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IN-VITRO STUDY TO EVALUATE VARIOUS DRUGS FOR POTENTIAL EFFECT ON DIFFERENT CANCER CELL LINES

Pharmacology

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ABSTRACT

Background and Objectives: With the increasing cost of new drug development, the concept of drug repositioning can be considered as an alternate method. The present study evaluated the effects of various non anti-cancer drugs for their possible effects on cancer cell lines.

Methods: The selected drugs in 4 graded concentrations were evaluated against cancer cell lines using Sulforhodamine B assay. Parameters evaluated were concentrations required for 50% growth inhibition (GI_{50}), total growth inhibition (TGI) and lethal concentration in 50% (LC_{50}) of various cancer cell lines.

Results: Doxycycline showed GI_{50} against most of the cancer cell lines at concentrations which are achieved in human at standard doses. Other drugs which showed GI_{50} included metformin, gentamicin and azithromycin. None of the drugs achieved the LC_{50}.

Conclusion: Known drugs with suitable pharmacokinetics and pharmacodyanamics can be explored for other indications including cancers. Further studies are needed to validate these drugs in clinical settings.

KEYWORDS

drug repositioning, sulforhodamine B assay, cancer cell lines, growth inhibition-50 GI_{50}

Introduction:

Drug repositioning is a process which includes investigating approved drugs for some different conditions, which is not initially approved for. The advantage of drug repositioning is that these drugs can be rapidly advanced into clinical trial for the "new indication"[1,2].

A well-known example of drug repositioning is the use of sildenafil for erectile dysfunction. Sildenafil was originally developed for the treatment of coronary artery disease but was later investigated and approved for treatment of erectile dysfunction [2]. Apart from the pharmacokinetic and pharmacodynamic information available, the information on adverse effects can also be utilized for investigating newer indication. One example is chloramphenicol which has the adverse reaction of bone marrow depression and was studied against leukemic cell lines which showed some beneficial effects in inhibiting these cell lines[3].

The current in-vitro study intended to screen selected approved drugs with known pharmacokinetic, pharmacodynamic and also adverse reactions for potential anti cancer effects through in-vitro test on various cancer cell lines using Sulforhodamine B assay. The various drugs were selected based on their actions on certain enzymes, accumulation of drugs in tissues or tissue specific adverse reactions as below.

Chloramphenicol: previous in-vitro studies showed inhibition of leukaemic cells by chloramphenicol[3]. In this study chloramphenicol was screened for its effect on some solid tumour cell lines.

Doxycycline: it is known that matrix metalloproteinases (MMPs) play a significant role in the growth, invasion and metastasis of many tumors. Doxycycline inhibited MMPs, inhibited cell proliferation and induced apoptosis in several cancer cell lines[4-8]. Hence in the present study, doxycycline was evaluated against many other cancer cell lines.

Azithromycin: the anti-proliferative and anticancer activity of azithromycin was examined previously using cervical and gastric cancer cells[9]. This effect was partly ascribed by activation of caspase enzymes playing essential roles in programmed cell death[9]. The present study evaluated azithromycin using the above cancer cell and few other cancer cell lines.

Metformin: studies demonstrated that metformin directly inhibits the enzymatic function of hexokinase (HK) I and II[10,11], which is one of a very important enzyme for the tumour. Taking into consideration of the above, metformin was evaluated against lung, breast, oral and colon cancer cell lines.

Gentamicin: it is known that aminoglycoside, especially gentamicin[12] causes nephrotoxicity by accumulating in proximal tubule lysosomes causing structural and chemical damage [12,13].

Moreover, some in-vitro studies have shown that gentamicin increased production of radical oxygen species (ROS) in the lung cancer cell line and increased the efficacy of anti-cancer drug[14]. In this study we intended to find out the effect of gentamicin on genito-urinary cancer cells where high concentrations are achieved and also some other cancer cells.

Metronidazole: few previous in-vitro studies showed metronidazole to possess some anti-cancer effects against breast cancers and also as radio-sensitizer agents in patients on radiotherapy[15,16]. Metronidazole is secreted in oral cavity hence was evaluated for oral cancer cell line.

Standard anti-cancer agents were selected for comparison. The study was initiated after obtaining a written approval from institutional ethics committee.

