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Study of the Cytotoxic effect of a Novel Ampicillin ZnO Nanocomposite Against Myeloma Cell Lines.

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Abstract *In the present work, the effect of the standard ampicillin, its ZnO nanocomposite or ZnO nanoparticles on myeloma cell line SP2OR in vitro was studied. Variable doses ranging from 10 to 100 µg /ml from the three compounds were used. The results indicated that the viability (%) of myeloma cells was not influenced by increasing doses (10-100 µg/ml) of ampicillin during the time of experiment. On the other hand the ampicillin ZnO nanocompoiste showed a significant effect on the viability of the myeloma cells. The viability was decreased by increasing doses from 10 to 100 µg /ml until reached its maximum at the third days of incubation of the cells with the ampicillin ZnO nanocomposite (zero) at dose 100 µg/ml. The results of the effect of Zinc Oxide on the myeloma cell lines at the same doses indicated a significant decrease by increasing doses which reached its maximum of viability of 2.7% at 100 µg/ml and it was less potential than the corresponding ampicillin ZnO nanocomposite which reached its maximum viability of zero at the dose.*

Keywords: Ampicillin, ZnO nanocomposite, ZnO nanoparticles, myeloma cell line

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INTRODUCTION

Nanotechnology and nanomedicine can provide the opportunity for the development of new nanomaterials in the size range of nanoparticles (NP). This make them differ from their counterparts to include an increase in relative surface area and quantum effects which can affect chemical reactivity and other physical and chemical properties **(**Lanone, S. et al., 2006 and, Nel, A., et al., 2006). Hence, a more targeted approach is obtained which promises significant improvements in cancer treatment **(**Nie, S., et al., 2007**)**. ZnO NPs showed cytotoxicity at 20 µg /ml without UVA-1. Due to their photocatalytic properties, ZnO NPs may induce cell death in human HNSCC cell lines in vitro

(Hackenberg, S., et al., 2010**).** ZnO NPs at higher concentrations exert differential effects on human astrocytoma (U87) cells and human fibroblasts (HFF-1) **(** Lai, M. B., et al., 2008). The putative cytotoxic effects of different types of nanoparticles on human astrocytes-like astrocytoma U87 cells proved that ZnO NPs were the most effective and cell death mechanisms included apoptosis, necrosis and possibly necrosis-like cell death types **(**Lai, J. C., et al., 2008**)**. ZnO NPs were evaluated in human colon-derived RKO cells and showed significant cytotoxicity with $1.C_{50}$ value of 15+ 1 Mg/cm^2. The mechanism of cell death includes the disruption of mitochondrial function, apoptosis, loss of mitochondrial potential, and increased penetration of superoxide **(**Philip, J., et al., 2010**)**. Nanaoparticles are increasingly being recognized for their potential utility in biological application including nanomedicine **(**Hanley, C., et al., 2008**)**. Metal oxide nanoparticles, including zinc oxide, are versatile platforms for biomedical application and therapeutic intervention. There is an urgent need to develop new classes of anticancer agents, and recent studies demonstrate that ZnO nanomaterials hold considerable promise (Rasmussen, J. W., 2010). Focusing on ZnO NPs and their proposed mechanisms of cytotoxic action, as well as current approaches to improve their targeting and cytotoxicity against cancer cells may be improved further to make them attractive new anticancer agents (Rasmussen, J. W., 2010). Functionalizing inorganic nanoparticles with natural designed biomolecules offer a root towards engineering responsive and multifunctional composite systems. Nanocomposite materials based on functionalized metal nanoparticles promise to transform the way cancer is diagnosed and treated (Minelli, C., et al., 2010). In a previous study under publication ampicillin ZnO nanocomposite was prepared to evaluate its effectiveness as antibacterial agents against Gram-negative short rods Salmonella typhimurium, Gram-positive cocci Staphylococcus aureus and another strain of Staphylococcus aureus referred as ampicillin resistant one. The results indicated that the antibacterial activity of the standard ampicillin was significantly enhanced by its ZnO nanocomposite. Also the new compound showed antibacterial activity against the clinically resistant isolate staphylococcus aureus. The aim of the present study is directed towards evaluating the role of the new compound ampicillin ZnO nanocomposite for the eradication of cancer cells.

MATERIALS AND METHODS

Myeloma cell line:

SP2OR myeloma cell line was used, Hammersmith Hospital, London, UK, (Chapman, R. S. ;1998).

