The Role of Hyperthermia in Potentiation of Chemotherapy and Radiotherapy in Mice Bearing Solid Tumor

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Abstract: Hyperthermia is procedure in which body tissue is exposed to a high temperature up to 41°C and is an effective tool in cancer treatment. Hyperthermia also is a therapy applied together with other modalities in the treatment of cancer. The aim of this study was to determine if there was a change in immunological and biochemical parameters after using each of hyperthermia, radiotherapy or chemotherapy separately and the combined treatments in mice bearing solid tumor. Seventy females Albino mice weighing (20-25g) were used in the current study. The animals were divided into five groups. Group I: served as a control animals. Group II: animals were cancered by solid tumor and were untreated. Group III: animals exposed to WBH alone. Group IV: animals administered doxorubicin (Dox) 3mg/kg body weight i.p. once a week. Group V: animals were exposed to fractionated whole body gamma rays (WB-γ) at a dose level of 0.5 Gy once a week. Group VI: animals were exposed to WBH and administered doxorubicin (Dox) 3mg/kg body weight (i.p.) once a week. Group VII: animals were exposed to WBH then fractionated whole body gamma rays (WB-γ) at a dose level of 0.5 Gy once a week. After four weeks (the end of treatments), blood samples were collected from orbital venous plexus in heparinized tubes from all animal groups. The results of the present study indicated that WBH with or without radio- and chemotherapy induced significant increase in TNF-α, IL-2 and HSP70 values as compared to cancered group. As well as WBH with or without radio- and chemotherapy induce significant increases of phagocytosis and killing cells percent as compared to untreated cancered group. On the other hand WBH alone or with radiotherapy and (Dox) induced significant decrease of α -FP as compared to cancered group. Also, the results revealed that WBH with or without radio- or chemotherapy induced apoptosis for cancer cells. It could be concluded that, WBH enhances the response of tumor cells to radiation and chemotherapy and it has an important role in potentiation of radio- and chemotherapy in solid tumor treatment. [Nature and Science. 2010;8(5):100-108]. (ISSN: 1545-0740).

Key Words: Hyperthermia, mice bearing solid tumors, doxorubicin, whole body gamma irradiation, apoptosis, immune responses.

1. Introduction

Hyperthermia is the use of therapeutic heat to treat various cancers on and inside the body. The purpose of this anticancer therapy is to shrink and hopefully destroy cancer without harming noncancerous cells. It can be used to treat cancer in many areas of the body, including brain [1] , thyroid [2], lungs [3], breast [4], and prostate [5]. It is thought that high temperatures, up to 40 °C, can help shrink cancerous tumors. It is applied alone or as an adjunctive with various established cancer treatment modalities such as radiotherapy and chemotherapy [6]. Hyperthermia is now being used more widely, because it does not have as many negative side effects as conventional forms of cancer treatment such as radiation or chemotherapy [7]. It's characters seemed to be mostly pronounced in the non proliferating tumor cells situated in the central area of solid tumors *in vivo* [8]. Moreover, **Chang et al.** [9] suggested that hyperthermia may augment vaccines delivery to tumors after systemic injection, as hyperthermia increases the permeability of the endothelial vasculature to nanoparticles. Also**,** they have demonstrated that the tumors that were treated with systemic vaccines under conditions of hyperthermia (41.5°C for 30 min) had significantly higher levels of vaccines marker gene activity and the (>100-fold) than those treated under normothermic conditions ($p < 0.05$) and that this effect was specific to tumor. Recent studies have confirmed and extended the old observation that heat may cause complete and selective tumor destruction of malignant cells [10] by activating of the immune system [11] .Radiation induces tumor cell apoptosis and necrosis, resulting in the release of tumor antigen and danger signals. Combined treatment with radiotherapy and Hyperthermia could induce a potent antitumor immune response, resulting in a significant decrease in the rate of local tumor relapse and might be associated with the production of apoptotic and necrotic tumor antigens and heat shock proteins after irradiation, phagocytosis and induction of more efficient tumor-specific cytotoxic T lymphocyte activity through a cross-presentation pathway [12].

Doxorubicin (DOX) is the most widely chemotherapeutic agent in the ultrasound-mediated drug delivery studies. This is because DOX is an intercalating drug that stacks between paired bases in DNA. However, like other anticancer drugs of anthracycline family, DOX is cardio toxic due to the induced production of active oxygen radicals [13].

Radiation therapy is the treatment of cancer with ionizing radiation. Radiation works by damaging the DNA (genetic material) within the tumor cells, making them unable to divide and grow. Radiation is often given with the intent of destroying the tumor and curing the disease (curative treatment). However, although radiation is directed at the tumor, it is inevitable that the normal, non-cancerous tissues surrounding the tumor will also be affected by the radiation and therefore damaged [14]. In general, cells are most radiosensitive in M and G2 phases and most radioresistant in S phase [15].