Materials and methods

A. Drugs and concentrations for evaluation

The list of drugs selected for evaluation and the positive control are given in the Table 1. Four incremental doses were selected for investigation in reference to the peak plasma concentration (Cmax) achieved in adult patients at standard doses. A total of 12 different cancer cell lines were selected for evaluation (Table 2).

Table 1: List of drugs with their concentrations selected for the in-vitro screening study.

Drugs	Conc. 1 (mcg/ml)	Conc. 2 (mcg/ml)	Conc. 3 (mcg/ml)	Conc. 4 (mcg/ml)
Chloramphenicol	5	10	20	40
Doxycycline	1	5	10	20
Azithromycin	0.5	1	2	4
Metformin	0.5	1	2	4
Gentamicin	1	5	10	20
Metronidazole	5	10	20	40
Vancomycin	10	20	40	80
Doxorubicin	1	5	10	20
Paclitaxel	0.5	1	2	4
Gemcitabine	5	10	20	40
Vinblastine	1	5	10	20
Etoposide	1	5	10	20
Oxaliplatin	0.5	1	2	4
Mitoxantrone	0.1	0.5	1	2
Methotrexate	1	5	10	20

Table 2: List of cancer cell lines used for evaluation and drugs selected for evaluation.

Cancer cell lines	Standard anti-cancer agent	Drugs evaluated			
		Drug 1	Drug 2	Drug 3	Drug 4
Human Lung Cancer Cell Line A-549	Etoposide	Chloramphenicol	Azithromycin	Doxycycline	Metformin
Human Breast Cancer Cell Line MCF-7	Methotrexate	Chloramphenicol	Doxycycline	Azithromycin	Metformin
Human Cervical Cancer Cell Line SiHa	Paclitaxel	Chloramphenicol	Doxycycline	Gentamicin	Azithromycin
Human Pancreatic Cancer Cell Line MIA-PA-CA2	Gemcitabine	Chloramphenicol	Doxycycline	Gentamicin	Azithromycin
Human Bladder Cancer Cell Line T-24	Gemcitabine	Chloramphenicol	Gentamicin	Doxycycline	Azithromycin
Human Ovarian Cancer Cell Line OVCAR-3	Paclitaxel	Chloramphenicol	Doxycycline	Gentamicin	Azithromycin
Human Prostate Cancer Cell Line DU-145	Mitoxantrone	Chloramphenicol	Doxycycline	Gentamicin	Azithromycin
Human Colon Cancer Cell Line HT-29	Oxaliplatin	Chloramphenicol	Doxycycline	Metformin	Azithromycin
Human Melanoma Cell Line SK-MEL-2	Vinblastine	Chloramphenicol	Doxycycline	Gentamicin	Azithromycin
Human Oral Cancer Cell Line KB	Paclitaxel	Chloramphenicol	Metronidazole	Metformin	Doxycycline
Human Ovarian Cancer Cell Line A-498	Vinblastine	Chloramphenicol	Gentamicin	Vancomycin	Azithromycin
Human Hepatoma Cell Line Hep-G2	Doxorubicin	Chloramphenicol	Gentamicin	Azithromycin	Doxycycline

B. Source of drugs: all the drugs were purchased from local pharmacy whereas Injection Doxorubicin was a kind donation from the pharmaceutical company Cipla Ltd.

C.Cancer cell lines: the list of cancer cell lines and the drugs used for evaluation are given in table 2. The cell lines were used from Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Mumbai.

D. Sulforhodamine B (SRB) Assay: The SRB assay was performed to assess growth inhibition of cancer cells. This assay estimates cell number indirectly by staining total cellular protein with the SRB dye. This test helps in calculation of the percentage of growth inhibition in the presence of the investigational drug after incubation for 48 hours[17].

E. Endpoint Measurement: The endpoints were based on calculating the percentage growth inhibition after incubating the cancer cell line with the drugs. Percentage growth inhibition (values below 100%) is where the growth of the cancer cells is inhibited after drug treatment. Based on the growth inhibition, the following parameters were evaluated:

- Growth inhibition-50 (GI_{50}) = Concentration of drug causing 50% inhibition of cell growth
- Total growth inhibition (TGI) = Concentration of drug causing total inhibition of cell growth
- Lethal concentration-50 (LC_{50}) = Concentration of drug causing 50% cell kill