Chemicals:

- Standard ampicillin was obtained from El Nasr Pharm. Chem. Co.'ADWIC'

- Ampicillin ZnO NPs was prepared as mentioned before (under publication)

- Zinc Oxide obtained from Aldrish Co.
- Tissue culture medium, RPMI-1640 with 15mM HEPES buffer, powder, sterile (Sigma, USA).
- Foetal bovine serum (FBS), liquid, sterile (Sigma, USA).
- Antibiotic antimycotic (10.000 U penicillin, 10 mg streptomycin and 25 µg amphotericin B, 1 ml in 0.9% NaCl), liquid, sterile (Sigma, USA).

- L-glutamine (200mM solution), Hybrid Max®, sterile filtered (Sigma, USA).
- Trypan blue dye (MOD), 0.5% W/V in normal saline (ICN Biological CA, USA).
- Dimethyle sulfoxide (DMSO), sterile, filtered, Hybrid Max® (Sigma, USA).

Preparation of RPMI culture medium:

The RPMI-1640 powder was reconstituted by one liter sterile distilled water and filtered by 0. 22 µm sterile membrane filters in a 100 ml clean sterile container then the following constituents were added: 86 ml RPMI-1640 solution, 10ml foetal bovine serum albumin (FBS), 1ml L-glutamine and 1ml antibiotic antimycotic reagent then the contents were mixed well to be ready for use.

Maintenance of myeloma cells:

The established myeloma cell lines were frozen as $1x10^7$ cell/ml as 0.5 ml aliquots in liquid nitrogen. One aliquot may be removed from liquid nitrogen. It was most convenient at this stage to handle only 10-15 ml RPMI culture medium in $25cm^2$ flasks until cell growth enter the log-phase and the viability more than 95% were achieved according to **(**Harlow **,**et al., 1988). Incubation was continued at 37°C in CO_2 incubator and the cells recounted with a viability check daily prior to adjustment until a cell doubling time of 18-24 hrs was achieved with viability more than 95%. Cell density should not be allowed to exceed 1-1.5 x 10^7cell/ml. At this storage, the total volume of cell can be expanded by transferring to 75 cm^2 flasks (max. vol. 50 ml) for freezing in liquid nitrogen to provide a secure stock of cells for future work (Chapman, R. S. ;1998).

Effect of standard ampicillin, ampicillin ZnO nanocomposite and ZnO nanoparticles on myeloma cell line SP2OR in vitro:

Preparation of standard ampicillin, its ZnO nanocomposite and zinc oxide np stock solution:

For both standard ampicillin trihydrate powder and its ZnO nanocomposite, two stock solution of 0.0373 gm ampicillin/ml was prepared. Both powder were dissolved in dimethyl sulfoxide (DMSO), Merk. Solutions were kept in dark glass containers and stored at 4°C. Also stock solution of 0.0373 gm zinc oxide/ml was prepared. It was dissolved in dimethyl sulfoxide and stored at 4°C. A series of dilutions were carried out to achieve a final ampicillin concentration of (10, 25, 50, 75 and 100 µg/ml) (Xiong W.J; et al., 2002)**.**

Preparation of variable count of myeloma cells and variable doses from standard ampicillin, ampicillin ZnO nanocomposite and ZnO nanoparticles:

Myeloma cells were cultured in the culture medium which prepared as mentioned before until cell growth enter log-phase and viabilities of ≥ 95% were achieved. Variable count was prepared for each dose of the drug. 24 well plate were prepared as follows: 1ml of media was added into all 24 well then 1ml of myeloma cells was added into the first row of the plate and mix well with the contents of the wells then, serial dilution was performed between wells of the first row and the wells of other rows after that 1ml of media was added into all wells of the plate and the plate mix well. Standard ampicillin, ampicillin ZnO nanocomposite and ZnO nanoparticles were added at variable doses (10, 25, 50, 75 and 100 µg/ml). Compared with count myeloma cells line control then the plate was incubated at 37°C for

4 days. The total count (T.C.), dead cells (D C) and viability cells (V C) were calculated after 24 hrs for 4 days.

RESULT

Effect of standard ampicillin on the growth of myeloma cell lines:

Results obtained from table (1) represent the effect of total cells, T.C., dead cells, D, living cells,V, and T.C./ml while the T. C./ml and viability (%) representing the effect of increasing dose of standard ampicillin on the life span of days compared to control. It is clear from the results that, after 24 and 48 hrs of incubation of the standard ampicillin at all doses with cell line, the viability (%) of the cell line does not influenced. Slightly decreasing in the viability was noticed after 4 days of incubation of the standard with the cell line if it compared with the control respectively (64.4 –76.2 %).

