Study the effect of hyperthermia ,laser and chemotherapy on white blood cell activity in cancers patient measuring by means ofLuminol dependentChemiluminescence

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ABSTRACT

Investigation the effects of low-level laser (532nm)irradiation and hyperthermia combination with chemotherapy on white blood cell activity in patient with different type of cancer, measuring by mean of luminol-dependent chemiluminescence(CL).Diminished function activity of peripheral blood neutrophils has been found in untreated human patients with several different types of cancer.As expected, cell viability decreases gradually with time . About 60 min, cell viability was about 75% in the control group, while in the group heated to 40^{o}C, only 30% of the cells are viable. Approximately 60% of cells died after a 30 min exposure at 40^{o}C. Exposure for 60-120 min at 40^{o}C causes a similar effect .Laser radiation causes significant enhancement of spontaneous chemiluminescence response($P<0.05$).Un expected result,the increase in CL functional activity has been found with laser treatment for all times of irradiation. A maximum enhancement in CL functional activity has been found at about 60 min of irradiation ($P<0.05$). A combination of laser and HT has similar behaviorof CL response($P<0.05$).The conclusion drown from this in vitro study demonstrated that diode laser radiation (at low power levels laser (λ = 532 nm, I = 150 mW/cm^2) combination with hyperthermia causes enhancement effects in phagocytosis activity of white blood cell in cancer patient.

KeyWord: laser,hyperthermia , chemotherapy. Chemiluminescence response

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Introduction

There are widespread applications of low intensity laser radiation in various areas of the medical field(wound healing, tissue repair, and vascular rest enosis)[1,2,3], and experimental medicine requires detailed information on the mechanisms of their biological effects[4.5]. The emitted laser light is polarized and coherent and may be absorbed by different tissues[6]. Tissue biostimulation is only possible if irradiated cells posses molecular photo acceptors that absorb the light and enter into state of excitation triggering intracellular cascade of signals leading to a measurable biological effect[6,7] . It is generally accepted that the mechanism of laser bio-stimulation is based on the absorption of monochromatic light by components of the cellular respiratory chain[6,7]. NADPH-oxidase is responsible for non-mitochondrial respiratory burst of phagocyticcells.Thisenzyme constitutes a redox chain that generates reactive oxygen species in response to activation and can react to laser irradiation[6,7].The term hyperthermia refers to raising the temperature of a part of or the whole body above normal for a defined period of time.The amount of temperature elevation is on the order of a few degrees above normal temperature (41 - 42.5 C°)[8]In 1898, a Swedish physician, Dr. Westermark described anecdotal incidences of local hyperthermia causing cervical cancer to regress. In1910, Moller described the potential of HT as an adjutant to radiotherapy (RT).[9] However the biologic rationale for applying HT with RT in cancer therapy was not investigated in depth until the 1970[10-12].Exposure of mammalian cells to temperatures higher than 40° C leads to reproductive cell death. This effect of hyperthermic treatment on cells' reproductive capacity depends on both the applied temperature and the duration of the exposure.[12]The aim of the present study is to assess the influence of light emitted by a low energy diode laser(532 nm) and hyperthermia combination with chemotherapy on white blood cell activity in patient with different kind of cancer measuring by mean of luminol-dependentchemiluminescence(CL).

Materials And Methods

1.Tris-HCl stock solution

Has been prepared usingthe following procedure : 24.2 g of Tris (FLUKAGARANTEE) dissolved in 1000 ml distilled water , 50 ml of Tris solution have been added to 41.4 ml of 0.2 M HCl (FLUKA - GARANTEE), and diluted to 200 Tris - ml with distilled water. The final of morality equal to solution HCL 0.015 M at pH = 7.4.

2.CL inducer :-

In order to activate granulocytes to burst luminol-dependent CL, a medium of 165 mMNaCl, 15 mM Tris - HCl solution , 2.25 mm CaCl and 25 mM $BaSO_4$ solution has been used . $BaSo_4$has been used inthis medium as a suspended agent.