Results

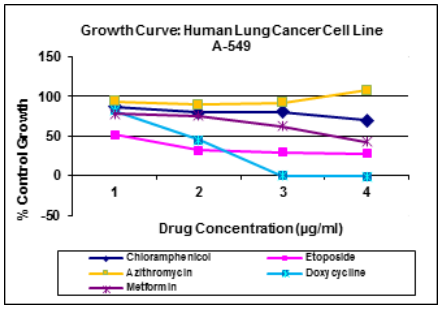
1. Human Lung Cancer Cell Line A-549: Doxycycline and metformin showed GI_{50} in this cell line (table 3 and figure 1). The GI_{50} achieved by doxycycline was at a concentration (4.6mcg/ml) which is generally achieved in the lung tissue after oral administration of this drug in patients[18]. Doxycycline also showed TGI, however the concentration achieved is very high which is not considered relevant in such in-vitro studies. Metformin also achieved the GI_{50} here at a higher concentration (3.3 mcg/ml) compared to its peak plasma levels (1.8 mcg/ml) achieved in the body. Both the drugs showed dose dependent effect in cell growth inhibition (Figure 1). As expected, etoposide which is one of the known drugs used for the treatment of lung cancers also showed GI_{50}. Azithromycin and chloramphenicol did not show any favourable effect.

Table 3: Effect of drugs on A-549 cell line representing lung cancer cell line (concentrations are extrapolated from figure 1 as per the methodology).

A-549	Drug concentrations (µg/ml) calculated from graph		
	LC_{50}	TGI	GI_{50}
Chloramphenicol	>40	>40	>40
Etoposide	>20	>20	<1
Azithromycin	NE	NE	NE
Doxycycline	>20	16.5	4.6
Metformin	>4	>4	3.3

GI_{50}- Growth inhibition-50; TGI - Total growth inhibition; LC_{50}- Lethal concentration-50; NE – no effect

Figure 1: Effect of drugs on A-549 cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.



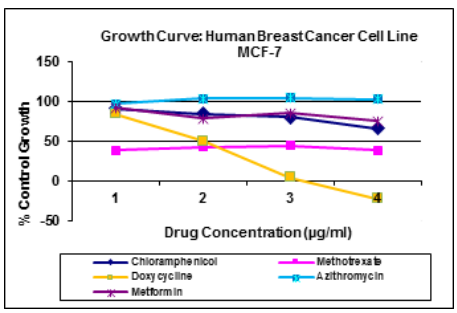
2. Human Breast Cancer Cell Line MCF-7: In this cancer cell line only methotrexate and doxycycline showed GI_{50} (Table 4). Doxycycline achieved GI_{50} at a concentration of 5.2 mcg/ml in this study.

The growth inhibition was again dose dependent for this cancer line (Figure 2). The TGI achieved by doxycycline was at a very higher concentration which cannot be interpreted as relevant through in-vitro method and also the concentration appears to be too high to be achieved by therapeutic dosage.

Table 4: Effect of drugs on MCF-7 cell line representing breast cancer cell line (concentrations are extrapolated from figure 2 as per the methodology).

MCF-7	Drug concentrations (µg/ml) calculated from graph		
	LC_{50}	TGI	GI_{50}
Chloramphenicol	>40	>40	>40
Methotrexate	NE	NE	<1
Doxycycline	>20	14.2	5.2
Azithromycin	NE	NE	NE
Metformin	>4	>4	>4

Figure 2: Effect of drugs on MCF-7 cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.

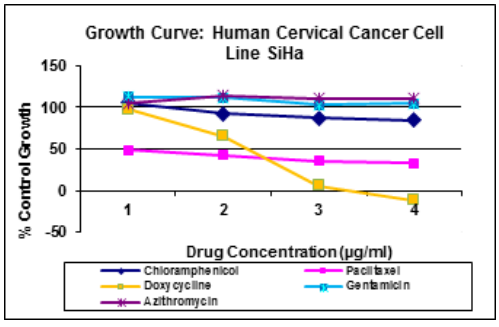


3. Human Cervical Cancer Cell Line SiHa: Doxycycline showed GI_{50} in this cancer cell line (Table 5). The effects appear to be dose dependent however the concentration at which the GI_{50} is on the higher side (Figure 3). Paclitaxel which is a known anti-cancer agent also achieved GI_{50}.

Table 5: Effect of drugs on SiHa cell line representing cervical cancer cell line (concentrations are extrapolated from figure 3 as per the methodology).