Material and Methods:

Chemicals:

Adriamycin: (Doxorubicin Hydrochloride)

Doxorubicin is a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius* var. *caesius*. Doxorubicin consists of a naphthacenequinone nucleus linked through a glycosidic bond at ring atom 7 to an amino sugar, daunosamine

Chemically, doxorubicin hydrochloride is: 5,12-Naphthacenedione,10-[(3-amino-2,3,6-trideoxy-alpha-L-*lyx*o-hexopyranosyl)oxy]-7,8,9,10-tetrahydro -6,8,11-trihydroxy-8-(hydroxylacetyl)-1-methoxy-, hydrochloride (8S-*cis*). Doxorubicin hydrochloride was used in the form of an injectable commercial product (Adriablastina, from Pharmacia Italy).

Irradiation Technique:

The animals exposed to whole body irradiation with a total dose level of 2 Gy fractionated into four doses. The animals received 0.5 Gy once a week. Gamma irradiation source was from ^{60}Co, in the Middle Eastern Regional Radioisotope Center for the Arab Countries in Dokki, Giza, Egypt, at a dose rate (15.26/ min.)

Induction of solid tumor:

The mice had been inoculated with syngeneic tumor cells (Ehrlich carcinoma) obtained from the National Cancer Institute – Egypt. Ehrlich carcinoma cells ($2x10^6$) were injected into interapritoneal cavity of the animal using a hypodermic syringe with a 18 gauge needle. 7-8 days following tumor transplantation, subcutaneous tumors had reached a palpable size.

Experimental Animals:

Seventy female Swiss Albino mice weighting 20-25gm were purchased from the National Cancer Institute Cairo University, Egypt. The animals were kept at constant environmental and nutritional conditions throughout the experimental period with room temperature ($21 \pm 2°C$) with a 12h light / 12h dark cycle. They were fed standred pellet rat diet and water *ad- libitum* throughout the experiment. Mice were divided into seven equal groups each of 10 mice. First group served as control.

As soon as solid tumor was palpable, the animals were classified into 6 groups, (each of 10 mice).

Second group: the animals carried with solid tumor without any treatment.

Third group: the animals carried solid tumor exposed to WBH 41-5°C once a week for four weeks.

Fourth group: animals administered doxorubicin (Dox) 3mg/kg body weight iteraperitoneally (i.p) once a week.

Fifth group: animals were exposed to fractionated whole body gamma rays (WB-γ) at a dose level of 0.5 Gy once a week

Sixth group: the solid tumor animals were exposed to WBH (41.5°C) and injected (i.p) with 3mg /kg body weight Doxorubicin (Dox) once a week for four weeks.

Seventh group: the solid tumor animals were exposed to WBH (41.5°C) and whole body gamma irradiation (0.5 Gy) once a week for four weeks.

The relative temperature of the mice was recorded using a thermocouple (Cole Parmer type T. thermocouple thermometer) connected with a rectal probe, which was inserted 2 cm beyond the anal sphincter.

After one month of the last treatment, the blood samples were withdrew from orbital venous plexus of experimental animals into fresh heparinized tubes. The blood samples divided into two parts, the first part was used for assaying the phagocytosis, killing .[16], interleukin 2 (IL2).[17] and heat shock protein 70 (HSP70) was assayed by an Hsp70 EIA Kit (StressGen Biotechnologies, British Columbia, Canada), which can detect and quantitate inducible HSP70 in samples originating from both human and mouse. ELISA was performed according to the manufacturer's instructions.The second part was centrifuged at 3000 rpm for 20 min. The plasma was separated and stored for tumor necrosis factor (TNF-α). [18] and alpha fetoprotein (α- FP) [19] determination · As well as apoptosis of cancer tissues can be detected. [20]

Statistical Analysis:

All results are expressed as mean ± SEM. The statistical analysis was carried out with Duncan's

multiple range test. A $P < 0.05$ was considered the level of statistical significance.

RESULTS:

WBH induced significant increase (P< 0.05) of phagocytosis % in cancered mice. The mean values of phagocytosis % were 51.70 ± 2.98 and 69.30 ± 1.77 before and after WBH treatment respectively. The increase was 20.52% Table (1&2). Combined treatments of WBH and Dox administration or whole body gamma rays induced highly significant increase amounting to 33.74% and 35.18%respectively. As well as there was a significant increase of percentage of blood concentration of killing cells after WBH combined with Dox or whole body γ- rays representing 43.41% and 51.11% respectively.

As apparent from table 1 the level of HSP70 which was increased remarkably after WBH alone as compared to untreated cancered mice. The mean values of HSP70 were 6.53 ± 1.01 and 3.65 ± 0.69 respectively. The increase was 78.90% Table (1&2). Furthermore, it shows a significant increase of HSP70 after WBH with Dox or with whole body γ-rays but the last combined was higher than that the first as compared to untreated cancered group 50.96% Table 2.

It is shown in table 1 that there was a significant increase of TNF-α after WBH with or without Dox or γ- rays as compared to untreated group. The maximum increase was detected in the cancer mice treated with combined WBH with Dox by 130.80 % table 2.