3.Luminol solution:-

Luminol solution has been prepared by 10^{-2}M of luminol (5-×dissolving 1.13 amino -2,3- dihydro -1,4- phthalaziuedione) in 2 ml of 0.2 M NaOH, this stock solution has beendiluted up to 100 ml with Deionized water and has beenkept prior to use.

Chemiluminescence of granulocytes

CL of granulocytes has been studied in full blood according to the method described earlier .Luminol -dependent CL has been assessed before and after chemotherapy. Light emission has been measured in multipurpose photon counting ; The system has been designed and built up in the department of physiology , college of medicine University of Basra (Iraq), with an option for CL. Results are shown as a (peak high per W.B.C counts) for 100 cell .

Laser irradiation: Green diode laser with a wavelength of 532 nm at (29.5)mwpower from(Shanghal Dream Laser Technology Co.)has been used as a power source.The power density is150mW/cm^2 at a distance of 6cm from blood inside the tube. During the experiments, the laser beam has beendirect delivered to the tubes of blood samples with an irradiation spot of a 5 mm diameter. Samples have been irradiated in different periods time (5,15,30,60,90,120) minute .

Preparation of Blood Samples:

Selection of patients

The protocol of our study on determination of activity of W.B.C for cancer patients with

laser and hyperthermia combination with chemotherapy, is clearly outlined .

It describes criteria for selection of cancer patients(different type)which includes only those don't treated with chemotherapy or radiotherapy ,and then themselves treated with chemotherapy(in vivo).Venous blood samples have been obtained from 50 healthy adults(control) and 50 cancer patients(different type of cancer) (aged 19 to 85 year)and their height and weight have been measured . Using 5 ml disposable syringe, (3ml) of blood has been transferred into EDTA tubes (EDTA is used as anticoagulant)and then kept at 4 $^{\circ}$C until the start of the assay (usually CL is measured within 1 hr.).Each blood sample has been divided into 4 groups.Group I to be treated without laser or rising temperature(NHNL).Group II includes incubation through water path at temperature (37,38,39,and 40)ºC without irradiated with laser(HNL).Group III includes irradiated with laser (wavelength 532 nm) only without rising temperature(LNH).Group IIII includes incubation through water path at temperature(37,38,39,and 40)ºC and irradiated with laser .The activity of W.B.C for all groups have been measured for different incubation time(5,15,30, 60,90 and 120)min using photon counting systemand the W.B.C have been counted using Hemocytometry method.

Statistical Analysis

The results have been evaluated by the analysis of the variance (ANOVA) ,mean± standerd deviation(SD) of mean ,P-values at levels ($P \leq 0.05$)has been considered to be statistically significant, this calculations have been carried out according to Statistical Package for Social Science (SPSS version 19).

Results:

EffectOfHyperthermia (HT) Combinations With Chemotherapy (CT) On W.B.Cs Activity.

At each time point studied, spontaneous CL of W.B.Cs(activity) is significantlyhigher in the control(NH-C)(no HT noCT)compared with cancer patient before andafter in vivo chemotherapy treatment(NH-B.T,NH-A.T) (noHT ,before and after CT respectively) at (P-value <0.05). We found that the activity of W.B.C has been increased in patient afterCT (no HT after CT (NH-A.T) compared with it before CT(NH-B.T)(no HTbefore CT).We next compare the exposure of blood at(37 ,38,39, 40)^{o}C for incubation time

(0,5,15,30, 60,90 ,120)min. The activity is greater in control group(H-C) in all temperature than the activity before and after CT and it has been increased in treated patient (H-A.T)compared with those before treatment(H-B.T) except at temperature 37°C , the activity before treatment has been increased .Figure (1)(A,B,C,D) illustrates means±SD (P-value < 0.05) of activity of W.B.Cs at different temperature for control and cancer patent before and after chemotherapy.Figure (2) shows theeffect of HT on activity of W.B.Cs for cancer patient at different temperature it is significantlyhigher (P< 0.05) after incubation with temperature(H) than that without risingtemperature(NH).

