molecules such as cytochrome c oxidase, singlet oxygen, or endogenous photosensitizer such as hemoglobin, myoglobin and porphyrines so that the second equation of Eq. (1), the reciprocity rule, should hold. The non-specific one is mediated by the non-resonant interaction of light with membrane proteins so that the first equation of Eq. (1) should hold and the reciprocity rule should not hold. In some cases, the intensity is so high that it can induced photodynamic damage by some of specific pathways such as endogenous photosensitizers[7]. Gu *et al.*[9] investigated the effect of 510.6-nm laser irradiation on apoptosis of vascular smooth muscle cells (VSMC). Rabbit normal abdominal aorta was irradiated with 510.6-nm laser and the intensity is 50, 100, 200, or 300 mW/cm^2. Irradiation time is 500 or 1000 s. They found that laser irradiation with the intensity of 100 mW/cm^2 or above might induce apoptosis of VSMC *in vivo*, which was confirmed by the experiment results of VSMC *in vitro* by Lu *et al.*[10]. Wang *et al.*[11] have showed that the treatment of low intensity He-Ne laser (40 mW, 62.5 mW/cm^2) would induce ASTC-a-1 cell apoptosis in a dose-dependent manner when the radiation time exceeded 16 minutes (60 J/cm^2). Stadler *et al.*[12] found that the effects of 660-nm laser (40 mW/cm^2) on blood *in vitro* were mediated by hemoglobin induced reactive oxygen species, which should be the mechanism of the effects of 632.8-nm (150 mW/cm^2, 540 J/cm^2) and 532-nm (150 mW/cm^2, 90 or 180 J/cm^2) laser irradiation on some rheological factors in human blood *in vitro*[3], and its proliferation on lymphocytes broke down at 5.0 J/cm^2, which might induce lymphocyte apoptosis. However, the tip intensity of the fiber-optic used in the present Chinese ILILT is over 1000 mW/cm^2[4]. ILILT induced lymphocyte apoptosis has been observed[13]. The further safety research should be done.

Whether there is photobiomodulation depends on the initial state of a cell or tissue. According to Karu 's theory[8], there is no effect of low intensity laser irradiation (LILI) on the cell which redox potential is so that the cell normally functions; the lower the redox potential of a cell comparing with the normal redox potential, the stronger the effect of LILI. Iijima *et al.*[14] have investigated the effect of the He-Ne laser (8.5 mW) irradiation on the deformability of human red blood cell (RBC). Blood samples were obtained from hematologically normal adult donors by venipuncture. RBCs were washed and adjusted to 30% with 0.9% NaCl solution (pH 7.00). RBC solution samples were assigned to three groups. Each sample was divided into seven 3-ml working aliquots. The aliquots in group 1 were irradiated for 0 (control), 1, 3, 5, 10, 15, and 30 minutes within 2 h after sampling. The aliquots in group 2 and group 3 were stored at 5 °C for 24 and 36 h, respectively, and received similar irradiations after 12 h (in both groups), 24 h (in group 2), and 36 h (in group 3) from sampling. The deformability shown as the filter filtration rate was unchanged in group 1 (fresh cell group) from the control value, but improved significantly in Groups 2 and 3 (damaged cell groups) after the irradiation.

As Tunér *et al.*[15] have summarized, the light energy is thought to reap the greatest benefit where it is most needed. Karu *et al.*[16] have studied the action of semiconductor laser radiation (λ = 820 nm) on the chemiluminescence of blood of clinically healthy humans, and found no significant effects. Wang *et al.*[17] have studied the effects of low-energy He-Ne laser irradiation of extracorporeally circulatory blood on ATPase activities of RBC membrane in 13 cases of patients with insulin dependent diabetes mellitus (IDDM). The results showed that ATPase were significantly lower in IDDM than that in control subjects ($P < 0.01$), low-energy He-Ne laser irradiation could markedly activate the Na^+/K^+-ATPase, Ca^{2+}, Ma^{2+}-ATPase of the patients with IDDM ($P < 0.05$ or 0.01), but could not significantly affect the ones of the control ($P > 0.05$).

If the radiation intensity is so low that the photodynamic effects of some specific pathways can not damage membrane or cell compartments, photobiomodulation should be dominantly mediated by the non-specific pathways[7]. The reciprocity law was not obeyed for almost all the studied photobiomodulation[18]. Li *et al.* have studied the non-resonant interaction of low intensity He-Ne laser irradiation with neutrophils[19]. Neutrophils were irradiated by He-Ne laser irradiation at doses of 800, 1000, 1800, and 2000 J/m^2, respectively, and the power was changed at each dose. They found the NADPH oxidase activity was different at different power for each dose of He-Ne laser irradiation, so that the reciprocal rule did not hold. Minkovich *et al.*[20] have analyzed the transmission spectra of diluted and nondiluted heparinized human blood before, after and in the course of irradiation. They found that no changes in the blood spectrum have been detected when irradiating diluted blood, and the reproducible variations of the blood transmission spectrum in the range of 640–805 nm have been observed when irradiating nondiluted blood.

There are many cellular studies on ILILT, such as the effects of near-infrared (810 nm) laser radiation on red blood cell ATPase activities and membrane structure[21], membranotropic photobiomodulation on red blood cell deformability[22], Low-level laser irradiation attenuates production of reactive oxygen species by human neutrophils[23], the effects of low intensity laser irradiation on nitric oxide and cytokines production by leukocytes[24] and the biostimulatory of blood platelet function[25]. Here is the only one signal transduction research on ILILT. Duan *et al.*[2] have probed signal transduction pathways of respiratory burst of bovine neutrophils which were induced by He-Ne laser at a dose of 300 J/m^2 by using the protein tyrosine kinases (PTKs) inhibitor, genistein, the phospholipase C (PLC) inhibitor, U-73122, and the protein kinase C (PKC) inhibitor, calphostin C, respectively, and found the inhibitor of PTKs can completely inhibit the He-Ne laser-induced respiratory burst of neutrophils, and PLC and PKC inhibitors can obviously reduce it, but not fully inhibit it. These results suggest that PTKs play a key role in the He-Ne laser-induced respiratory burst of neutrophils and [PTK-PLC-PKC-NADPH oxidase] signal transduction pathways may be involved in this process.

Although the basic research of ILILT is very progressive, the *in vivo* research of ILILT is rather delayed. Xiao *et al.*[26] reported the first and only clinic research presented in the annual meeting of American Society for Lasers in Medicine and Surgery. Zvereva *et al.*[27] reported the first and only randomized placebo-controlled study on ILILT clinical efficacy in patients suffering from rheumatoid arthritis. It should be pointed out that the present *in vivo* research is of problem as the tip intensity of the fiber-optic used in the present Chinese ILILT is over 1000 mW/cm^2[4] and it would induce the apoptosis or necrosis of the blood cells near to the tip. Obviously, the *in vivo* research should be further done.

This work was supported by the 2000 summer student research grants of the American Society of Lasers in Surgery and Medicine; the National Natural Science Foundation of China (No. 60478048, 6017800, and 6027812); the Group Project (No. 20003061), Key Project (No. 20011480), and General Project (No. 20031526) of Guangdong Science Foundation of China; the Visiting Foundation of State Key Laboratory of Laser Technology, Huazhong University of Science and Technology; and the Guangdong Qianbaishi Fellow Foundation (No. Q02087). T. Liu's e-mail address is liutcy@scnu.edu.cn.

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