It can be noted from table 1 the level of serum tumor marker α-FP significantly decreased after WBH alone. This decrease was 64.80%. As shown as from table 1 the combined treatment of WBH and Dox or with γ-rays induced highly significant decrease of α-FP amounting to 74.18% and 75.84% respectively
Table (1) shows that after WBH combined with Dox or with γ- rays there was highly significant increase of IL-2 representing 38.15% and 41.38% respectively as compared to untreated group (table 2). Table (3) demonstrates a significant increase of apoptosis in the tumor cells (Ehrlich carcinoma) after WBH either as alone or combined with γ rays or (Dox) . DNA ladder and hallmarks of apoptosis were observed post three treatments (Fig. 1a lanes 3-8 &b lanes 1-3). Hyperthermia increased the sensitivity of cancer cells to chemotherapy or radiotherapy and affected the mode of cancer cells death then DNA ladder formation was observed. These results suggest that heat is required for the induction of apoptosis.

Table (3) demonstrates a significant increase of apoptosis in the tumor cells (Ehrlich carcinoma) after WBH either as alone or combined with γ rays or (Dox) . DNA ladder and hallmarks of apoptosis were observed post three treatments (Fig. 1a lanes 3-8 &b lanes 1-3). Hyperthermia increased the sensitivity of cancer cells to chemotherapy or radiotherapy and affected the mode of cancer cells death then DNA ladder formation was observed. These results suggest that heat is required for the induction of apoptosis.

Table (1): Effect of WBH, Dox, whole body γ- rays alone or combined on various biological parameters in mice bearing solid tumor.

Groups / Parameters	Control mice	Cancered mice	Cancered mice+ WBH	Cancered mice+ Dox	Cancered mice Rad.	Cancered mice WBH+ Dox	Cancered mice WBH+ Rad.
Phagocytosis%	82.02 ± 1.62 [a]	57.7 ± 2.98 [bc]	69.30 ± 1.77 [b]	55.70 ± 2.16 [c]	47.1 ± 1.44 [c]	76.90 ± 1.19 [a]	77.70 ± 1.64 [a]
Killing cells%	78.6 ± 1.84 [a]	49.50 ± 2.17 [g]	63.80 ± 2.09 [d]	53.3 ± 2.70 [f]	56.25 ± 2.10 [e]	70.99 ± 2.32 [c]	74.80 ± 2.10 [b]
HSP70 (ng/ml)	2.59 ± 0.28 [e]	3.65 ± 0.69 [cd]	6.53 ± 1.01 [a]	3.48 ± 0.40 [d]	1.79 ± 0.48 [f]	4.26 ± 1.28 [b]	4.60 ± 0.44 [b]
TNF-α (pg/ml)	354.40 ± 7.11 [a]	135.70 ± 11.10 [f]	289.78 ± 8.13 [c]	265.46 [bc] ± 7.30	238.8 ± 6.64 [d]	313.06 ± 9.10 [c]	280.54 [c] ± 7.93
α -FP(ng/ml)	1.11 ± 0.48 [d]	28.10 ± 1.86 [a]	9.89 ± 2.69 [d]	13.40 ± 0.63 [b]	12.20 ± 1.40 [bc]	5.85 ± 0.82 [c]	6.79 ± 1.38 [c]
IL2 (pg/ml)	651.86[a] ± 19.07	433.45 [c] ± 13.85	554.30 ± 7.74 [b]	446.8 ± 15.20 [c]	466.78 [c] ± 17.21	598.79 [ab] ± 11.30	612.80 ± 14.304[a]

Means with different small letters within each column are significant at α 0.05

Dox: doxorubicin, WBH: whole body hyperthermia, Rad: radiation

Table (2): Effect of WBH, Dox, whole body γ- rays alone or combined on various biological parameters in mice bearing solid tumor (% change).

Groups / Parameters	Cancered mice+ WBH	Cancered mice+ Dox	Cancered mice WBH+Dox	Cancered mice Rad.	Cancered mice+ WBH
Phagocytosis %	20.10	3.47	33.28	18.37	34.66
Killing cells%	28.89	8.08	43.41	13.64	51.11
HSP70 (ng/ml)	78.90	4.66	16.71	50.96	26.03
TNF-α (pg/ml)	113.54	95.62	130.80	75.98	106.74
α -FP(ng/ml)	-64.80	-52.31	-74.18	-56.58	-75.84
IL2 (pg/ml)	27.88	3.08	38.15	7.69	41.38

Table (3) Apoptosis in the tumor cells (Ehrlich carcinoma) after WBH either as alone or combined with γ rays or (Dox)