	Drug concentrations (µg/ml) calculated from graph		
SiHa	**LC_{50}**	**TGI**	**GI_{50}**
Chloramphenicol	>40	>40	>40
Paclitaxel	23.1	11.3	<0.5
Doxycycline	>20	15.8	7.2
Gentamicin	>20	>20	>20
Azithromycin	NE	NE	NE

Figure 3: Effect of drugs on SiHa cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.

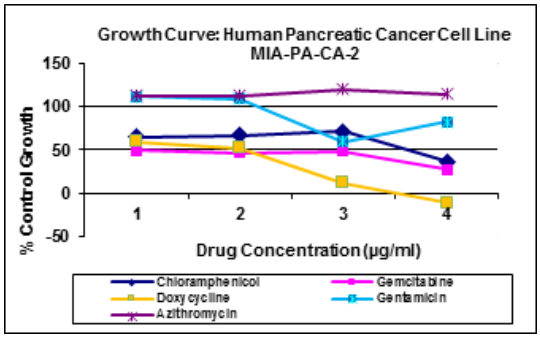


4. Human Pancreatic Cancer Cell Line MIA-PA-CA2: In this cancer cell line, gentamicin and doxycycline achieved GI_{50}(Table 6 and Figure 4). As expected, gemcitabine also showed cancer cell line growth inhibition effects.

Table 6: Effect of drugs on MIA-PA-CA2 cell line representing pancreatic cancer cell line (concentrations are extrapolated from figure 4 as per the methodology).

MIA-PA-CA-2	Drug concentrations (µg/ml) calculated from graph		
	Lc_{50}	**TGI**	**Gi_{50}**
Chloramphenicol	>40	>40	30.1
Gemcitabine	>40	>40	7.0
Doxycycline	>20	16.1	3.4
Gentamicin	>20	>20	2.7
Azithromycin	NE	NE	NE

Figure 4: Effect of drugs on MIA-PA-CA2 cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.

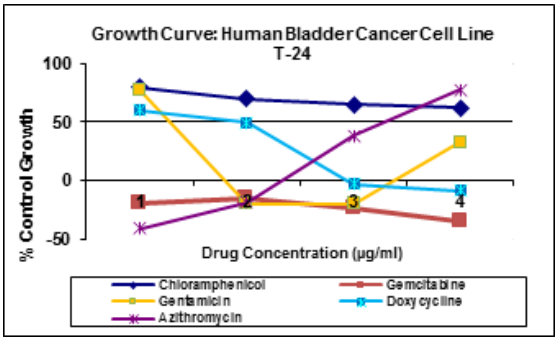


5. Human Urinary Bladder Cancer Cell Line T-24: all the drugs except chloramphenicol produced GI_{50} in this human bladder cancer cell lines T-24 (Table 7 and Figure 5). Azithromycin showed particularly good result with induction of TGI at lower doses. Also doxycycline caused dose dependent inhibition of cancer cell line (Figure 5). The TGI achieved by doxycycline is at a relatively higher concentration which may not be relevant for interpretation. The GI_{50} achieved by gentamicin also was at a lower concentration (<1µg/ml). Overall, Bladder Cancer Cell Line T-24 appears to be sensitive to many agents.

Table 7: Effect of drugs on T-24 cell line representing bladder cancer cell line (concentrations are extrapolated from figure 5 as per the methodology).

T-24	Drug concentrations (µg/ml) calculated from graph		
	LC_{50}	**TGI**	**GI_{50}**
Chloramphenicol	>40	>40	>40
Gemcitabine	>40	<5	<5
Gentamicin	>20	>20	<1
Doxycycline	>20	15.3	2.4
Azithromycin	NE	1.5	2.9

Figure 5: Effect of drugs on T-24 cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.

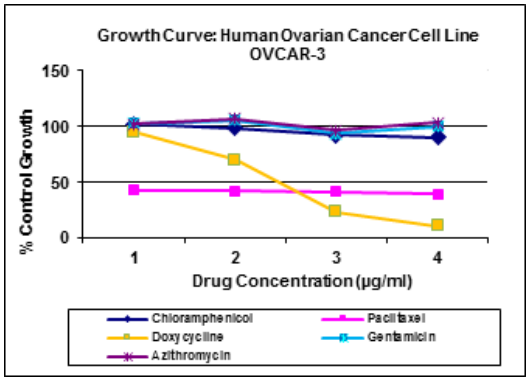


6. Human Ovarian Cancer Cell Line OVCAR-3: In this cancer cell line only doxycycline and paclitaxel showed GI_{50} within the concentrations evaluated (Table 8 and Figure 6). However the concentration required for doxycycline to bring about GI_{50} appears to be higher.