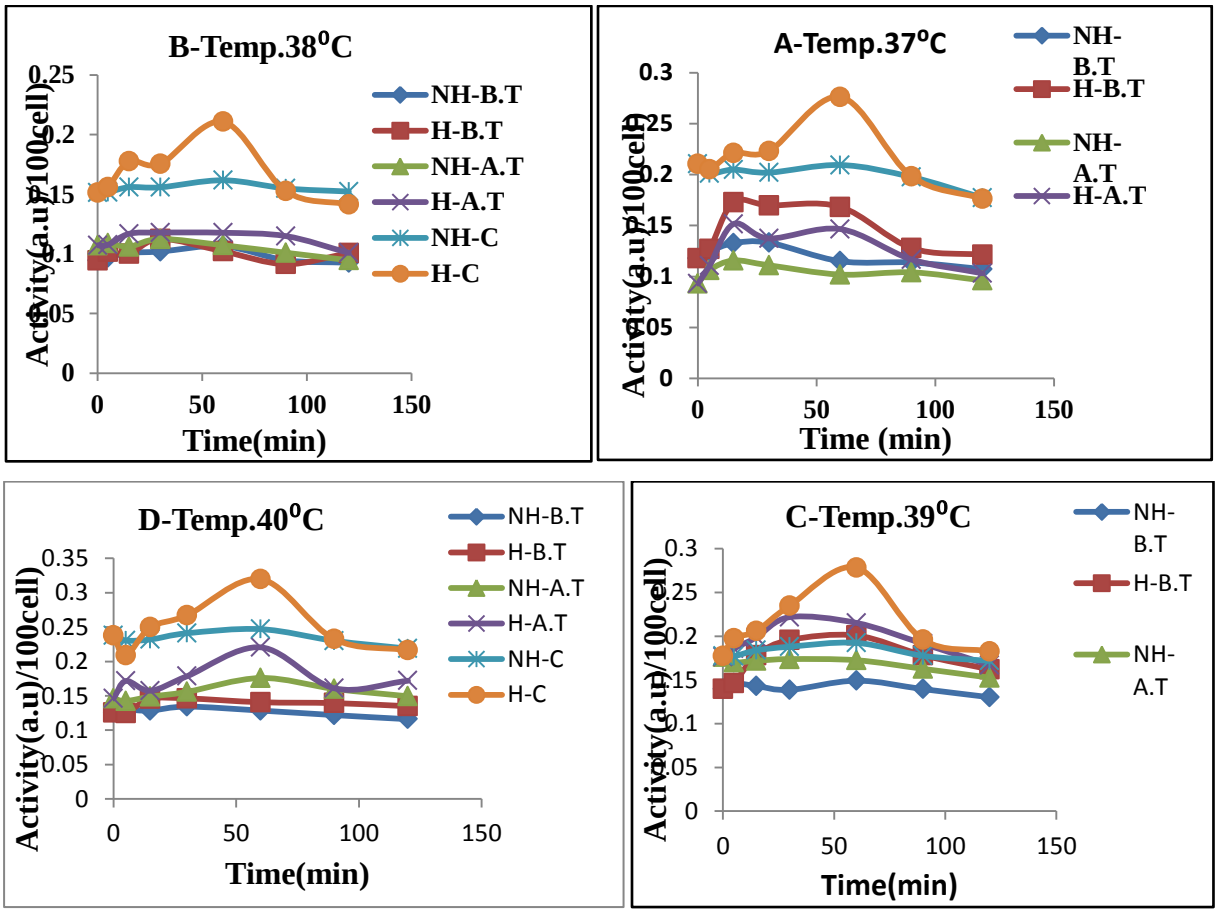

Figure (1) Effect of hyperthermia on activity of W.B.Cs for cancer patient before and after chemotherapy compared with control(P<0.05) .A: 37 ºC , B :38 ºC , C : 39 ºC , D:40 ºC , NH :NO Hyperthermia. H :Hyperthermia B.T :Before Chemotherapy Treatment ,A.T: After Chemotherapy Treatment. C:Control. (data shown as mean of activity (per 100 cell))for each time at certain temperature)