Table (1): Effect of variable doses of standard ampicillin on the growth of myeloma cell line Sp2OR compared to control

Days	Variable count of myeloma cell	Control	Variable doses of standard ampicillin (µg/ml)				
			10	25	50	75	100
Day 1	T.C	110	108	106	105	103	100
	D	6	8	6	5	7	5
	V	104	100	100	100	96	95
	T.C/ml	$55x10^4$	$54x10^4$	$53x10^4$	$52.5x10^4$	$51.5x10^4$	$50x10^4$
	V (%)	95.0	93.0	94.3	95.3	93.2	95.0
Day 2	T.C	128	125	120	110	105	110
	D	16	18	25	15	13	15
	V	112	107	95	95	92	95
	T.C/ml	$64x10^4$	$62.5x10^4$	$60x10^4$	$55x10^4$	$52.5x10^4$	$55x10^4$
	V (%)	88.0	85.6	79.2	86.4	87.6	86.4
Day 3	T.C	150	145	140	144	141	135
	D	38	40	45	50	43	50
	V	112	105	95	94	98	85
	T.C/ml	$75x10^4$	$72.5x10^4$	$70x10^4$	$72x10^4$	$70.5x10^4$	$67.5x10^4$
	V (%)	74.7	72.0	68.0	65.3	69.5	63.0
Day 4	T.C	168	160	155	170	158	160
	D	40	52	51	60	51	57
	V	128	108	104	110	107	103
	T.C/ml	$84x10^4$	$80x10^4$	$77.5x10^4$	$85x10^4$	$79x10^4$	$80x10^4$
	V (%)	76.2	67.5	67.0	65.0	67.7	64.4

Effect of ampicillin ZnO nanocomposite on the growth of myeloma cell lines:

Results obtained from table (2) represents the effect of T.C, D cells, V cells and T.C./ml. The data indicated that T. C./ml and viability (%) decreased by increasing dose of ampicillin ZnO nanocomposite during life span of the incubation period, compared to control. It is clear from the results, that after 2 days of incubation no viable count was recorded. This denotes that ZnO nanocomposite of ampicillin exert very significant cytotoxic effect on myeloma cell line. Data in table (2) revealed that on day 2,3 and 4 viable count was zero for 100 µg/ml ampicillin ZnO nanocomposite while data in table (3) showed that viable count from ZnO nanoparticles was 50, 13.2 and 2.7 (for the same dose 100 µg/ml) on day 2,3 and 4. Standard ampicillin only, showed 86.4, 63 and 64.4 corresponding viability.

Table (2): Effect of variable doses of ampicillin ZnO nanocomposite on the growth of myeloma cell line Sp2OR compared to control.

Days	Variable count of myeloma cell	Control	Variable doses of ampicillin ZnO nanocomposite (µg/ml)				
			10	25	50	75	100
Day 1	T.C	108	96	65	45	35	20
	D	4	10	25	22	20	18
	V	104	86	40	23	15	2
	T.C/ml	$54x10^4$	$48x10^4$	$32.5x10^4$	$22.5x10^4$	$17.5x10^4$	$10x10^4$
	V (%)	96.3	89.6	61.54	51.11	42.86	10.0
Day 2	T.C	130	75	44	33	25	8
	D	19	23	28	25	14	8
	V	111	52	16	8	11	0
	T.C/ml	$65x10^4$	$37.5x10^4$	$22x10^4$	$16.5x10^4$	$12.5x10^4$	$4x10^4$
	V (%)	85.38	69.3	36.36	24.24	44	0
Day 3	T.C	126	46	35	28	18	6
	D	28	18	20	10	8	6
	V	98	28	15	18	10	0
	T.C/ml	$63x10^4$	$23x10^4$	$17.5x10^4$	$14x10^4$	$9x10^4$	$3x10^4$
	V (%)	77.8	60.9	42.9	64.3	55.6	0
Day 4	T.C	168	33	28	25	19	4
	D	40	22	18	15	19	4
	V	128	11	10	10	0	0
	T.C/ml	$84x10^4$	$16.5x10^4$	$14x10^4$	$12.5x10^4$	$9.5x10^4$	$2x10^4$
	V (%)	76.2	33.3	35.7	40	0	0

Table (3): Effect of variable doses of Zinc Oxide nanoparticles on the growth of myeloma cell line Sp2OR compared to control.

