In vitro anti-cancer activity of Doxycycline on some human cancer cell lines

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Abstract:

Doxycycline (DC) is an antibiotic that is used in the treatment of number types of infections diseases caused by bacteria and protozoa. Doxycycline is a kind of second-generation tetracyclines which is commonly used to treat a variety of infections. Three cancer cell lines include: human cerebral glioblastoma-multiforme (AMGM) at passages 75-84, human cervical cancer (HeLa) at 70 passages, and human pelvic rhabdomyosarcoma (RD) at 75 passages cell lines were used in this study. The results showed that DC exerted significant cytotoxic effects with all concentrations used (50,100,150,200,250,300,350 and 400) µg/ml on all types of cell lines. Because of cytotoxic activity, good pharmacokinetic characteristics and the safety of drug which used for many years in the treatment of infectious disease, we can conclude that these characteristics make DC a valuable treatment for many types of cancer.

Key Words: Doxycycline, Anticancer, Cell Line, Glioblastoma, cervical cancer, rhabdomyosarcoma.

Introduction:

Tetracyclines are a family of antibiotics with effectiveness against several diseases caused by microbial infection. They were discovered in 1953 and feature a 4-ring structure to which a variety of side chains is attached. Doxycycline (DC) is a member of the tetracycline family. It has good water and lipid solubility and in bacteria, it is known to inhibit protein synthesis of pathogens [1]. As such it has found applications in the treatment of a large number of diseases, including Lyme disease [2], Legionnaire's disease [3] and malaria [4]. Treatments are well tolerated by patients [5] with little side effects. In addition to its anti-microbial action, DC has also shown therapeutic potential against rheumatoid arthritis [6], abdominal aortic aneurysms [7,8], malignant pleural effusions[9] and mesenteric ischemia [10]. Doxycycline are also becoming known as anti-tumor properties in the case of prostate, breast and colon cancer [11,12,13,14]. Its role as a nonspecific matrix metalloproteinase and angiogenesis inhibitor is well established [15,16,17,18,19]. Its pro-apoptotic effects are also known as in the case of T-lymphocytes [20]. Apoptosis is a

gene-directed form of cell death. It is important in a number of physiological processes like in normal development and maintenance of tissue homeostasis [21,22]. It also plays a role in tumor regulation because dysregulation of apoptosis may cause cancer cells to increase in numbers. Also chemotherapeutic agents may exert their anti-tumor action via activation of apoptosis in target cells [23,24,25]. Doxycycline is an antibiotic that is used in the treatment of a number of types of infections caused by bacteria and protozoa [26]. Current studies have demonstrated that DC is a pluripotent drug that affects many anticarcinogenic functions, including anti-tumor growth effect on human. It is useful for bacterial pneumonia, acne, infections, early Lyme disease, cholera and syphilis. It is also useful for the treatment of malaria when used with quinine and for the prevention of malaria. Doxycycline can be used either by mouth or intravenously [26].It is a broad spectrum antibiotic of the tetracycline class [27] .Like other agents of this class it kills bacteria and protozoa by inhibiting protein production[26,28]. Although chemotherapy plays significant role in the treatment of cancer, morbidity and mortality due to drug resistance and cancer metastasis are yet to be eliminated. Doxycycline has been reported to have cytotoxic and anti-proliferating properties in various cancer cells [29]. In a study whether DC was apoptosis threshold lowering agent in colorectal can-

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cer cells by targeting mitochondria. The tetracycline family have been used clinically since the mid twentieth century. Since then, they have found application beyond their anti-microbial activity in both the clinic and biomedical research [30,31]. Tetracycline controlled transcriptional activation the method of inducible gene where transcription is reversibly turned on or off in the presence of the antibiotic tetracycline or one of its derivatives (e.g. Doxycycline)[32]. To investigate the potential of DC as a therapeutic agent, this study aimed to determine the effect of DC on three human cancer cell lines growth including (AMGM, HeLa and RD) cell lines and study its cytotoxic effect *in vitro*.

Methods:

Cells and Cell Culture

Three cancer cell lines, human cerebral glioblastoma-multiforme (AMGM, at passages 75-84), human pelvic rhabdomyosarcoma (RD, at 75 passages), and human cervical cancer (HeLa, at 70 passages) cell lines were kindly provided by ICCMGR (Baghdad, Iraq) and used throughout this study. AMGM was propagated and maintained on Rosswell Park Memorial Institute medium (RPMI-1640, US biological, USA), while HeLa and RD cultured on minimal essential medium (MEM, Sigma) [33]. To these media, 10% fetal bovine serum (Cellgro, USA) and 1% penicillin/streptomycin (Cellgro, USA) were added and incubated in a humidified 5% CO_2 incubator (Heracell 150, Thermo Electron Corp.) at 37ºC. The cells were subculture after they had achieved 80-90% confluency which can be observed under inverted microscope (Nicon Eclipse TS100). Cell viability was assessed by using trypan blue (Pharma, Sweden) exclusion test and found to be greater than 99% [34].