Lanes:	M		Cancer tissues				Radiation only				Dox only				WBH only			
			3		15		1		5		6		11		2		4	
Bands	Bp	%	bp	%	bp	%	bp	%	bp	%	bp	%	bp	%	bp	%	bp	%
1	1000	32.15	1782	61.3	1845	85.8	1824	59.41	1890	53	1824	48.58	1815	45.11	1755	50.4	1755	43.4
2	700	12.7	540	21.2	540	4.5	540	15.24	540	18.93	450	25.68	540	18.4	540	16.9	540	43.4
3	500	13.2	360	13.2	360	4.5	360	15.24	360	14.54	360	11.79	360	26.9	540	16.9	540	15.3
4	300	13.8	180	4.29	180	4.28	180	10.12	180	13.54	180	13.98	180	9.6	360	17.3	360	23.60
5	200	11				5.4									180	15.5	180	17.7
6	100	17.1																
Sum		100.0				100.0		100.0						100.0				
In Lane		100				100		100						100				

Lanes:	M		WBH only 10		WBH + Dox 8		9		12		WBH + Rad 7		13		14	
Bands	Bp	%	bp	%	bp	%	bp	%	bp	%	bp	%	bp	%	bp	%
1	1000	32.1	1754	51.28	1854	49.9	1805	43.7	1769	45.26	1854	48.18	1828	44.88	1750	46.3
2	700	12.7	540	17.58	540	8.724	540	14.88	540	19.28	540	24.78	540	13.6	540	27.31
3	500	13.2	360	15.215	360	11.72	360	22.1	360	15.74	360	11.59	360	15.8	360	12.21
4	300	13.8	180	16.251	180	29.62	180	19.3	180	19.73	180	15.48	180	15.7	180	14.16
5	200	11.07												15.7		
6	100	17.1														
Sum		100.0								100.0				90.0		100.0
In Lane		100								100				100		100

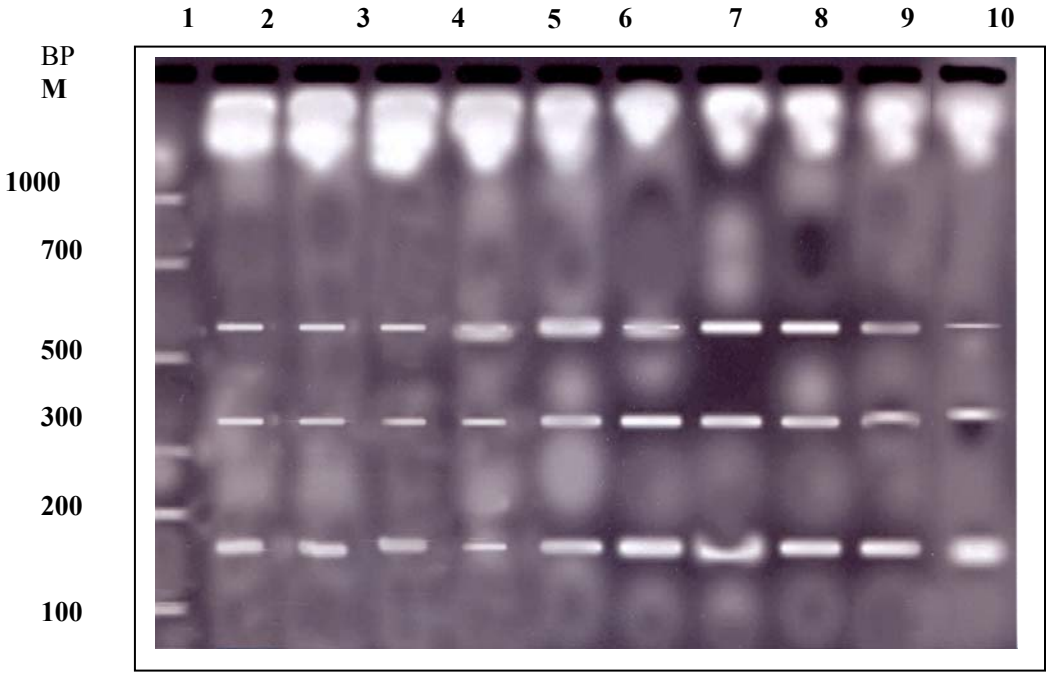

Fig. 1 a
The effect of WBH alone (41.5^{O}C) and combined with Dox or γ rays treatments on DNA ladder formation.

