Estimation of IgM & IgG values in the serum after intravenous irradiation of blood with diode laser

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Date of acceptance 11/10/2004

Summary

The present study is designed to evaluate the effect of low level laser irradiation on the immune system when administered intravenously. (16) Adult local rabbits used in this study, they were divided in to two equal groups (control & treated with low level laser) they were anaesthetised generally and the site of the operation which was the medial aspect of the left thigh prepared to reach the femoral vein from where blood samples obtained from all the animals and considered as (standard readings). The blood of the animals of the treated group irradiated with a diode laser, its energy reached the blood via a fibre optic introduced to the femoral vein through a fine canula fixed in its end, after that a fine catheter fixed in to the femoral vein of all the animals and blood samples obtained for the periods (0.5, 1, 2, 2.5, 3, 6, 9, 12, 15, 18, 21, 24 hours) from the beginning of the operation in the control group and after irradiation of the blood in the treated group. The readings obtained for the periods from (0.5- 12 hours) revealed gradual significant increase in the level of the immunogloblines (IgM & IgG) in the serum followed by a plateau continued to the readings of (21 hours), and little decrease at the readings of (24 hours). The study showed that irradiation of the blood enhanced the immunological properties significantly and this appeared through the increase of the level of the (IgM & IgG) which are the most important members in the primary and secondary immunological response respectively.

Introduction

The immune system protects the body against the effects of a great variety of foreign cells and substances, the response of the immune system to these assaults involves interaction between the ones own and foreign cells That are mediated by foreign antigens and cellular receptors,(David,1996),(1)

Cellular receptors found on membranes of lymphocytes (B & T), they bind to foreign antigens, B- cell recognition molecules are called immunoglobulines or antibodies, (Warren & Ernest, 1998).(2).

The main immunoglobulin classes are IgG, IgM, IgE, IgA & IgD, IgM is the

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main immunoglobulin produced early in the primary response while the IgG is the predominant antibody in the secondary immunological response and constitutes an important defence against bacteria and viruses, (Helen, et. al., 1999), (3) &(Saimah & Arjmand, 1998), (4).

To expand the clinicians developing efficient manner to avoid toxicity and achievement of perfect treatment and therapies, series of trials carried out to study the role of the modern discoveries in medicine at the molecular and cellular levels in addition to their clinical applications, one of these modern discoveries is laser, both laser irradiation and the resultings from its primary and secondary effects enhance the neuro – humoral reactions leading to activation of the immune system and increase the adaptive hormones concentration , (Aleksandrov, 1992), (5).

Laser can be used for initiation intrinsic resistance against the extrinsic factors (antigens) through increase the viability of monoclonals or isotypes of the antibodies directly or indirectly by releasing of mediators such as; Enkephalins, Endorphins, Prostaglandins and others, (Danilov, et. al., 1992), (6)& (Ohshiro &Calderhead, 1988), (7).

Many medications which are swallowed, rubbed on or injected in to the patient to help him to reject or fight infections or diseases, sometimes these routs of medications may cause excessive immune system activity leading to allergic reactions, L.L.L.T. proved effective in restoring system balance in such cases, (Ohshiro &Calderhead, 1988), (7).

This experiment is designed to study the immunological response after irradiation of the blood with low level laser intravenously, the value of IgM & IgG are considered as a ratio of the immunological response because they are the major component of the primary and secondary immune response.

Materials & Methods

(16) Adult local rabbits with average weight of (1.5-2Kg) used in this study, they were divided into two equal groups (control and treated with low-level laser) each one consisted of (8 rabbits). Surgical operation carried out under general anaesthesia using a mixture of (Acepromazine malet [1] 10mg/Kg B.W.) with both of (Ketamine hydrochloride [2] 10mg/Kg B.W.) and (Xylazin [3] 5mg/Kg B.W.) injected all intramuscular, surgical anaesthesia obtained after 10 minutes and continued for 30-45 minutes, (Nelson, et. al., 1989), (8), then the skin incised, the muscles separated, the femoral vein exposed and samples of blood (2 ml) from each animal collected using a fine syringe [4] with fixed needle in its end, the samples of the blood transmitted to test tubes (without anticoagulant) which placed in a slant position in the refrigerator for half an hour, then transmitted to the centrifuge[5] with 1500 rpm/min for 5 min., thus the serum is separated and sent for examination using HPLC[6] these

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standard readings

[1] Calmivet, 0.5mg, magny – vernois – 70200, Lura-France.