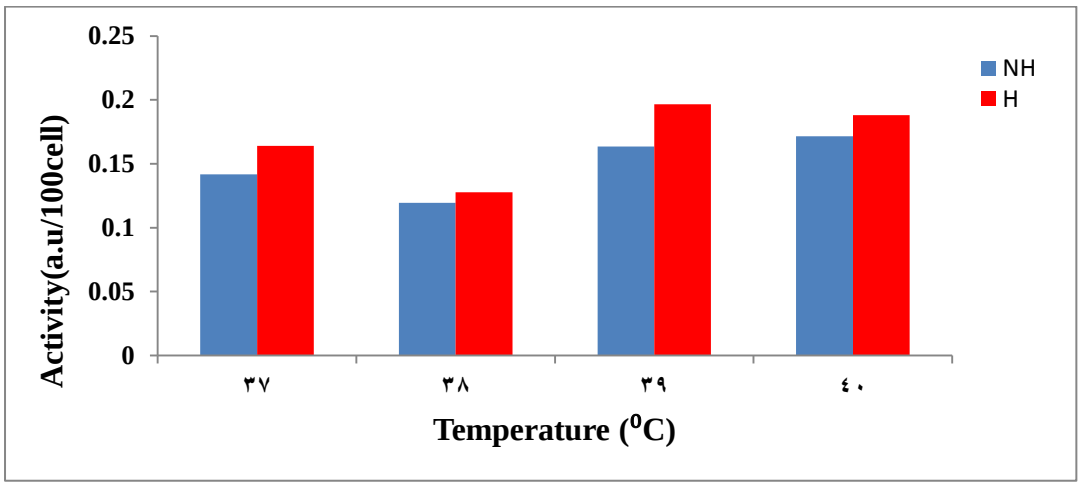

Figure (2): Effect of hyperthermia on activity of W.B.Cs for cancer patient atdifferent temperature(P< 0.05)(data shown as mean of activity (per 100 cell) in different time of incubation).

Effect Laser Irradiation On Activity Of W.B.Cs.

Figure (3) shows the effect of diode laser (532) nm irradiation on activity of W.B.Cs for cancer patients before and after in vivo chemotherapyand controlthrough different exposuretime.The activity has been increased in all groups after irradiation with laser compared with the activity in same groups before irradiation.The activity in treated group is lower than that in control and higher than unirradiated group .

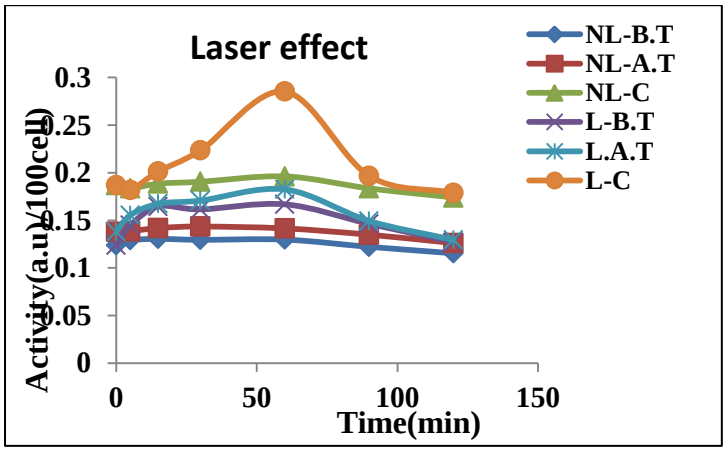

Figure (3) Effect of laser (532 nm) on activity of W.B.Cs for cancer patient and control(P<0.05) .NL : NO Laser . L : Laser, B.T :Before Chemotherapy Treatment ,A.T: After Chemotherapy Treatment, C:Control.

Effect Of Combinations Laser And Hyperthermia With Chemotherapy On Activity Of W.B.Cs

The activity of W.B.Cs before and after in vivochemotherapy treatment of cancer patient combined with hyperthermia (37,38,39,40)ºC and irradiated by diode laser (532) nm is significantly(P<0.05)increased compared with the same group without laser irradiation and hyperthermia Figure (4)(A,B,C,D) shows the activity at each temperature through different time of incubation .