Cytotoxicity Measurement of Doxycycline

The cytotoxicity of DC on cancer cell lines was examined according to the inhibition of proliferation rate (IR %) [35]. In a 96 well tissue culture plate, seed 2×10^4 cells per well in 200 µl antibiotic-free normal growth medium supplemented with FBS. Incubate the cells at 37° C in a CO_2 incubator until the cells are 60-80% confluent. This will usually take 18-24 hours. Then it will exposure with different concentrations of DC (50,100,150,200,250,300,350 and 400) µg/ml in 100 µl and incubated at 37Cº for 24, 48 and 72 hrs. After incubation, 20 µl of MTT [3-(4, 5- dimethylthiazol-2-yl-2-2.5- diphenyl tetrazolium bromide)] was added and incubated for further 3 hrs at 37Cº. The untreated cells were also done as control. The absorbance of treated and untreated cells was measured at 492 nm. The inhibitory rate of cell proliferation was calculated according the equation:

$IR\% = A–B/A×100$

Where A represents the absorbance of untreated cells, while B represents the absorbance of treated cells. The results of IR% was plotted on Y-axis while the concentration of DC where plotted on X-axis. The concentrations that caused a 50% reduction in cell growth (IC 50) was determined by non-linear regression analysis using Graph pad

Prism software [36].

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors (concentration, cell lines and time) in study parameters (inhibition rate and Knock-down percentage). Least significant difference –LSD test was used to significant compare between means in this study [37].

Results:

According to our knowledge this is the first research which study the cytotoxic effect of DC on (AMGM, HeLa and RD) cell lines. The results showed that DC decreased the growth of AMGM cells significantly as compared to untreated control cells; it appeared that the growth inhibition was concentration and exposure time dependent. The inhibition rates for the concentrations 50, 100, 150, 200, 250, 300, 350 and 400 µg/ml were 32.87, 33.79, 44.55, 41.08, 51.22, 57.14, 60.40 and 62.64% respectively after 24 hrs of exposure. When the exposure time increased to 48 hrs, the inhibition rates for these concentrations reached 34.82, 42.82, 43.36, 42.87, 56.06, 65.62, 78.71 and 71.48% respectively. However after 72 hrs exposure the inhibition rates increased to 31.08, 42.49, 46.00, 50.84, 53.36, 67.54, 72.33 and 69.42% respectively (Figure 1).
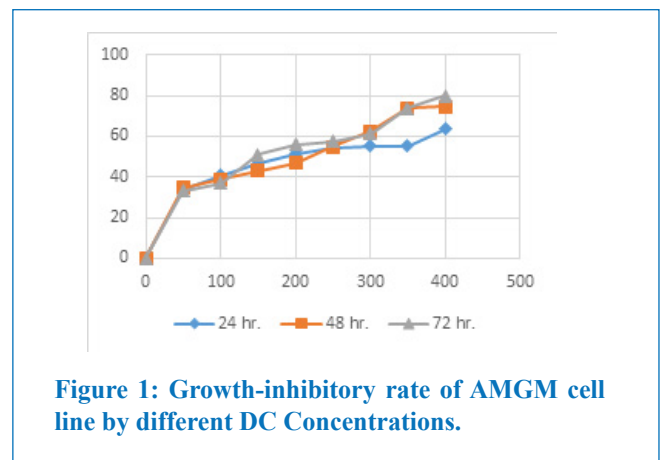

Figure 1: Growth-inhibitory rate of AMGM cell line by different DC Concentrations.