Days	Variable count of myeloma cell	Control	Variable doses of ZnO nanoparticles (µg/ml)				
			10	25	50	75	100
Day 1	T.C	110	106	103	100	90	89
	D	5	5	7	15	18	25
	V	105	101	96	95	72	64
	T.C/ml	$55x10^4$	$53x10^4$	$51.5x10^4$	$50x10^4$	$45x10^4$	$44.5x10^4$
	V (%)	95.5	95.3	93.2	85	80	72
Day 2	T.C	100	63	67	66	63	60
	D	10	20	25	25	28	30
	V	90	43	42	41	35	30
	T.C/ml	$50x10^4$	$62x10^4$	$34x10^4$	$33x10^4$	$32x10^4$	$30x10^4$
	V (%)	90.0	68.3	63	62	56	50
Day 3	T.C	128	77	76	69	73	76
	D	16	41	42	43	46	66
	V	112	36	34	26	27	10
	T.C/ml	$64x10^4$	$38.5x10^4$	$38x10^4$	$35x10^4$	$37x10^4$	$38x10^4$
	V (%)	88.0	46.8	44.7	37.7	37.0	13.2
Day 4	T.C	170	96	88	81	78	75
	D	43	50	53	51	54	73
	V	127	46	35	30	24	2
	T.C/ml	$85x10^4$	$48x10^4$	$44x10^4$	$41x10^4$	$39x10^4$	$38x10^4$
	V (%)	75.0	48.0	40.0	37.0	31.0	2.7

Effect of ZnO nanoparticles on the growth of myeloma cell lines:

Results obtained from table (3) represent the effect of ZnO nanoparticles T.C, D cells, V cells and T.C./ml. The results indicated that T. C./ml and viability (%) decreased by increasing dose of ZnO nanoparticles during life span of the days compared to control. It is obvious from the results that after 2, 3 and 4 days of incubation of ZnO nanoparticles with the myeloma cell line the viability was decreased significantly until it reached 50 %, 13.2 % and 2.7 % at 100 µg/ml concentration of ZnO nanoparticles. Accordingly, it is clear from table 1,2,3, that ampicillin ZnO nanoparticles was found to be the most effective one on myeloma cell line if it compared with ampicillin and ZnO nanopartices. Also the viable count recorded the lowest value (zero) at100 µg/ml. Accordingly it is clear from the previously mentioned results illustrated in tables 1, 2 and 3 that on days 1, 2, 3 and 4 the viable count reached 95%, 86.4%, 63.0% and 64.4% for standard ampicillin, while for ampicillin ZnO nanocomposite 10%, 0, 0 and 0, for ZnO np 72%, 50%, 13.2% and 2.7 respectively. The above results indicated that ampicillin ZnO nanocomposite is more potential against myeloma cell line than ZnO nanoparticles.