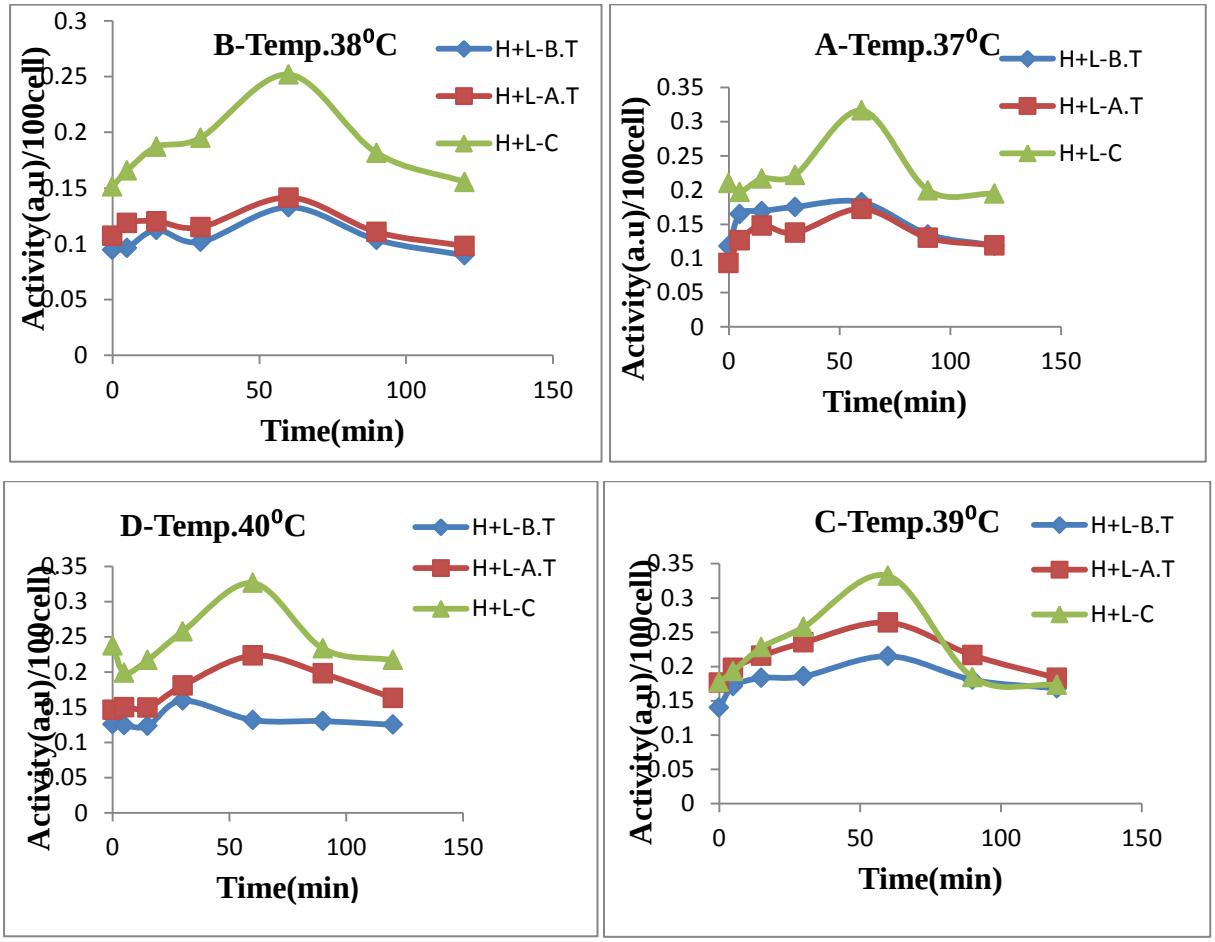

Figure (4) Effect of combination of laser (532 nm) and hyperthermia with chemotherapy on activity of W.B.Cs for cancer patient and control (P<0.05). A: 37ºC , B :38 ºC, C : 39 ºC, D : 40ºC . H+L: Laser + Hyperthermia, B.T :Before Chemotherapy Treatment ,A.T: After Chemotherapy Treatment. (data shown as mean of activity (per 100 cell))for each time at certain temperature)

The comparison between the activity of W.B.Cs of cancer patient at different temperatures HT (37,38,39,40)ºC and irradiated using diode laser (532nm) after CT for different incubation time issignificantly at(P-value < 0.05). This is shown in figure(5) , all graphs areillustrated as mean± standard deviation of activity at different time and temperatures.significantly at(P-value < 0.05).