Using low concentration of DC was used, cells continued to proliferate for 48 hrs, as shown by the comparison of 48 hrs to 24 hrs (P<0.05). There was a trend for MTT values to increase from 48 hrs to 72 hrs under low concentration conditions, the P values were a significant, including comparison with untreated as control. This suggests that cell proliferation slowed considerably by 72 hrs in this assay condition. Thus, there was a growth-inhibitory but not a cytotoxic effect from DC on AMGM cell line treated in serum-free culture conditions. The cell viability rate of DC effect on AMGM cell line for 24, 48 and 72 hrs for treated and untreated cells as shown in figure 2.
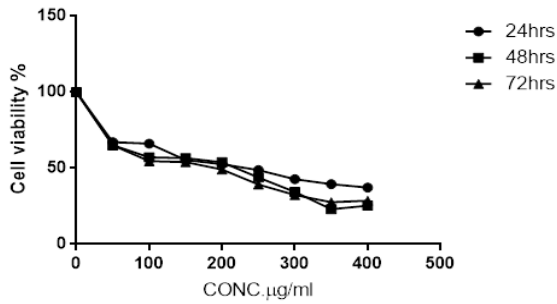
**Figure 2: The Cell viability rate of DC effect on AMGM cell line.**
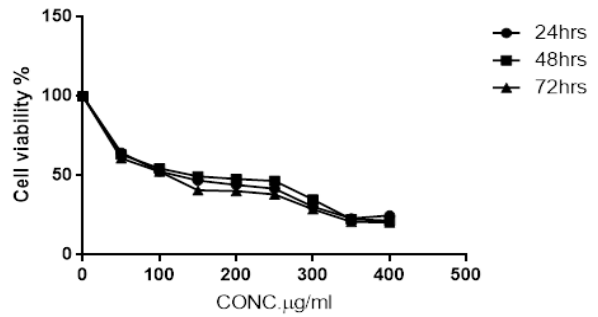
Figure 4: The Cell viability rate of DC effect on HeLa cell line.

Doxycycline also significantly decreased the growth of HeLa cells in comparison to untreated control cells with a concentration and exposure time dependent manner. When HeLa cell line exposed to 50, 100, 150, 200, 250, 300, 350 and 400 μg/ml concentrations of Doxycycline, the growth rates inhibited by 40.52, 49.31, 55.60, 57.97, 61.31, 70.91 and 76.97% respectively after 24 hrs exposure. The same concentrations of the drugs exerted inhibition of growth rates 32.05, 43.63, 46.51, 50.42, 52.37, 61.07, 75.53 and 75.53%, when the exposure time increased to 48 hrs. While after 72 hrs of exposure, the growth rates inhibition reached 39.10, 39.10, 47.43, 59.21, 59.73, 61.76, 71.17, 79.02 and 77.56% for the same concentrations respectively (Figure 3).

The results also showed that cimetidine decreased the growth of RD cells significantly as compared to untreated control cells with a concentration and exposure time dependent manner. The inhibition rates for the concentrations 50, 100, 150, 200, 250, 300, 350 and 400 μg/ml were 33.92, 40.85, 46.29, 51.38, 54.54, 55.20, 55.02 and 63.41 respectively after 24 hrs of exposure. When the exposure time increased to 48 hrs, the inhibition rates for these concentrations reached 34.81, 38.72, 43.15, 46.81, 54.68, 62.38, 73.79 and 74.51% respectively. After 72 hrs exposure the inhibition rates increased to 33.20, 37.04, 50.95, 55.92, 57.41, 61.09, 73.59 and 79.84% respectively (figures 5).
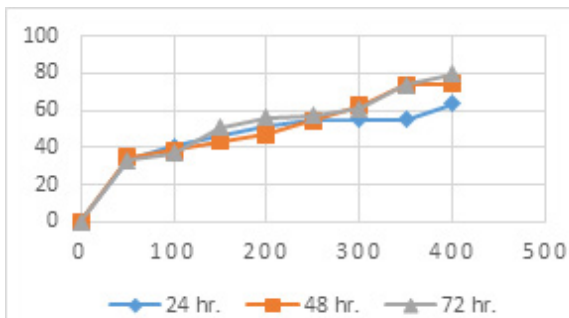

Figure 3: concentration-dependent growth-inhibitory effect of DC on HeLa cell line.
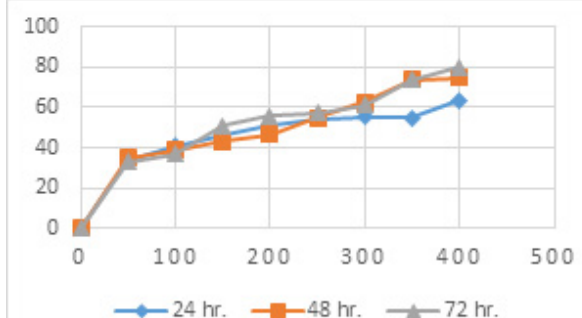
Figure 5: concentration-dependent growth- inhibitory effect of DC on RD cell line.
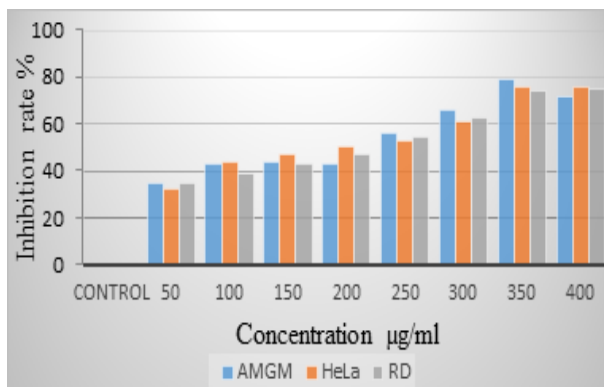
Based on the above observations, the highest inhibition effect by using 350 μg/ml concentration of the DC after 72hrs of the treatment duration on HeLa cell line where it was inhibited by 79.02%. Figure 4 represents the cell viability rate of DC effect on HeLa cell line for 24, 48 and 72 hrs for treated and untreated cells.