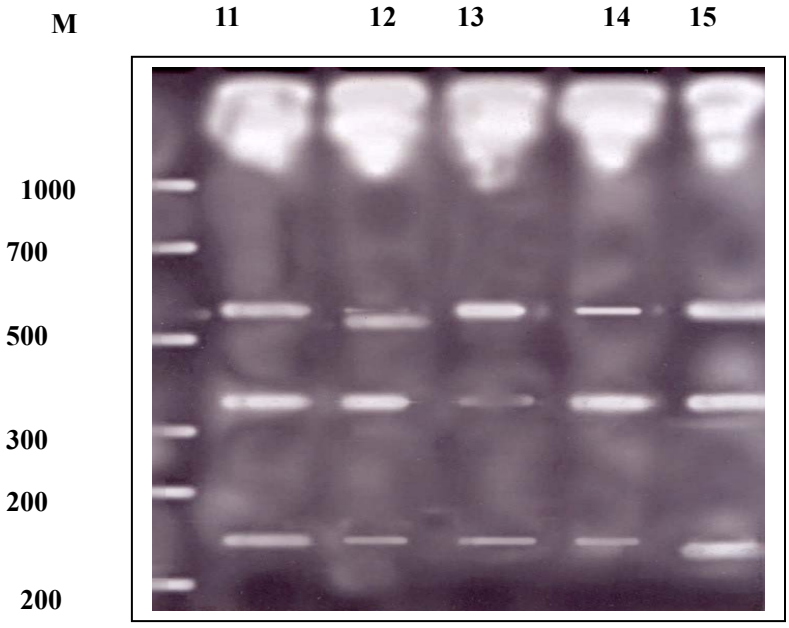

Fig. 1 b
Fig 1 a& b: The effect of WBH alone (41.5^{O}C) and combined with Dox or γ rays treatments on DNA ladder formation. Lane 1,5: γ rays alone. Lane 3&15: untreated cancer. Lane 6& 11: Dox alone. Lane 2, 4& 10: WBH treatment alone. Lane 8, 9&12: WBH treatment combined with Dox. Lane 7, 13&14: WBH treatment combined with γ rays.

Discussion:

Hyperthermia, a therapeutic method by increasing tissue temperature (range 40- 43C°), potentially induces tumor cell death by a spectrum of molecular, metabolic, cellular and tumor tissue changes [21] .Moreover, a raised body temperature raises the metabolic rate and makes the immune response more efficient [22] .

In the present study showed that WBH 41.5°C given in combination with doxorubicin or whole body-γ irradiation showed decrease significantly of FP-α,and increased phagocytosis and killing cells. Reflecting the frequency of cure mice bearing solid tumors these results were supported by the published data of other authors [23, 24] , who found that tumor cells increase the accumulation of doxorubicin under hyperthermic conditions. In general, mild hyperthermia (HT) at 39-42^{o}C induces an increase in tumor tissue blood flow volume [25]. Thus, the effect of HT on tumor tissue and tumor drug accumulation varies with thermal dose. Furthermore, it is supposed to be applicable in the development of less toxic regimens to reverse drug resistance by using relatively low concentrators of reversal agents combined with HT in order to reduce their side effects [26] . Tumor necrosis factor alpha (TNF- α), is a potent mediator of inflammatory and metabolic function and it was originally detected as a highly cytotoxic cytokine for tumor cells, it causes tumor necrosis *in vivo* [27] . One of the causes of cancer progression is the failure of tumor cells recognition by the immune system [28]. Thus, in the present study WBH alone or combined with γ irradiation or Dox induced significant increase of IL-2 and TNF-α as compared to cancer group. This result in accordance with **Tarner el al**. [29] showed that HT caused induction of all cytokines by 40-50% such as TNF-α & IL-2.

In the present study, the elevated level of HSP 70 level after WBH alone and combined with radiation or Dox treatments, studies of other authors have shown that hyperthermia (HT) can be applied successfully in an antitumor protocol based on TNF and HSP70 leading to a significant inhibition of lethality but not to a reduction of antitumoral capacity [30] .Stress induced HSP70 effectively protects cells against apoptosis. In the present study HSP70 released from solid tumor, this result is in a harmony with those obtained by **Salamatu et al.** [31] suggested that HSP70 can be released from tumor cells and stimulate a potent antitumor immune response. Up-regulation of HSPs in various cancers suggests that they might be involved in tumorigenesis. [32] Enhancement of tumorigenesis by overexpression of HSP70 has been implicated in a rodent model [33]. HSPs are known to be essential for the survival of cancer cells in different cancers [34, 35]. Also, HSPs as molecular chaperones might sustain cancer cells by modulating the activity of different proteins involved in cell cycle and apoptosis. . As well as, **Park et al**. [36] suggested that a subsequent accumulation of heat shock proteins (HSP) is likely to contribute to the malignant progression of hypoxic tumor cells. However, HSP70 does not contain a consensuses secretory signal and thus can not transverse the plasma membrane by conventional mechanisms [37]. The data of the present study shows statistically highly significant higher mean of HSP70 in the group treated with WBH alone or combined with WB-γ rays or (Dox) than in normal. It has reported that many HSPs are known to regulate apoptosis and even prevent apoptosis induced by anticancer drugs [32]. Also, they protects cellular elements from injury by reducing oxidation inflammation and apoptosis and by refolding damage proteins [38]. Moreover, it is also well established that heat shock or elevated HSP70 alters the regulation of signaling cascades and transcription factors and potently sensitizes tumors to radiation. [39]