DISCUSSION

Cancer is a group of diseases characterized by uncontrolled growth of tissue cells in the body and the invasion by these cells into nearby tissue and migration to distant sites. Cancer results from alternation in genes that make up DNA, the master molecule of the cell. Genes make proteins, which are the ultimate workhorses that permit humans to breath, think and move, among other functions. Some of these proteins control the orderly growth, division, and reproduction of normal tissue cells. Gene mutations can produce faulty proteins, which in turn produce abnormal cells that no longer divide and reproduce in an orderly manner. These abnormal cells divide uncontrollably and eventually from a new growth known as a tumor or neoplasm (Janes, H. et al., 2002). Multiple myeloma is a progressive hematological disease. It is a cancer of the plasma cell, an important part of the immune system that produces antibodies to help fight infection and disease(Greipp, P. R., et al., 2005). The aim of cancer treatment is to remove or destroy all or as much of the primary tumor as possible and to prevent its recurrence or metastases. While devising a treatment plan for cancer, the likelihood of curing the cancer has to be weighed against the side effects of the treatment. If the cancer treatment is always tailored to the individual. The treatment choice depends on the type and location of cancer, the extent to which it has already spread, and the age, sex, and general health status of the individual. The major types of treatment are: surgery, radiation, chemotherapy, immunotherapy, hormone therapy, and bone-marrow transplantation **(**Woznick, G, et al., 2002**).** Chemotherapy is the use of drugs that kill rapidly dividing cells to treat cancer. Chemotherapy drugs are toxic to cancer cells, which take in the drugs as they multiply. Once inside the cells, the drug kills the cell or prevents it from dividing and forming new Cells. Chemotherapy may consist of a single medication or a combination of drugs administered intravenously or orally. Alkylating drugs kill cancer cells by directly attacking DNA, the genetic material of the genes **(**Skeel, T. K., 2003). Radiotherapy is the use of high-energy radiation from x-rays, gamma rays, neutrons and other sources to kill cancer cells and shrink tumors. Radiation may come from a machine outside the body (external-beam radiation therapy), or it may come from radio active material injected into the body near cancer cells (internal radiation therapy). Systemic radiation therapy uses a radioactive substance (Steven, A. R, et al., 2005). Nanoparticles are uncreasingly recognized for their utility in biological applications including nanoparticles. Cytotoxicity of ZnO to mammalian cells was studied using human myeloblastic leukemia cells HL60 and peripheral blood mononuclear cells (PBMC). Antibacterial activity of ZnO was also tested against Gram-negative bacteria, Escherichia coli and Pseudomonas aeruginosa as well as Gram-positive bacterial Staphylococcus aureus and the effect was more pronounced with the positive bacterial than negative ones. ZnO nanoparticles exhibited a preferential ability to kill cancerous HL60 cells as compared to normal PBMC (Premanathan, M., et al., 2010). Nanocomposite materials based on functionalized metal nanoparticles promise to transform the way cancer is diagnosed and treated (Minelli, C., et al., 2010 **)**. Recent reports have demonstrated that some anticancer drugs could be readily self-assembled on some biocompatible nanomaterials which may play an important role in the relevant biological and biomedical system. Combination of different sized ZnO nanoparticles and daunorubicin under UV irradiation could have synergistic cytotoxic effect on leukemic, cancer cells, indicating the great potential of ZnO nanoparticles in relevant clinical and biomedical application (Guo, D., et al., 2008). Mechanisms of ZnO nanoparticles toxicity involve the generation of reactive oxygen species with monocytes displaying the highest level and inducing the production of proinflammatory cytokines, 1FN-gamms, TNF-alpha, and 1L-12 at concentration below those causing appreciable cell death (Hanley, C., et al., 2009). ZnO nanoparticles exerted a cytotoxic effect on the human myeloma cell lines whereas no cytotoxic effect was observed on normal human astrocytes. Similarly the ZnO

nanoparticles induced cell death in breast and prostate cancer cell lines while no major effect was observed in the respective normal breast and prostate cell lines. These results suggest that ZnO nanoparticles may by employed for the eradication of cancer cells **(**Ostrosky, S., et al., 2009). Zinc Oxide nanoparticles exhibit a strong preferential ability to kill cancerous T cells compared to normal cells. Mechanisms of toxicity appear to involve the generation of reactive oxygen species, with cancerous T cells producing higher inducible levels than normal cells**(**Hanley, C., et al., 2008**)**. Zinc Oxide nanoparticles showed cytotoxicity at 20 µg /ml without UVA-1. Due to their photocatalytic properties, ZnO nanoparticles may induce cell death in human HNSCC cell lines in vitro **(**Hackenberg, S., et al., 2010**).** The response of normal human cells to ZnO nanoparticles under different signaling environment and compare it to the response of cancerous cells was previously investigated in **(**Hanley, C., et al., 2008**)**. The results indicating a novel findings of cell selective toxicity towards potential diseases causing cells, indicating a potential utility of ZnO nanoparticles in the treatment of cancer and/ or autoimmunity. It is clear from the previously mentioned results that ampicillin ZnO nanocomposite was found to be the most effective compound in eradication of myeloma cell line. It seems that ampicillin potentiates the antitumor activity of ZnO np by possible increase in disruption of mitochondria function, loss of mitochondria potential and increase penetration of superoxide.

CONCLUSION

Functionilizing ZnO nanoparticles with ampicillin offer a root towards potential responsive composite systems. Mechanisms underlying the cytotoxicity of ZnO nanoparticles and its composite with ampicillin could be: (i) involvement of the generation of reactive species. (ii) induction of apoptosis (iii) inhibiting p-gp function and change the special composition and properties of the membrane of drug resistant myeloma cells. Nanocomposite ampicillin based on functionalized ZnO nanoparticles promise to transform the way cancer is treated.

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