Table 8: Effect of drugs on OVCAR-3 cell line representing ovarian cancer cell line (concentrations are extrapolated from figure 6 as per the methodology).

OVCAR-3	Drug concentrations (µg/ml) calculated from graph		
	LC_{50}	**TGI**	**GI_{50}**
Chloramphenicol	>40	>40	>40
Paclitaxel	NE	NE	<0.5
Doxycycline	>20	20.2	9.0
Gentamicin	NE	NE	NE
Azithromycin	NE	NE	NE

Figure 6: Effect of drugs on OVCAR-3 cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.

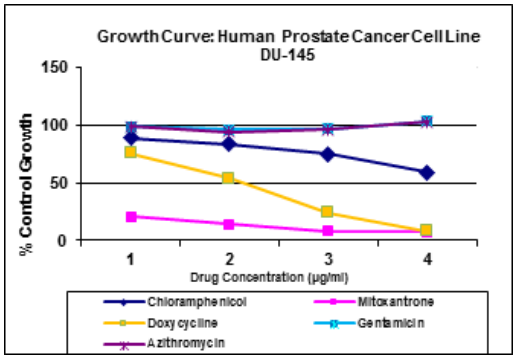


7. Human Prostate Cancer Cell Line DU-145: In this prostate cancer cell line, doxycycline and the standard drug mitoxantrone showed GI_{50} (Table 9 and Figure 7). Doxycycline showed dose dependent inhibition of this cancer cell line (Figure 7).

Table 9: Effect of drugs on DU-145 cell line representing ovarian cancer cell line (concentrations are extrapolated from figure 7 as per the methodology).

DU-145	Drug concentrations (µg/ml) calculated from graph		
	LC_{50}	TGI	GI_{50}
Chloramphenicol	>40	>40	>40
Mitoxantrone	>2	>2	<0.1
Doxycycline	>20	20.5	6.2
Gentamicin	NE	NE	NE
Azithromycin	NE	NE	NE

Figure 7: Effect of drugs on DU-145 cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.

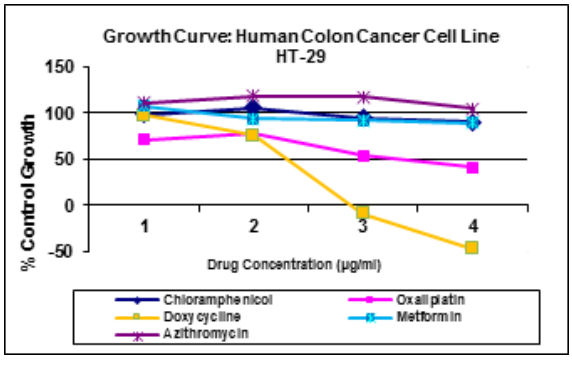


8. Human Colon Cancer Cell Line HT-29: In this colon cancer cell line, doxycycline and the standard oxaliplatin showed GI_{50} (Table 10 and Figure 8). Doxycycline showed dose dependent inhibition of this cancer cell line.

Table 10: Effect of drugs on HT-29 cell line representing ovarian cancer cell line (concentrations are extrapolated from figure 8 as per the methodology).

HT-29	Drug concentrations (µg/ml) calculated from graph		
	LC_{50}	TGI	GI_{50}
Chloramphenicol	NE	NE	NE
Oxaliplatin	>4	>4	3.0
Doxycycline	19.0	12.7	6.4
Metformin	NE	NE	NE
Azithromycin	NE	NE	NE

Figure 8: Effect of drugs on HT-29 cell line. The four points for each drugs (1, 2, 3, and 4 on x axis) corresponds to the 4 incremental concentrations as mentioned in table 1.



The other cancer cell lines evaluated were human melanoma cell line (SK-MEL-2), human oral cancer cell line (KB), human ovarian cancer cell line (A-498) and human hepatoma cell line (Hep-G2). However in all these cell lines only the standard anticancer agent showed growth inhibition and not the investigational drugs. Hence the details have not been included in the article.