Figure 6 shows the gradually decreasing in the viability rate with increasing the DC concentration, which it is inverse the inhibition rate in RD cell line.
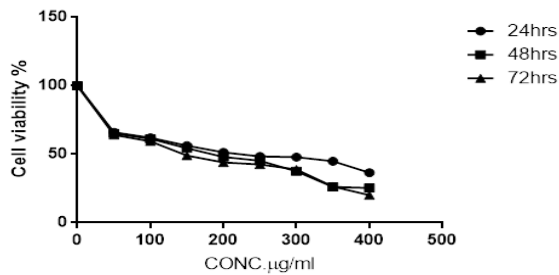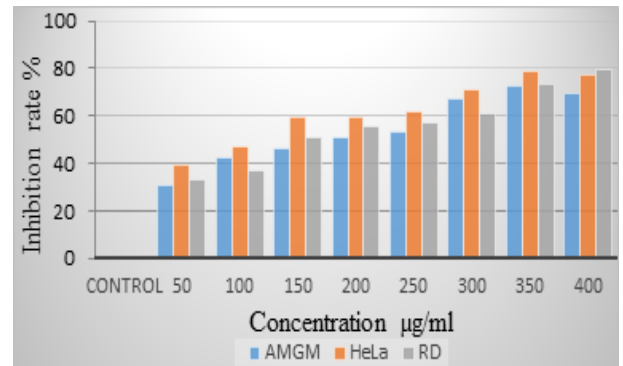
Figure 6: The Cell viability rate of DC effect on RD cell line.

Figure 8: Compare between AMGM, HeLa and RD cell line in Inhibition rate in 48 hour.

Finally, the comparison between AMGM, HeLa and RD cell lines inhibition rates for treated and untreated cells for the same day presented in figure 7, it was clear that there were a significant effect for each concentration to all cell lines under study after 24 hrs. The highest inhibition rate were noticed for HeLa cell line using 350 μg/ml and the least one for RD cells.
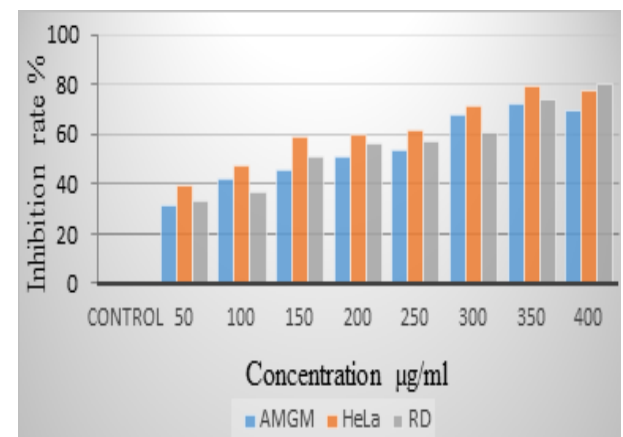

Figure 7: Compare between AMGM, HeLa and RD cell line in Inhibition rate in 24 hr.

After 48 hour there were no significant effect (P>0.05) of each concentration for the three cell lines except for the concentration 200 μg/ml, there were a significant effect (P<0.05). As shown in figure 8 below. The higher DC concentration was noticed in AMGM cell line using 350 μg/ml by 78.71% (P>0.05), then HeLa cells with 75.58% using 400 μg/ml (P>0.05).

Figure 9 showed the compression between AMGM, HeLa and RD cell line with the inhibition rates after 72 hour for treated and untreated cells. From the figure could concluded that there were a significant effect in each concentration for all the studied cell lines (P<0.05), except in concentration 350 μg/ml, there were no significant effect (P>0.05). The highest inhibition rate was noticed for RD cells 79.84% in 400μg/ml, and the least one for HeLa cell line 79.02% in 350μg/ml.

Figure 9: Compare between AMGM, HeLa and RD cell line in Inhibition rate in 72 hr.

From the above results the most DC concentrations effectives on the cells viability is 350 and 400 μg/ml after 48 and 72 hours. The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The values are typically expressed as molar concentration. It is commonly used as a measure of antagonist

drug potency in pharmacological research. According to the FDA, IC50 represents the concentration of a drug that is required for 50% inhibition in vitro. It is comparable to an IC50 for agonist drugs. IC50 also represents the plasma concentration required for obtaining 50% of a maximum effect in vivo [38].

