Effect of Low Level Laser on Induced Pulmonary Hypertension in Rats

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Type of Participation: Abstract and search study.

Abstract

Background: After the success of the first trials of this experiment which were done on rabbits, a new study were conducted on dogs to ensure the past results; in a step forward to use low level LASER (LLL) therapy in the treatment of congenital septal defects in infants.

Purpose The aim of this study was to investigate the effect of LLL irradiation on congenital septal defects in experimental dog model and explain the mechanisms and consequences of the heart exposure to LLL irradiation.

Subjects and Methodology: six male dogs who have congenital septal defects in their hearts - with age ranged 6-10 months- enrolled in this study for one and half months. They were assigned into two groups: Group (A): The study group consisted of 3 canine hearts who received routine animal care associated with LLL irradiation. Group (B): The control group consisted of 3 canine hearts who received only routine animal care. Sizes of the septal defects were measured by echocardiogram for both groups at the beginning and after the end of the study.

Results: Dogs exposed to LLL irradiation showed increase in their body weight and level of activity with increase of the cardiac output of the right ventricle in echocardiogram 3 months after the irradiation. Histological analysis demonstrated an increase in angiogenesis and augmented cell recruitment in the irradiated sites. This cell recruitment was caused by increased activation of circulating fibroblasts, and vascular endothelial cells. However, LLL exposure did not increase any circulating leukocytes or macrophages, proving that there is no any inflammatory process. Consistently, levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were within normal values after 3 months of LLL exposure. In addition, there was significant decrease of size of the diameter of the congenital septal defect with this group by 42.19%. Accordingly, in vivo depletion of leukocytes and macrophages with the abundance of fibroblasts and endothelial cells indicating that the latter group of cells are the prime cause of the shunt healing.
Abstract

Background and purpose: After the success of the first trials of this experiment which were done on rabbits, a new study were conducted on dogs to ensure the past results; in a step forward to use low level LASER(LLL) therapy in the treatment of congenital septal defects in infants. Subjects and Methodology: six dogs who underwent induction for ventricular septal defects by cardiac puncture technique with age ranged 6-10 months enrolled in this study for one and half months. They were assigned into two groups: Group (A): The study group consisted of 3 dogs who received routine animal care associated with LASER irradiation. Group (B): The control group consisted of 3 dogs who received only routine animal care. Size of the septal defects were measured for both groups at the beginning and after the end of the study. Results: There was significant decrease of size of the diameter of the induced ventricular septal defect with study group (percentage of improvement was 42.19%) when compared with control group. Conclusion: It was concluded that low level LASER therapy can be considered as a promising therapy for congenital heart defects in animals and to be examined on children with similar congenital lesions after then.

Key words: LASER, VSD, dogs.

1. Introduction

Left-to-right shunt lesions are among the most common CHD lesions that the anesthesiologist will encounter. The level of shunting can occur at any location between intracardiac chambers (i.e., ventricular septal defect [VSD] or atrial septal defect [ASD]), or extracardiac structures (i.e. patent ductus arteriosus [PDA]). The pathophysiologic consequences of L-R shunt depend on several factors: the size of the defect, pressure gradient between chambers or arteries, the pulmonary/systemic vascular resistance (PVR/SVR) ratio, the relative compliance of right and left ventricles, and blood viscosity.

Ventricular septal defect (VSD) is the most common congenital heart defect seen in children. Defects can occur at various locations in the septum but most commonly occur in the membranous or muscular portions. Small defects often close spontaneously during childhood. One type of defect, the outflow (or supracristal) VSD, can be spontaneously occluded by one of the aortic leaflets prolapsing into it. This can result in the development of significant aortic insufficiency.
Small VSDs are usually asymptomatic, whereas larger defects are more likely to manifest during childhood with heart failure. VSD is the most common cause of Eisenmenger’s syndrome.²

Eisenmenger syndrome refers to any untreated congenital cardiac defect with intracardiac communication that leads to pulmonary hypertension, reversal of flow, and cyanosis.³ ⁴ ⁵