DISCUSSION:

Drug repositioning is a very important tool for research into new indications of already approved drugs. This gives an advantage to not only the pharmaceutical companies but also to the academic institutes as ours to venture into research with minimum financial resources. One study indicated that academic groups are becoming increasingly interested in drug discovery and development through this method [1]. In the current study we evaluated the effects of various drugs against different cancer cell lines.

Chloramphenicol: In this study, Chloramphenicol did not show and activity against any solid tumour cells even though previous study did show its efficacy in leukaemic cell lines[3].

Doxycycline: GI_{50} was achieved against all cell lines with doxycycline except in melanoma cell line, oral cancer cell line, ovarian cancer cell line and hepatoma cell line which did not response to any investigational agents. TGI achieved by doxycycline were at higher concentrations which may not be interpreted as relevant for in-vitro studies as the concentration were too high. The effects of doxycycline appears to be dose dependant and seen at a concentration nearby to the peak plasma concentration achieved in humans with usual therapeutic doses. Also there are many other experimental studies published which evaluated the effects of doxycycline in various types of tumour cell lines with positive outcomes[4 - 7]. The current study confirmed the previous results. This indicates that the beneficial effects may also be seen in patients. The possible mechanisms of anti cancer effects of doxycycline were also identified including inhibition of matrix metalloproteinases and induction of apoptosis[4, 6]. Further clinical studies should give better information of doxycycline in various types of cancers.

Gentamicin: Gentamicin showed GI_{50} against human pancreatic cancer cell line MIA-PA-CA2 (at a very low concentration; 2.7mcg/ml) and against human bladder cancer cell line T-24 (<1mcg/ml). No effects of gentamicin on other cancer cell lines were seen. Gentamicin is commonly used in patients with gram negative infections. Clinical studies with gentamicin in these cancer patients requiring antibiotic coverage should be undertaken to evaluate its efficacy in humans.

Azithromycin: In the present study azithromycin showed GI_{50} and TGI in bladder cancer line at a very low concentration. No effect of azithromycin was seen on other cancer cell lines. As previously investigated, the apoptosis induced by azithromycin in cancer cells was partly through a caspase-dependent mechanism with an up-regulation of apoptotic protein cleavage PARP and caspase-3 products, as well as a down-regulation of anti-apoptotic proteins, Mcl-1, bcl-2 and bcl-X1[9].

Metformin: In this study it was seen that metformin bring about GI_{50} in lung cancer cell lines A-549 at a concentration of 3.3mcg/ml, which was within the range of concentration evaluated but it was above the plasma concentration in humans (1.8mcg/ml)[19]. Even though previous study had showed inhibition of growth of breast cancer cell with metformin, the current study did not show this effect on human breast cancer cell line MCF-7. This may be due to the fact that previous study had used a different cell line with different evaluation method. Apart from the other anti-diabetic action, metformin is known to cause inhibition of the enzymatic function of hexokinase (HK) I and II which is an important enzyme for tumour cells[10].

Metronidazole and Vancomycin did not show any favourable effects on any cancer cell lines. Even though previous studies[15, 20] on metronidazole showed some anti-cancer effects against breast cancer cells.

In summary, doxycycline showed 50% growth inhibition in all the cancer cell lines studied except the human melanoma cell line, human oral cancer cell line, human ovarian cancer cell line (A-498) and human hepatoma cell line. In most cancer cell lines, the GI_{50} with doxycycline was within the concentration range studied and can be correlated approximately with the peak plasma level in human. The other drugs which showed GI_{50} in the cancer cell were metformin in

human lung cancer Cell Line (A-549), gentamicin in human pancreatic cancer cell line (MIA-PA-CA2) and human bladder cancer cell line (T-24) and azithromycin in human bladder cancer cell line (T-24). Azithromycin also brought about TGI at a low concentration in bladder cancer cell line.

Further clinical studies may be undertaken to evaluate the role of these drugs in cancer patients in combination with standard anti-cancer drugs especially in patients requiring antibiotic coverage.

Conclusion:

With the current increase in the information available for the existing drugs there is an opportunity for the researchers to explore newer indications by drug repositioning. The present in-vitro screening study took into consideration of the pharmacokinetics, pharmacodyanamics and also the adverse effects for exploring newer benefit of the existing agents. In this study it can be seen that doxycycline, metformin, gentamicin and azithromycin has some potentials for their development of anti-cancer agent. Further clinical studies are necessary to confirm the utility of these drugs for these new indications.