The results reveal that the mechanism of enhancement of WBH induced apoptosis by Dox or γ irradiation .A biochemical hallmark of apoptosis is characteristic from of DNA degradation in which the genome is cleaved at inter-nucleosomal sites, generating a ladder of DNA fragments [40]. The present study observed that WBH alone induced apoptosis in solid tumor cells *in vivo* in accordance with previous studies indicated that relatively mild heat shock induces apoptosis [41]. The sensitizing effect on killing the tumor cells can be related to an enhanced inhibition of nucleic acid synthesis, as both modalities are known to have that effect [42,43] An inhibited repair of DNA damage, which is known to be one of the factors in the heat-sensitizing effect of irradiation and doxorubicin [44] , may also be a possible mechanism.. Heat induced apoptosis has been studied in normal cells or tissues and in a variety of tumor cells or tissues. These studies demonstrate that cellular ATP level may be involved in determining the mode of heat – induced cell death i.e. apoptosis or necrosis, since cellular ATP levels are indicated to be an important determinant of apoptosis [45]. Also DNA Ladders and hall marks of apoptosis were observed post WBH combined with Dox or radiation. The appearance of DNA fragmentation was increased post WBH combined radiation treatment. Hyperthermia can modulate the action of many anticancer drugs and lead to temperature dependent DNA damage [28]. Apoptosis may be due to change in expression of some genes in the cancer cells transplanted into nude mice such as p53, Bcl-2, and Bax [46]. As well as, the mechanisms of cell killing are explained that the influences of tumor

cells may be due to the impairment of a DNA, RNA and proteins synthesis [46, 47]. Moreover, the promotion of apoptosis is an important component of the antitumor activity of traditional anticancer therapies, including chemotherapeutic drugs and radiotherapy [48]. Furthermore, the creation of a functional blood supply from the normal tissue vasculature via the process of angiogenesis is critical for the continued growth and development of solid tumors [49]. Hyperthermia has been identified as angiogenesis inhibitor [50, 51] . However, it has been proposed that when massive apoptosis occurs, the normally efficient phagocytic system is overwhelmed, resulting in secondary necrosis in vivo, release of proinflammatory mediators [52]. In other studies reported that, apoptosis was manifested by classical changes in cell morphology and activation of caspase -3, both considered the hall marks of apoptotic mode of cell death [40]. **Sosman and Puzanov** [53] reported that ,in MCF-7 breast cancer cells, tumor necrosis factor α(TNFα) and TNF-related apoptosis-inducing ligand inhibit overall translation by a mechanism that requires caspase (but not necessarily caspase-3) activity.

Conclusion:

WBH enhances the response of tumor cells to radiation and chemotherapy and it has an important role in potentiation of radio- and chemotherapy in solid tumor treatment.

References

1- Emanuel NM, Bogdanov GN, Orlov VS. Free-radical mechanisms in the cytotoxic action of antitumor antibiotics, Russian Chem. Rev. 1984; 53:1121–1138.

2- Trieb K, Sztankay AA, Amberger Lechner H, Grubeckloebenstein B. Hyperthermia inhibits profileration and stimulates the expression of differentiation markers in cultured thyroid carcinoma cells, Cancer Lett. 1994;87 :65–71.

3- Sekins KM, Leeper DB,Hoffman JK, Wolfson MR Shaffer TH. Feasibility of lung cancer hyperthermia using breathable perfluorochemical (PFC) liquids. Part I: Convective hyperthermia, Int. J. Hyperthermia 2004; 252–277.

4- Guo B, Xu LZ , Li J. Time reversal based microwave hyperthermia treatment of breast cancer, Microwave Opt. Techn. Lett. 2005; 47: 335–338.

5- Ahmed S, Lindsey B, Davies J. Emerging minimally invasive techniques for treating localized prostate cancer, BJU Int. 2005; 96: 1230–1234.

6-Habash RW, Bansal R, Kerwski D, Alhafid HT. Thermal therapy, part 2: hyperthermia techniques. Critical Rev. Biomed. Eng. 2006; 34(6):461-542.

7- Sakurai H, Hayakawa K, Mitsuhashi N, Tamaki Y, Nakayama, Y, Kurosaki H, Nasu HS ,Ishikawa J, Saitoh I , Akimoto T, Niibe H. Effect of hyperthermia combined with external radiation therapy in primary non-small cell lung cancer with direct bony invasion, Int. J. Hyperther. 2002;18 **:** 472–483.

8- Gannon CJ, Patra CR, Bhattacharya R, Mukherjee P, Curley SA. Intracellular gold nanoparticles enhance non-invasive radiofrequency thermal destruction of human gastrointestinal cancer cells Journal of Nanobiotechnology. 2008; 6 (2):1-9.

9- Chang E, Chalikonda S, Friedl J, Giao QHX, Francesco PM, Marincola H, Alexander R, David L ,Targeting B. Vaccinia to Solid Tumors with Local Hyperthermia Human Gene Therapy. 2005;16 (4): 435-444.

10- Makrin V, Vagner I. Clinical hyperthermia by resonant selective tumor destruction tuned by hyperfine interaction: I. Basic model Journal of Science and Engineering A, 2006 ; 3, (1,):162-168.