Surgical closure of the ventricular septal defect is the most commonly performed procedure in pediatric cardiac surgery.⁶ Postoperative Complications may include heart block and junctional ectopic tachycardia (in infants). Residual VSD’s may also remain.⁵

LLLT uses low-powered laser light in the range of 1-1000 mW, at wavelengths from 632-1064 nm, to stimulate a biological response. These lasers emit no heat, sound, or vibration. Instead of generating a thermal effect, LLLT acts by inducing a photochemical reaction in the cell, a process referred to as biostimulation or photobiomodulation.⁷ (See NOTE 2)

Purpose of the study:
Was to investigate whether low level Laser had any effect on the healing of moderate sized induced ventricular septal defects, to investigate whether low level Laser had any side effects on the patient, and to investigate whether treatment with low level Laser could be an adjunctive method of treatment for the traditional surgical choices. According to our knowledge, there is no past studies conducted in this field but other studies were conducted on the thermal effect of argon laser in palliation of obstructive congenital lesions such as aortic stenosis and coarctation of the aorta and it was found to be effective.

This study is considered A NOVEL TRIAL to conduct that method of treatment for induced ventricular septal defects. (See NOTE 1)

2. Materials
2.1. Animals:

Six dogs (See NOTE 11) who underwent surgical induction of moderate sized septal defects in their hearts via cardiac puncture technique were included in the study, aged from 6-10 months and recruited from the Department of physiology at the faculty of veterinary medicine, Cairo University. All subjects of the study were hospitalized and housed in a conditioned environment (22±1°C, 55±5% relative humidity, 12 h light/dark cycles) and were fed standard laboratory chow and water. This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996). They were randomly assigned into two groups:
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The study group:
The study group consisted of 3 dogs, which received low level LASER therapy at the site of the induced shunt percutaneously plus routine animal care (feeding, and psychological support).

The control group:
The control group consisted of 3 dogs, which received the same induction of the shunt and routine animal care only (feeding, and psychological support).

Inclusion criteria:
1- Dogs who underwent surgical induction of moderate sized septal defects in their hearts.
2- Their age ranged from 6-10 months old.
3- Dogs with clinically and medically stable conditions.

Exclusion criteria:
Dogs that are apparently not healthy or known to have any kind of illness.

2.2. Instrumentations:

1. Induction equipment:
Surgical needle: its size is 24 guage used in the induction of the lesion within the heart whilst the heart is exposed.

2. Anesthesia protocol:
General anesthesia with ketamine hydrochloride and xylazine 0.1mg/kg.

2.3. Evaluating equipment:
Echocardiography:
(PHOX PLUS C 402103020, America)

2.4. Therapeutic equipment:
The LASER device:
(laserklasse2M, EN60825-1,+A2:2002,w.l=635-670nm,p<6mW, German)

3. Methods:
Demographic data, clinical characteristics and all medical history were collected from dogs’ file.

3.1 Induction procedure (induction of septal defects): has been done for all rabbits as follows:
1. for each dog before the study, the induction of the ventricular septal defects via opening of the chest via lateral thoracotomy incision.
2. Whilst the heart is exposed a needle is inserted directly into the heart with a cardiac puncture technique to induce moderate sized induced septal defects.
3. The induced septal defect was defined as moderate sized when we measured the annulus of the aortic valve by the echocardiography and divided
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by 2, the diameter of the annulus of the aortic valve was 1.2+/-.0.2cm, so the diameter of the induced shunt was 0.6+/-.0.1cm in the hearts of the dogs. (See NOTE 3,5)

4. The shunt is left for 2 weeks before the start of the treatment phase to make sure that it will not heal spontaneously as dogs have much higher healing characteristics than humans. (See NOTE 4)

3.2 Evaluation procedure:
Echocardiography: has been done for all dogs as follows:
For each dog before, during, and after the study, the echocardiography was used