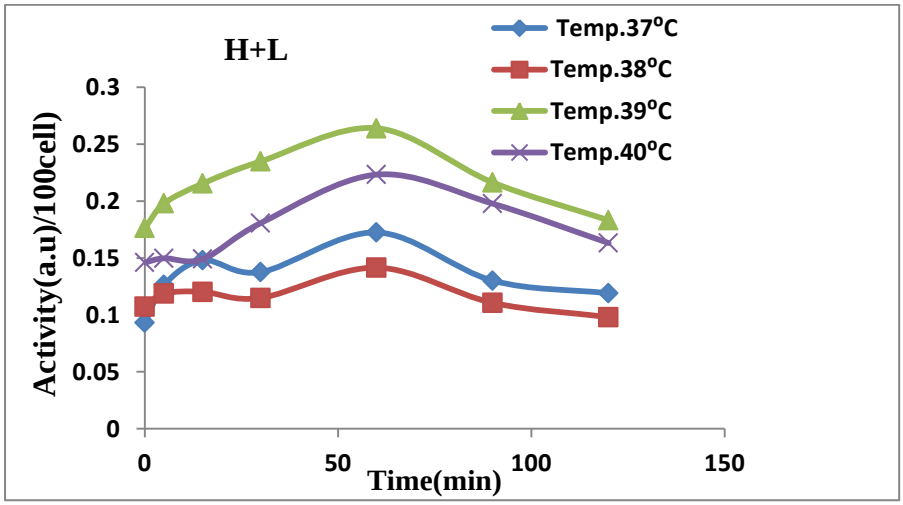

Figure (5) The comparison between the activity of W.B.Cs of cancer patient at different temperatures(hyperthermia) (37,38,39,40)ºC and irradiated using diode laser (532nm) after chemotherapy for different incubation time. (P<0.05) (data shown as mean of activity (per 100 cell))for each time at certain temperature)

Figure (6) shows the comparison between mean of activity(per 100cell) of W.B.Cs of cancer patient at different temperatures(HT) (37,38,39,40)ºC and irradiated using diode laser (532nm).The activity at(39 ºC) issignificantly higher compared with other temperatures when irradiated with diode laser (532nm). Figure (7) shows the comparison between mean of activity(per 100cell) of W.B.Cs of cancer patient at different temperatures(HT) (37,38,39,40)ºC and /or irradiated using diode laser (532nm) in different study condition .The activity when irradiated with laser and incubation at different temperatures(H+L) is significantly higher compared with other conditions (NHNL,HNL, and LNH)