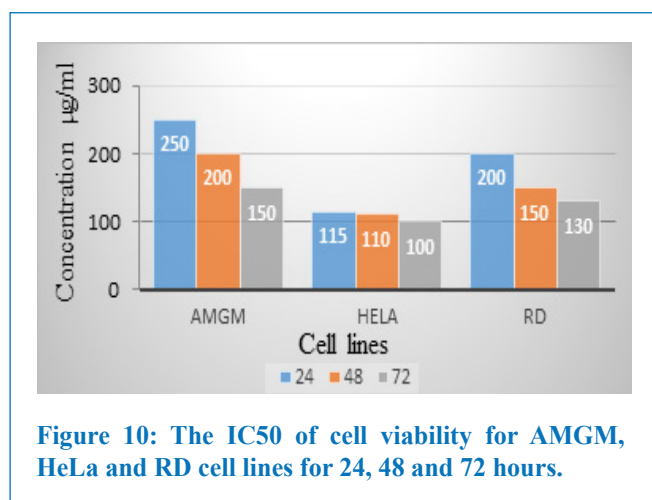Table 1 represents the mean IC50 values for AMGM, HeLa and RD cell lines after 24, 48 and 72hour treatment with DC.

Table 1: The Mean IC50 of DC concentrations (µg/ml) of AMGM, HeLa and RD cell lines for 24, 48 and 72 hours.

Time (hour)	Mean IC$_{50}$ µg/ml		
	AMGM	**HeLa**	**RD**
24	250	115	200
48	200	110	150
72	150	100	130

From the data above, it was clear that for AMGM cells, the higher concentration noticed in 24 hour by 250 µg/ml but the lower one after 72 hour was 150 µg/ml, that is mean the concentration decreased with increasing the days. Which noticed the same thing for HeLa cell line, where after 24 hour, the IC50 was 115 µg/ml and in 72 hour was 100 µg/ml. But for the RD cell line, it's clear that after 24 hour the IC50 was 200 µg/ml, but in 72 hour, the IC50 was 130 µg/ml. The highest IC50 values were for AMGM cell line, but the lower one was for HeLa cell line, as shown in figure 10.

Figure 10: The IC50 of cell viability for AMGM, HeLa and RD cell lines for 24, 48 and 72 hours.

Discussion:

Doxycycline belongs to the tetracycline family of drugs, members of which function as anti-microbial agents by preventing the binding of amino acyl tRNA to the ribosome, thereby inhibiting protein synthesis [39]. It has been known for years that members of the tetracycline family, such as DC and minocycline, inhibit the growth of various tumor cells in vitro [40]. Doxycycline was found to be the most effective analogue of tetracycline at inhibiting cell survival in a human adenocarcinoma cell line

[41]. The mechanisms for DC anti-proliferative effects have been reported, and include impairment of mitochondrial protein synthesis [42,43], proliferation arrest in the G1 phase of cell cycle [44], and induction of apoptosis by caspase-3 activation in human T-lymphoblastic leukemia cells [45]. However, the serum concentrations of DC required to achieve these cytotoxic effects are generally higher (20 to 50) µg/ml than the typical therapeutic serum concentration used to treat infections, generally from 0 to 5 µg/ml and occasionally as high as 10 µg/ml [46], making DC less desirable as a cytotoxic agent. There are reports showing that different cancer cell lines have different sensitivities to DC. Doxycycline at 10 µg/ml was sufficient to induce G0/G1 arrest, and a concentration of 20 µg/ml was able to provoke mitochondrion mediated apoptosis in HT 29 cells [47,48]. In prostate cancer cell culture, necrosis and apoptosis were not apparent until DC concentrations exceeded 20µg/ml [49]. In clinical settings, GBM is highly resistant to cytotoxic intervention as compared to other solid tumors, and this may be a clinical reflection of our observation that glioma cells require higher concentrations of DC in vitro to achieve its effect on growth inhibition [50].