1. Before the study, to investigate the exact site of the shunt to apply the laser therapy on it. (See NOTE 6) (Fig.1)
2. During the study, to measure the shunt size before the start of the treatment period to make sure that the shunt did not heal spontaneously
3. After the study, to investigate the differences happened to the shunt after the application of the laser therapy at the end of the study. (Fig.2)

3.3 Treatment procedure
Each dog in the study group received laser therapy at the site of the shunt percutaneously for 15 minutes with two sessions daily for four consecutive weeks.
Laser therapy was introduced to the rabbits as follows:

1. Shaving of the dog hair (See NOTE 7)
2. Adjustment was done for the wave length and intensity of the device. (wave length=635-670nm,p=6mW, 0.5 j/cm2,15minutes) (See NOTE 8,9,10)
3. The dogs had received anesthesia (ketamine hydrochloride and Xylazine 0.1/kg) to decrease their movements during laser application.
4. The dog lied comfortably while holding the laser probe tightly at the site of the shunt which was seen under echocardiography (echo guided) and a marker was put on that site to apply laser at the same point in the first session.
5. The dogs vital signs was closely monitored as the vet was taking the heart rate, respiratory rate and body temperature of the dog at regular intervals every session during the application of the laser.
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4. Notes:
1. This study aimed to find an alternative or even adjunctive non-surgical method for some congenital heart defects like septal defects whether it is ventricular or atrial, in order to avoid or decrease -at least- open heart surgeries complications, which is still till now the only method for treatment of such cases.

2. The present study revealed that post induction of induced septal defect, a treatment of LASER, has a beneficial effect in increasing of the healing process of the cardiac structures and that came in support with the work of Mitsos and his colleagues, (2014) who reported that the application of LASER had been shown to be effective in increasing the revascularization after induced myocardial infarction in rabbits.9

3. The size of the shunt is Based on the maximum measured diameter of the defect as compared to a normal aortic valve annulus as follow: Small VSD: < 1/3 of the diameter of the aortic valve annulus, moderate sized VSD: about ½ of the diameter of the aortic valve (which is the only included shunt in the study), and Large VSD: near the same diameter of the aortic valve annulus.10

4. The size of the induced shunt was measured via echocardiography after 2 weeks from the induction to give a time for the shunt to heal if it can be healed spontaneously, if you left the shunt for shorter period and started treatment very soon that may be a false indicator about the healing susceptibility of the shunt.

5. The induced septal defect was defined as moderate sized when we measured the annulus of the aortic valve by the echocardiography and divided by 2, the diameter of the annulus of the aortic valve was 1.2+/-0.2cm, so the diameter of the induced shunt was included in the study only if it falls in the range 0.6+/-0.1cm and excluded if less (because that means that it may be a small sized shunt which can heal spontaneously) or more (because that means that it may be large sized shunt which mostly cannot be healed with this procedure).

6. Echocardiography will be applied on the dog after the induction to localize the exact site of the induced shunt, and we put a marker on the skin to know the exact site of laser application, because it was not available for us to use
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echocardiography every session, if it is will be applicable for us that would much easier to determine the site where you will apply the laser every session.

7. Before laser application, you have to shave the hair of the dog in—at least— the area where you will apply laser to avoid beam absorption by the hair and therefore decrease of the delivered dose to the site of the shunt.

8. We tried many wave lengths, intensities, and treatment durations during the experiment but finally it was found that high intensities and long wave lengths hinder the healing process, so the optimal dose is 15 minutes of application (with wave length=635-670nm, p<6mW, and energy 0.5 j/cm2), pulsed wave not continuous as the latter induce vasospasm which lead to vascular damage.

9. Avoid using thermal lasers like acute continuous argon-laser because it has ablation and destructive effects rather than healing effects.

10. This class of laser is called class 2M which is very safe for the eye so wearing goggles during application is not needed.

11. It is recommended to study to conduct that research on larger samples.

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Figures

Fig. (1): Echocardiography showing the mosaic appearance that happened after induction of the shunt at the beginning of the study
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Fig. (2): Echocardiography showing marked improvement and decrease in the amount of the mixed blood after laser therapy for the shunt (at the end of the study)