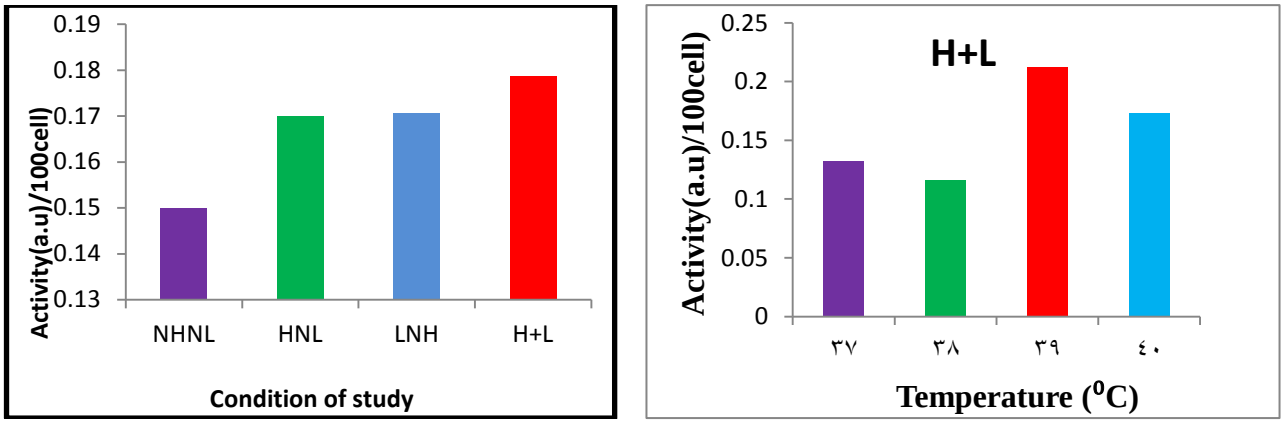

Figure (6) Activity(per 100cell) of W.B.Cs of cancer patient at different temperatures(hyperthermia) (37,38,39,40)ºC and irradiated with diode laser (532nm)(P-value <0.05).

Figure (7) Mean of activity(per 100cell) of W.B.Cs of cancer patient at different temperatures(hyperthermia) (37,38,39,40)ºC and/or irradiated using diode laser (532nm) in different study condition. NHNH: No Hyperthermia No Laser, HNL: Hyperthermia NO Laser, LNH: Laser No Hyperthermia, H+L: Hyperthermia with Laser .

Discussion

CL has been widely used to estimate granulocytesactivity. However, CL does not reflect only the phagocytes function of cells, but also the intracellular oxidative metabolic response .[13]In the present study we attempt toevaluate neutrophil function activity of adult human cancer patientsbefore and after receiving chemotherapy. Diminished function activity of peripheral blood neutrophils has been found in untreated human patients with several different types of cancer ,this finding correspond to the studies of Shirai, Ueta,KastelanBaskic,Tullgren,Hara,Lukac,Mi

kiro[14-22] Hyperthermia therapy is a medical modification treatment in which body tissue is exposed to slightly higher temperatures to damage and kill cancer cellslocally or to make cancer cells more sensitive to the effects of radiation and certain anti-cancer drugs[23-27]. In the present studyH.T plus C.T already has been taken by the patient appears to increased cell toxicity due to net increase in cell damage after exposure to hyperthermia and chemotherapy. The thermal dosecytotoxicity damage is related not only to temperature of exposure, but also toduration of exposure.Fig(1)(A,B,C,D),which are in agree with results ofOvergaard and Oliveira-Filho[28.29] assure our methodology.The exposure of granulocytes cells at(37,38,39 and 40)^{o}C for periods of time (5,15,30,60,90and120)min is to detect the cell viability. As expected, cell viability decreases gradually with time . About 60 min, cell viability is about 75% in the control group, while in the group heated at 40^{o}C only 30% of the cells are viable (P<0.05). As mentioned above, approximately 60% of cells died after a 30-min exposure at 40^{o}C. Exposure for 60-120 min at 40^{o}C caused a similar effect(data not shown).In these experiments, we have been evaluated cell death using a CL activity as a function of cell survival, is a late event in the biochemistry of cell death [30].Thus, in these CL assays the cytotoxic effects of hyperthermia are clearly estimated. We have been studied cell behavior after heat exposure and observed a progressive decrease in cell viability for up to 2 h after cessation of hyperthermia . However, a number of cells areexposure resistant up to 40^{o}C, for up to 30 min (thermoresistant) exposure time as shown in figure(1)(A,B,C.D). The cell activity isincreased for each temperature though about 60 min and then losses its viability (reduced CL factional activity),according to fig(1)(A,B,C,D).CL activity has shown similar effect at different temperature for different time of incubation .The effect of diode laser radiation (λ = 532 nm, I = 0.118 W/cm^{2}), irradiation time from (5 to 120min) on kinetics of spontaneous stimulated chemiluminescence of white blood cells has been studied. It is found that laser radiation caused significant