Wang et al. [51] found that the glioma cells under serum-free culture conditions were exposed to low concentrations of DC displayed a similar behavior to those cultured in 5% serum. However, viability of U87 and LN229 cells tended to be reduced in response to high concentrations of DC treatments. A similar, but less obvious trend was observed with lower concentrations as well. For example, the cell viability of U87 cells after 72 hrs was reduced to 79% compared to that after 48 hrs in the higher concentration group (P < 0.05), indicating a cytotoxic effect of DC on U87 cells in serum-free conditions. In U251HF cells, the curve for cell viability was essentially the same between 48 hrs and 72 hrs for all treated groups including the untreated control cells. These data are consistent with the study by Sagar et al [52], they demonstrated cytotoxic activity of DC in colorectal

cancer cell (HT29 cell line). Zhao et al [53] found that in HeLa and CaLo cell lines, DC inhibited proliferation in a dose-dependent manner with IC50 (10–20 µmol/L). While Chen et al [54] explained that DC significantly inhibited the proliferation of many cancer cells in a dose-dependent manner. IC50 of most cells was less than 5 µM, which was quite low. This result demonstrated that many cancer cell lines were very sensitive to DC. Wei et al [55] found after 48 hrs treatment with DC in different concentrations, cell viability was decreased in a dose-dependent manner in HO8910, SKOV3 and SKOV3/DDP. The IC50 of DC in SKOV3 was compared with that in SKOV3/DDP. The results showed that the IC50 was 16.33 mg/ml in SKOV3

and 8.53 mg/ ml in SKOV3/DDP respectively, which indicated that SKOV3/DDP cells were more sensitive to DC treatment than SKOV3 cells. But Hector et al [56], when they study on HuTu-80 human duodenal adenocarcinoma cells 1x104, after being exposed to DC for 72 h, a cellular viability of 55±2% and 10±1.6% at 5 and 10 µM, respectively, was determined. These concentrations corresponded to 2.22 and 4.44 µg/ml. The maximum plasma concentration attainable in humans varies from 1.5 to 3.6 µg/ml after therapeutic doses of DC. Thus, the concentrations employed in this study were similar to those that can be obtained in human plasma at therapeutic doses.

References:

1. Prescott LM, Harley JP, Klein DA. (2002). Microbiology, 5th edn. New York: McGraw Hill. 807–15.
2. Loewen PS, Marra CA, Marra F.(1999). Systematic review of the treatment of early Lyme disease. Drugs; 57:157–73.
3. Amsden GW. (2005).Treatment of Legionnaires' disease. Drugs; 65: 605–14.
4. Keystone JS. Prevention of malaria. Drugs 1999;39:337–54.
5. Cunha BA.(1997). New uses for older antibiotics. The rediscovery of four beneficial and cost-effective antimicrobials. Postgrad Med; 101: 68–70, 73, 74, 79, 80.
6. Ryan ME, Greenwald RA, Golub LM. (1996) Potential of tetracyclines to modify cartilage breakdown in osteoarthritis. Curr Opin Rheumatol; 8:238–47.
7. Petrinec D, Liao S, Holmes DR, Reilly JM, Parks WC, Thompson RW. (1996).Doxycycline inhibition of aneurysmal degeneration in an elastase-induced rat model of abdominal aortic aneurysm: preservation of aortic elastin associated with suppressed production of 92 kDa gelatinase. J Vasc Surg; 23:336–46.
8. Curci JA, Petrinec D, Liao S, Golub LM, Thompson RW.(1998). Pharmacologic suppression of experimental abdominal aortic aneurysms: a comparison of doxycycline and four chemically modified tetracyclines. J Vasc Surg; 28:1082–93.
9. Fenton KN, Richardson JD. (1995).Diagnosis and management of malignant pleural effusions. Am J Surg; 170:69–74.
10. Smith JR, Gabler WL. (1995).Protective effects of doxycycline in mesenteric ischemia and reperfusion. Res Commun Mol Pathol Pharmacol; 88:303–15.
11. Fife RS, Sledge GW, Jr, Roth BJ, Proctor C. (1998).Effects of doxycycline on human prostate cancer cells in vitro. Cancer Lett; 127:37–41.
12. Fife RS, Sledge GW, Jr.(19950. Effects of doxycycline on in vitro growth, migration and gelatinase activity of breast carcinoma cells. J Lab Clin Med; 125:407–11.
13. Lokeshwar BL. (1999).MMP inhibition in prostate cancer. Ann N Y Acad Sci; 878:271–89.
14. Onoda T, Ono T, Dhar DK, Yamanoi A, Nagasue N.(2006). Tetracycline analogues (doxycycline and COL-3) induce caspase-dependent and -independent apoptosis in human colon cancer cells. Int J Cancer; 118:1309–15.
15. Richardson M, Wong D, Lacroix S, Stanisz J, Singh G. (2005). Inhibition by doxycycline of angiogenesis in the chicken chorioallantoic membrane (CAM). Cancer Chemother Pharmacol; 56:1–9.
16. Lee CZ, Xu B, Hashimoto T, McCulloch CE, Yang GY, Young WL. (2004).Doxycycline suppresses cerebral matrix metalloproteinase-9 and angiogenesis induced by focal hyperstimulation of vascular endothelial growth factor in a mouse model. Stroke; 35:1715–19.
17. Fife RS, Sledge GW, Jr, Sissons S, Zerler B. (2000).Effects of tetracyclines on angiogenesis in vitro. Cancer Lett; 153(1–2):75–8.
18. Hanemaaijer R, Visser H, Koolwijk P, Sorsa T, Salo T, Golub LM, Van Hinsbergh VW. (1998).Inhibition of MMP synthesis by doxycycline and chemically modified tetracycline (CMTs) in human endothelial cells. Adv Dent Res; 12:114–8.
19. 23. G, Mitchell M, Ledbetter S, Poorman R. (1995). The tetracycline analogs minocycline and doxycycline inhibit angiogenesis in vitro by a non-metalloproteinase-dependent mechanism. Cancer Chemother Pharmacol; 36:418–24.
20. Liu J, Kuszynski CA, Baxter BT. (1999).Doxycycline induces Fas/Fas ligand-mediated apoptosis in Jurkat T lymphocytes. Biochem Biophys Res Commun; 260:562–67.
21. Hengartner MO. (2000).The biochemistry of apoptosis. Nature; 407(6805): 770–76.
22. Desagher S Martinou J. (2000).Mitochondria as the central control point of apoptosis. Trends Cell Biol; 10(9):369–77.
23. Kaufmann SH, Gores GJ. (2000).Apoptosis in cancer: cause and cure. Bioessays; 22:1007–17.
24. Kaufmann SH, Hengartner MO. (2001).Programmed cell death: alive and well in the new millennium. Trends Cell Biol; 11(12):526–34.
25. Petros X.E. Mouratidis*, Kay W. Colston and Angus G. Dalgleish. (2006). Doxycycline induces caspase-dependent apoptosis in human pancreatic cancer cells. Int. J. Cancer: 120, 743–752.
26. "Doxycycline calcium". The American Society of Health-System Pharmacists. Retrieved Aug 18, 2015.
27. Sledz CA et al. (2003).Activation of the interferon system by shortinterfering RNAs. Nat Cell Bio; 5: 834–839.
28. Nelson, ML; Levy, SB (2011). "The history of the tetracyclines." Annals of the New York Academy of Sciences 1241: 17–32.
29. Chopra I, Hawkey PM, Hinton M (1992) Tetracyclines, molecular and clinical aspects.J Antimicrob Chemother 29: 245–277.
30. Gossen M, Bujard H (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci USA 89: 5547–5551.
31. Roberts MC. (2003) .Tetracycline therapy: update. Clin Infect Dis 36: 462–467.