[2] Ketaller, 50mg/ml, Park Davis & Co. Gwent, U.K

[3] Rompon, 20mg/ml, De, Hoeve 28, PANTEX Holland.

[4] Micro-Fine plus, u-100, Deetom Dickinson, Dublin-Irland

[5] Kotterman Laboratory Instrument Co., West Germany.

[6] Schmsu Analytical Laboratory Instrument, Japan, 45 J-4002.

reading were considered as lood of the animals of the treated group were irradiated using a Ga-Al-As diode laser [1] with wave length (904nm) continuos beam and power (10 mw) once, the energy of the laser was transmitted through a fine fibre optic [2] passed into the femoral vein across a canula [3] fixed in its end.

The femoral vein of all the animals catheterised using a fine catheter [4] then the area was washed with normal saline and the muscles sutured using continuos stitches of (4-0) catgut [5] while the skin is sutured using simple interrupted stitches of (4-0) surgical silk [6].

Samples of blood collected from the animals of both groups at the times (0.5,1,1.5,2,2.5,3,6,9,12,15,18,21,24 hours) after the operation interval, the serum separated and sent for detection of the level of both IgM & IgG using HPLC by anion exchanges depending upon Brooks & Steven method, (Brooks & Steven, 1985), (9).

HPLC is a sensitive and selective chromatographic method, it is equipped with two pumps, precolumn, analytical column, UV detector, mobile phase, citrate buffer pH (4) and flow rate was set at 2ml/min .

[1] Russian – Polish Joint venture "Moskovesky Polisib", G bld.3 Novoslobod skaya str., 103030 Moscow, Russa.

[2] Light guide probe with outer diameter of (0.8mm).

[3] 20G 1 ¼ mm, B. Braun melsungen AG, D-34209, Vasofix – Germany.

[4] VYGON, 95440 ECOUE FRANCE, L.30cm, page (6).

[5] Chordasw resor, bills aseptica, pH-Eue, B. Berlin-Germany.

[6] Ethicon, Ltd. P.O. Box 408 Bankhead Ave. Edinburgh, Scotland, U.K.

Results

Concentration (µg/ml)/Time (hours) relation of both. IgM & IgG reveal gradual significant increase ($p < 0.05$) for the readings from (0.5 to 12 hours) followed by insignificant increase (plateau) ($p>0.05$) up to the time of (24 hours) when the readings began to decrease n the treating group. The readings of the control group showed insignificant hanges $p>0.05$) in all the times (from 0.5 up to 24 hours) for both the IgM & IgG. Concentration/time elationship f oth IgM & IgG in the treated group were significantly higher ($p < 0.05$) than those of control group, (Table 1&2)

Time/Hours	Concentration (µg/ml)	
	Control	Laser
0.5	0.16	0.28
1	0.20	0.31
1.5	0.21	0.39
2	0.20	0.48
2.5	0.09	0.53
3	0.03	0.72
6	0.05	0.79
9	0.03	0.92
12	0.06	0.93
15	0.04	0.88
18	0.03	0.89
21	0.08	0.89
24	0.04	0.73

Table (1): Concentration / Time relationship of IgM of both groups

Time/hours	Concentration (µg/ml)	
	Control	Laser
0.5	5.72	6.39
1	5.9	6.75
1.5	6.54	40.90
2	6.9	12.56
2.5	6.1	18.66
3	6.01	22.48
6	6.0	22.84
9	5.81	22.80
12	5.79	22.86
15	5.28	21.17
18	5.60	21.95
21	5.41	21.72
24	5.61	21.02

Table (2): Concentration / Time relationship of IgG of both groups

Discussion

This is first study that systemically analyses serum responses to laser, the study revealed fundamental role of laser in potentiation of immune system to elaborate high levels of antibodies, low level laser activities the immune responses, (Zeuv&Rybachenco,1992) ,(10), activation f immune system toward enhanced immune response passed through stimulation the differentiation of the B-cells to reach such levels of antibody production or through positive egulation f he humoral responses,(Barnu&Hxi,2001), (11).

Results of this study showed over expression of both IgM & IgG molecules by B-cells in treated group that leaded to increase of the humoral immunity, (Warren & Ernest, 1998), (2). IgG remained predominant component of the immune response with 4 folds higher when compared with the level of IgM, the levels of both IgM & IgG in the treated group were higher when compared with the control group, this results is in agreement with those obtained by, (Zeuv&Rybachenco, 1992), (10), while the IgM/IgG ratio in the control group agreed with that reported by, (Saimah & Arjmand, 1998), (4).