enhancement of spontaneous chemiluminescence response .Fig(3) shows the increasing in granulocytes functional activity may be related to the stimulation activity of cells rapidly in short time within in about 60 min time interval of laser irradiation .After irradiation, production of reactive oxygen species by neutrophils has been measured using luminol-dependent chemiluminescence. The CL response of neutrophilsis enhanced by laser irradiation at about 60 min time interval.Laser radiation may acts on whole cells. The results of the some studies suggest that more reactive oxygen speciesare generated in the electron transfer system of the cell. Mitochondria are themajor source of intracellular free radicals. Un expected result increasing in CL functional activity is found with laser treatment only for all time of irradiation .A maximum enhancement in CL functional activity has been found in about 60 min of irradiation . A combination of laser and HT has similar behavior of CL response .A comparison between treatment using laser alone and laser with HT treatment has significant response($P<0.05$) this mean that laser modified the viability of the cells and acts against heating damage. Diode laser light may cause a simulative effect as a result of the excitation of NADH molecule, which leads to increase the Oxidation –Reduction reactions. High-energy electrons flow may occur through the respiratory chain leading to the high-energy production via ATP molecules, which as a result, increase the cell activity,generation of singlet oxygen O^1 . These species are $^{\bullet}_2$ or hydroxyl radical OH cytotoxic because they are strong oxidizing agents, they can oxidize luminal and we detect increasing in CL functional activity.

Conclusion

The conclusion drown from this in vitro study demonstrated that diode laser radiation (at low power levels laser (λ = 532 nm, I = 150 mW/cm^2).combination with hyperthermia causes enhancement effects in phagocytosis activity of white blood cell in cancerpatient.

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دراسة تأثيرالعلاج بالحرارة والليزر والعلاج الكيميائي على نشاط خلايا الدم البيضاء لمرضى السرطان مقاسة بطريقة اللمعان الكيميائي المنوط باللومينول

غنية سالم الظاهر, علي حسين الهاشمي , عبد المنعم خليل الكامل

الخلاصة

تم دراسة تأثير الليزر منخفض الطاقة بطول موجي (532nm)والعلاج بالحرارة بالاشتراك مع العلاج الكيميائي على فعالية كريات الدم البيض (W.B.Cs) لمرضى السرطان. وقد تم قياس الفعالية بطريقة اللمعان الكيميائي المنوط باللومينول . وجد نقص في الفعالية الحيوية لكريات الدم البيض (العدلات) للمرضى قبل العلاج مقارنة مع مجموعة السيطرة (الأصحاء). وكما هو متوقع , فان قابلية الخلايا على البقاء حية تقل تدريجيا بمرور الوقت . في زمن حوالي 60 دقيقة فان قابلية الخلايا على البقاء حية حوالي 75% لمجموعة السيطرة ,بينما في المجموعة التي عرضت لدرجة حرارة 40ºم ,فقط 30% من الخلايا بقيت حية.تقريبا 60% من الخلايا ماتت بعد تعرضها لمدة 30 دقيقة لدرجة حرارة 40º م .إن التعرض لفترة من 60-120 دقيقة لحرارة 40ºم له نفس التأثير السابق .

إن التعرض لليزر أدى إلى زيادة في فعالية كريات الدم البيض التي تم قياسها بدلالة اللمعان الكيميائي وبدلالة إحصائية معنوية عند مستوى احتمالية ($P<0.05$) .وكنتيجة غير متوقعة فان الفعالية الحيوية تزداد عند العلاج بالليزر لكافة أوقات التعرض لليزر. وان أعلى زيادة حصلت عند زمن تعرض 60 دقيقة وبدلالة إحصائية معنوية عند مستوى احتمالية ($P<0.05$.) وجد انه عند استخدام الليزر (بطول موجي 532 نانومتر وقدرة منخفضة 150 ملي واط / سم^2) وبالاشتراك مع الحرارة له نفس التأثير وبدلالة إحصائية معنوية عند مستوى احتمالية ($P<0.05$) . نستنتج من هذه الدراسة انه تم إثبات إن استخدام الليزر الثنائي مع الحرارة أدى إلى زيادة الفعالية البلعمية لكريات الدم البيض لمرضى السرطان.

الكلمات الدلالية : ليزر ,العلاج الحراري , العلاج الكيميائي, اللمعان الكيميائي , عملية البلعمة .