11- Maria D , Thomus B, Peter W, Hanno R, Herwig G, Thoralf K. Stress induced changes in lymphocyte subpopulations and associated cytokines during whole body hyperthermia of 41.8 ^{o} C -42.2 degree C . European J. of Applied Physiology. 2005; 95(4):298-306.

12-Chen Z , Xia D, Bi X, Saxena A, Sidhu N, El-Gayed A , Xiang J. Combined radiation therapy and dendritic cell vaccine for treating solid tumors with liver micro-metastasis. J. Gene Medicine. 2004; 7(4):506-517.

13- Hattori T, Kokura S , Okuda T,Okayama T, Takagi T, Handa, O, NaitoY, Yoshida N, Yoshikawa T. Antitumor effect of whole body hyperthermia with alpha- galactosylceramide in a subcutaneous tumor model of colon cancer. Int, J. Hyperthermia. 2007; 23(6):591-598.

14- Burnet NG, Wurm R, Nyman JJH. Peacock,J.H.: Normal tissue radiosensitivity-how important is it? Clin Oncol (R Coll Radiol). 1996;8(1):25-34.

15- Quitet CA, Weichselbaum RR, Gradina DJ. Variation in radiation sensitivity during the cell cycle of two human sauamous cell carcinomas. Int J Radia Oncol Bio Phys. 1991; 20(4): 733-738.

16- Woldehiwet Z. Some observations on the effect of age of calves on the phagocytosis and killing of

staphylococcus aureus by polymorphonuclear leucocytes. Br.Vet.J. 1990;146:156.

17- Tigges MA, Casey LS, Koshland ME. Mechanism of interluken-2 signalling: mediation of different outcomes by a single receptor and transduction pathway. Science. 1989; 243:781.

18- Kwon J, Chung IY, Benveniste EN. Cloning and sequence analysis of the tumor necrosis factor encoding genes. Gene.1993 ;132:227-236.

19- Watanabe A, Mori O, Takeda K, Kosaka K. Purification and immunochemical characterization of alpha-fetoprotein from rat fetal serum and liver. Acta Medica Okayama. 2007;29 (5),

20- Hale A, Smith CA, Sutherland LC, Stoneman VE, Laongthorne VL, Culhane AC, Williams GT. Apoptosis :molecular regulation of cell death. Cancer and Metastasis Rev. 1992;11(2):105-119.

21- Song CW, **Park HJ**, **Lee CK**, **Griffin R**. Implication of increased tumor blood flow and oxygenation caused by mild temperature hyperthermia in tumor treatment. Int. J. Hyperthermia. 2005; 22(5):433-437.

22- Broom M. Physiology of fever. J Paediatr Nurs. 2007;19 (6): 40-44.

23- Kawasaki S, Sasaki K, Nagaoka S et al: Increased accumulation of adriamycin in mouse 3T3 cells with hyperthermia Nipp. Act. Radiol. 1984; 44:727-731.

24- Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, Felix R, Schlag PM. Hyperthermia in combined treatment of cancer. Lancet (Oncol) 2002; 3(8): 487-497.

25- Zhang L, Yang Y, Wei XY, Shi YR, Liu HY Niu RF, Hao XS. Reversing adriamycin resistance of human breast cancer cells by hyperthermia combined with Interferon alpha and Verapamil. J Exp Clin .Cancer Res. 2007;26(2):201-207.

26- Takahashi I, Emi Y, Hasuda S, Kakell Y, Maehara Y, Sugimachi K. Clinical application of hyperthermia combined with anticancer drugs for the treatment of solid tumors. Surdery 2002; 131(1):S78-S84.

27-Whittle B, Varga C, Posa A, Molnar A, Collin M, Thiemermann

C. Reduction of experimental colitis in the rat by inhibitors of glycogen synthase kinase-3b. British J Pharmacol 2006; 12:147-575.

28-Kubes J, Svoboda J, Rosina J, Starec M, Fiserova A. Immunological response in the mouse melanoma model after local hyperthermia. Physiol Res. 2008;57: 459-465.

29- Tarner IH, Ladner MU, Uhlemann C, Lange U. The effect of mild whole-body hyperthermia on systemic levels of TNF-alpha, IL-1beta, and IL-6 in patients with ankylosing spondylitis . Clinical Rheumatology. 2009; 28(4): 397-402.

30- Von-molle W, Wielockk B,Mahieu T,Takada M, Taniguchi T, Sekikawa K Libert C. HSP70 protects against TNF induced lethal inflammatory shock. Immunity 2002;16(5):685-695.

31- Salamatu S M, Calderwood SK. Heat Shock Protein 70 Is Secreted from Tumor Cells by a Nonclassical Pathway Involving Lysosomal Endosomes. The Journal of Immunology. 2006; 177: 7849-7857.