Conflict of Interest: There is no conflict of interest.

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REFERENCES

1. Sukhai MA, Spagnuolo PA, Weir S, Kasper J, Patton L, Schimmer AD. New sources of drugs for hematologic malignancies. Blood. 2011;117(25):6747-6755.
2. Shim JS, Liu JO. Recent Advances in Drug Repositioning for the Discovery of New Anticancer Drugs. International Journal of Biological Sciences. 2014;10(7):654-663.
3. Lokhande J, Juvekar AS and Kulkarni KP. Chloramphenicol: Screening and Review to evaluate its potential beneficial effects in leukaemia. J Indian Med Assoc. 2007;105: 224-228.
4. Fife RS, Sledge GW Jr, Roth BJ, Proctor C. Effects of doxycycline on human prostate cancer cells in vitro. Cancer Lett. 1998;127(1-2):37-41.
5. Saikali Z, Singh G. Doxycycline and other tetracyclines in the treatment of bone metastasis. Anticancer Drugs. 2003;14(10):773-8.
6. Yang B, Lu Y, Zhang A, Zhou A, Zhang L, Zhang L, et al. Doxycycline Induces Apoptosis and Inhibits Proliferation and Invasion of Human Cervical Carcinoma Stem Cells. PLoS One. 2015;10(6):e0129138.
7. Wu W, Yu LH, Ma B, Xu MJ. The inhibitory effect of doxycycline on cisplatin-sensitive and -resistant epithelial ovarian cancer. PLoS One. 2014;9(3):e89841
8. Son K, Fujioka S, Iida T, Furukawa K, Fujita T, Yamada H, Chiao PJ, Yanaga K. Doxycycline induces apoptosis in PANC-1 pancreatic cancer cells. Anticancer Res. 2009;29(10):3995-4003
9. Zhou X, Zhang Y, Li Y, Hao X, Liu X, Wang Y. Azithromycin Synergistically Enhances Anti-Proliferative Activity of Vincristine in Cervical and Gastric Cancer Cells. Cancers. 2012;4(4):1318-1332.
10. Marini C, Salani B, Massollo M, Amaro A, Esposito AI, Orengo AM, Direct inhibition of hexokinase activity by metformin at least partially impairs glucose metabolism and tumor growth in experimental breast cancer. Cell Cycle. 2013;12(22):3490-9.
11. Qu Z, Zhang Y, Liao M, Chen Y, Zhao J, Pan Y. In vitro and in vivo antitumoral action of metformin on hepatocellular carcinoma. Hepatol Res.2012;42(9):922-33.
12. Lopez-Novoa JM; Quiros Y; Vicente L; Morales AI; Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int. 2011;79(1):33-45
13. Olbricht CJ, Fink M, Gutjahr E. Alterations in lysosomal enzymes of the proximal tubule in gentamicin nephrotoxicity. Kidney Int. 1991;39(4):639.
14. Cuccarese MF, Singh A, Amiji M, O'Doherty GA. A Novel Use of Gentamicin in the ROS-Mediated Sensitization of NCI-H460 Lung Cancer Cells to Various Anticancer Agents. ACS chemical biology. 2013;8(12):10.
15. Sadowska A, Prokopiuk S, Miltyk W, Surażyński A, Konończuk J, Sawicka D, et al. Metronidazole affects breast cancer cell lines. Adv Med Sci. 2013;58(1):90-5.
16. Acharya DK. Role of metronidazole in radiation therapy (a review of 717 cancer cases). Indian J Med Sci. 1994;48(5):111-6.
17. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst 1990; 82:1107 12.
18. Thadepalli H, Mandal AK, Bach VT, Oparah SS. Tissue levels of doxycycline in the human lung and pleura. Chest. 1980;78(2):304-5.
19. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P, Williams KM. Clinical pharmacokinetics of metformin. Clin Pharmacokinet. 2011;50(2):81-98
20. Luo Y, Li Y, Qiu KM, Lu X, Fu J, Zhu HL. Metronidazole acid acyl sulfonamide: a novel class of anticancer agents and potential EGFR tyrosine kinase inhibitors. Bioorg Med Chem. 2011;19(20):6069-76