32. Bujard, Hermann; M. Gossen. (1992). "Tight Control of Gene Expression in Mammalian Cells by Tetracycline-Responsive Promoters.". Proc. Natl. Acad. Sci. U.S.A. 89 (12): 5547–51.

33. Freshney, R. I. (2002). Cell line provenance. Cytotechnology, 39, 3-15.

34. Phelan, M. (1988). Basic techniques for mammalian cell tissue culture. Current Protocols in Cell Biology, 7, 15-35.

35. Xu-Dong, L., Xiao-Lan, Z., Cheng, C., Guang-Bo, Z., Xue-Guang, Z., & Jian-An, H. (2009). Inhibition of lung cancer cell proliferation by CD40 signaling through tumor necrosis factor I. Chinese J Cancer, 28(1), 20-23.

36. Stover,E. (2002). In vitro cytotoxity of the epothilone analog, BMS 247550, in pediatric malignacies. Cantaurus, 10, 31-37.

37. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

38. Cheng Y, Prusoff WH (1973). "Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (IC50) of an enzymatic reaction". Biochem Pharmacol. 22 (23): 3099–108.

39. M. Ehtesham, K.Y. Mapara, C.B. Stevenson, et al. (2009). CXCR4 mediates the proliferation of glioblastoma progenitor cells, Cancer Lett. 274:305– 312.

40. Clark JM, Chang AY. (1965). Inhibitors of the transfer of amino acids from aminoacyl soluble ribonucleic acid to proteins. J Biol C hem 240: 4734-4739.

41. Fife RS, Rougraff BT, Proctor C, Sledge GW. (1997). Inhibition of proliferation and induction of apoptosis by doxycycline in cultured human osteosarcoma cells. J L a b Clin M e d 130: 530534.

42. Duivenvoorden WC, Hirte HW, Singh G.(1997). Use of tetracycline as an inhibitor of matrix metalloproteinase activity secreted by human bone-metastasizing cancer cells. Invasion Metastasis 17: 312-322.

43. Kroon AM, Dontje BH, Holtrop M, van den Bogert C. (1984). The mitochondrial genetic system as a target for chemotherapy: tetracyclines as cytostatics. Cancer Lett. 25: 33-40.