Laser priming and boosting the production and function of induced antibodies, that means efficient increment of immunisation and/or vaccination, this fact can be attributed by the irreversible promotion of the laser therapy on the Glutathione System that required in injuries tissues, Halliwell ,1988), (12), others attributed he significant increase of IgM & IgG to free radicals; mainly ö and increase tissue destruction which stimulates the immune responses, (Weiss,1989),(13) &(Von Boehmer &Kisielow, 1991) ,(14).

Increasing the levels of both IgM & IgG can be explained by promotive effect of low level laser on the Arachidonate hydrolysis and inducing production of kinase, Prostaglandines, and types of Leukotrines, which acts as chemotachtic factors, these factors are biological mediators secreted by the leukocytes, these substances are synthesised and stored in special granules and released in response to external stimuli, the function of secretion probably stem from the activation of contractile proteins in the cytoplasm, this phenomenon in turn is due to a transmembranal influx of ca^{++} ions, possibly elicited by conformational changes in certain membrane proteins in response to external stimuli, this sequence aggregates B-cells and triggered them by complement that provokes responses of local and systemic immunologic responses, (Van& Betting , 1987) ,(15) & (Sell, 1987) , (16).

The cell membrane structure has a great importance in the formation of response to the laser emission, their high sensitivity explained through the fact that they represent natural boundaries of phase division, laser emission reorient the lipid biolayer polar groups and since there is a close contact between the lipids and proteins, these orientations influence the processes connected with membranes, cell energy production and enzyme reaction, but the immunological behavior of the cell depend not only the membrane structure and function states but also on the pre- membrane-layer – glycocalsis – constituted mainly of calcium ions, the physical and chemical changes in the cell membrane and the pre- membrane layer in particular after the laser emission explain the stimulation of phagocytic activity of leukocytes in blood , T & B lymphocyte rosette – formation ability,(Golovin ,1992),(17) .

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تقدير أقيام الغلوبولينان المناعية IgG و IgM في البلازما بعد تشعيع الدم بالليزرات الواطئة الطاقة عن طريق الحقن الوريدي

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الخلاصة

تم تصميم الدراسة الحالية للتعرف على تأثير التشعيع بالليزرات الواطئة الطاقة على الجهاز المناعي في حال إعطائها عن طريق الحقن الوريدي. استخدمت في هذه الدراسة (١٦ أرنبا) محليا بالغا تم تقسيمهم إلى مجموعتين متساويتين (السيطرة والمعالجة بالليزرات الواطئة الطاقة)، تم تخدير الحيوانات تخديرا عاما وتهيئة منطقة العملية وهي الجانب الإنسي من الفخذ الأيسر وصولا إلى الوريد الفخذي حيث أستحصلت نماذج دم من كافة الحيوانات واعتبرت (قراءات دم قياسية)، وتم تشعيع الدم في حيوانات مجموعة المعالجة بواسطة ليزر ثنائي الصمام، تم ايصال طاقته بواسطة ليف بصري تم تمريره إلى داخل الوريد الفخذي من خلال مبزل دقيق مثبت في نهايته، أعقبت هذه العملية تثبيت قسطرة دقيقة في الوريد الفخذي للحيوانات كافة واستحصلت نماذج دم للفترات (٠,٥ ، ١ ،١,٥، ٢، ٢,٥، ٣، ٦، ٩، ١٢، ١٨، ٢١ و٢٤ ساعة) من بداية العملية في حيوانات مجموعة السيطرة وبعد تشعيع الدم في حيوانات مجموعة المعالجة.أوضحت نتائج القراءات المستحصلة للفترات من(٠,٥-١٢ ساعة) وجود زيادة تدريجية ملحوظة في مستوى الغلوبولينات المناعية (IgGو IgM) في مصل الدم أعقبتها فترة سكون امتدت لغاية قراءة (الساعة ٢١) ثم انخفاض طفيف عند قراءة الساعة (٢٤). نستنتج من هذه الدراسة إن تشعيع الدم بالليزر عن طريق الدم يحسن الصورة المناعية بصورة ملحوظة تجلت بارتفاع مستوى الغلوبولينات المناعية (IgG,IgM) وهما العنصران الأكثر أهمية في الاستجابة المناعية الأولية والثانوية على التوالي.