32-Garrido C, Fromentin A, Bonnotte B, Favre N, Moutet M, Arrigo AP, Mehlen P, Solary E. Heat shock protein 27 enhances the tumorigenicity of immunogenic rat colon carcinoma cell clones. Cancer Res. 1998; 58: 5495-5499

33- Gibbons NB, Watson RW, Coffey RN, Brady HP, Fitzpatrick JM. Heat-shock proteins inhibit induction of prostate cancer cell apoptosis. Prostate .2000; 45: 58-65.

34-Conroy SE, Latchman DS. Do heat shock proteins have a role in breast cancer? Br J Cancer. 1996; 74: 717-721

35- Elisabetta P, Marco F, Alessandro G. Hyperthermia as an adjunctive treatment for soft-tissue sarcoma. Expert review of anticancer therapy. 2009;9(2):199-210.

36- Park KM, **Byun JY**, **Kramers C**, **Kim JI**, **Huang PL**, **Bonventre JV**. Inducible nitric-oxide synthase is an important contributor to prolonged protective effects of ischemic preconditioning in the mouse kidney. J Biol Chem. 2003;278(29):27256-27266.

37- Hashemi S M, Hassan ZM, Soudi S, Ghazoenfari T, Kheirandish M, Shahabi S. Evaluation of antitumor effects of tumor cell lysate enriched by HSP70 against fibrosarcoma tumor in BALB / c mice. Int. Immunopharmacology. 2007;7(7):920-927.

38-SakaguchiY,Stephens LC, Makino M, Kaneko T, Strebel FR, Danhauser LL, Jenkins GN, Bull JM. Apoptosis in tumors and normal tissues induced by whole body hyperthermia in rats. Cancer Res. 1995; 55:5459-5464.

39- Yamada T, Hayashi Y, Kaneko R, Tohnai I,

Ueda M. Ito M. Effect of the combination of a local OK 432 injection and hyperthermia on SCC VII tumors in mice. J Radiat Res. 1998; 39:101-109.

40- Miyazaki N, **Kurihara K**, **Nakano H**, **Shinohara K**. Role of ATP in the sensitivity to heat and the induction of apoptosis in mammalian cells Int J Hyperthermia. 2002 ;18(4):316-331

41- Blasiak J, Widera K, Pertynski T. Hyperthermia can differentially modulate the repair of doxorubicin-damaged DNA in normal and cancer cells Acta Biochimica Polonica. 2003; 50 (1): 191-195.

42. Dickson J A , Shah D M. The Effects of Hyperthermia (42°C) on the Biochemistry and Growth of a Malignant Cell Line. European J. Cancer. 1972;8: 561-571.

43. Di Marco A. Adriamycin (NSC-123127): Mode and Mechanism of Action. Cancer Chemotherapy. 1975;Rept Part 3, 6: 91-106,

44. Ben-Hur E, Elkind M M, Bronk B V. Thermally Enhanced Radi-oresponse of Cultured Chinese Hamster Cells: Inhibition of Repair of Sublethal Damage and Enhancement of Lethal Damage. Radiation Res.1974; 58: 38-51.

45- Liang H, Zhan HJ, Wang BG, Pan Y, Hao XS. Change in expression of apoptosis genes after hyperthermia,chemotherapy and radiotherapy in human colon cancer transplanted into nude mice . World J Gastroenterol . 2007; 13 (32): 4365-4371.

46-Ghobrial IM, Witzig TE, Adjei AA. Targeting apoptosis pathways in cancer therapy. CA Cancer J. Clin. 2005;55:178-194.

47- Jin Z, El-Deiry WS. Overview of cell death signaling pathways. *Cancer* Biol Ther.2005; 4:139-163.

48-Curry HA, Clemens RA, Shah S ,Bradbury CM, Botero A, Goswami P, Gius D. Heat shock inhibits radiation-induced activation of NF-kappaB via inhibition of I-kappaB kinase. J Biol Chem. 1999; 274:23061-23067.

49- Michael H. Angiogenesis and vascular targeting: Relevance for hyperthermia Int J Hyperther. 2008; 24, (1): 57-65.

50- Fajardo L F, Prionas S D. Endothelial cells and hyperthermia. Int. J. Hyperther.1988;10: 347-353.

51- Roca C, Primo L, Valdembri D, Cividalli A, Declerck P, Carmeliet P, Gabriele Bussolino F. Hyperthermia Inhibits Angiogenesis by a Plasminogen Activator Inhibitor 1-dependent Mechanism. Cancer Res. 2003; ***63:*** 1500-1507.

52- Nowak AK, Lake*RA,* Marzo,*AL,* Scott *B,* Heath *WR,* Collins*EJ,* Frelinger FA Robinson *BWS*. Apoptosis In Vivo Increases Tumor Antigen Cross-Presentation, Cross-Priming Rather than Cross-Tolerizing Host Tumor-Specific CD8 T Cells .*The* Journal of Immunology. 2003; 170: 4905-4913.

53- Sosman JA, Igor Puzanov I. Molecular Targets in Melanoma from Angiogenesis to Apoptosis. American Association for Cancer Research. 2006; 12, 2376s-2383s

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