44. van den Bogert C, Dontje BH, Holtrop M, Melis TE, Romijn JC, van Dongen JW, Kroon AM.(1986) Arrest of the proliferation of renal and prostate carcinomas of human origin by inhibition of mitochondrial protein synthesis. Cancer Res 46: 3283-3289.

45. Van den Bogert C, van Kernebeek G, de Leij L, Kroon AM.(1986). Inhibition of mitochondrial protein synthesis leads to proliferation arrest in the G1-phase of the cell cycle. Cancer Lett 32: 41-51.

46. Iwasaki H, Inoue H, Mitsuke Y, Badran A, Ikegaya S, Ueda T.(2002). Doxycycline induces apoptosis by way of caspase-3 activation with inhibition of matrix metalloproteinase in human Tlymphoblastic leukemia CCRF-CEM cells. J Lab Clin Med 140: 382-386.

47. Rolain JM, Boulos A, Mallet MN, Raoult D. (2005). Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. A n timicrob A gents Chemother 49: 2673-2676.

48. Onoda T, Ono T, Dhar DK, Yamanoi A, Fujii T, Nagasue N.(2004). Doxycycline inhibits cell proliferation and invasive potential: combination therapy with cyclooxygenase-2 inhibitor in human colorectal cancer cells. J L a b Clin M e d 143: 207-216.

49. Onoda T, Ono T, Dhar DK, Yamanoi A, Nagasue N. (2006). Tetracycline analogues (doxycycline and COL-3) induce caspase-dependent and -independent apoptosis in human colon cancer cells. In t J Cancer 118: 1309-1315.

50. Lokeshwar BL, Selzer MG, Zhu BQ, Block NL, Golub LM.(2002). Inhibition of cell proliferation, invasion, tumor growth and metastasis by an oral non-antimicrobial tetracycline analog (COL-3) in a metastatic prostate cancer model. Int J Cancer, 10; 98(2):297-309.

51. Andrea Wang-Gillam, Eric Siegel, Debra A. Mayes, Laura F. Hutchins, and Yi-Hong Zhou. (2007).Anti-Tumor Effect of Doxycycline on Glioblastoma Cells. J. Cancer Mol. 3(5): 147-153.

52. Sagar J, Sales K, Dijk S, Taanman J, Seifalian A and Winslet M.(2009). Does doxycycline work in synergy with cisplatin and oxaliplatin in colorectal cancer? World J Surg Oncol 7: 2.

53. Yan Zhao, Xinyu Wang, Lei Li, and Changzhong Li. Can. J. (2016). Physiol. Pharmacol. 94: 526–533.

54. Chen,B.,Zhou,H.,Wang,W.,Gu,W.,Zhao,D.,Wang,P. (2016). Studies on antitumor activity spectrum of doxycycline. Journal of Solid Tumors, Vol. 6, No. 1.

55. Wei Wu, Li-hua Yu, Bei Ma, Ming-juan Xu.(2014). The Inhibitory Effect of Doxycycline on Cisplatin Sensitive and -Resistant Epithelial Ovarian Cancer. PLOS ONE, Volume 9 | Issue 3, 1-12.

56. Hector,R. Galvan-Salazar, Alejandro, D., Soriano-Hernandez, Daniel, A., Montes-Galindo, Gabriel, C. E., Jose, G. E., Iram, P. R., Oscar A. N., and Ivan D.E., (2016).Preclinical trial on the use of doxycycline for the treatment of adenocarcinoma of the duodenum. Molecular and clinical oncology. 5: 657-659.

فعالية عقار الدوكسيسيكلين على بعض الخطوط السرطانية البشرية (في الزجاج)

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الخلاصه:

إلدوكسيسيكلين مضاد حيوي من الجيل الثاني للتتراسيكلينات يستخدم في علاج العديد من الامراض التي تسببها البكتيريا والبروتوزوا. استخدمت في هذه الدراسة ثلاثة خطوط لخلايا سرطانية هي: glioblastoma الدماغ البشري- متعدد الأشكال (AMGM) عند تمريرة ال84-75, سرطان عنق الرحم البشري (HeLa) عند مقطع ال70 و سرطان الخلايا العضلية المخططة البشرية (RD) عند تمريرة ال75. أشارت النتائج الى ان الدوكسيسيكلين يظهر تأثير معنوي للسمية عند التراكيز المستخدمة وهي (50, 100, 150, 200, 250, 300, 350 و 400) $\mu g/ml$ على جميع انواع الخلايا قيد الدراسة. نظرا'' للفعالية السمية والخواص الدوائية الجيدة وسلامة الدواء حيث استخدم لسنوات عديدة في علاج العديد من الامراض المعدية, يمكننا الاستنتاج بان إلدوكسيسيكلين علاج جيد لأنواع عديدة من السرطان.