ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
Index Copernicus Value: 49. 23

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 3, Issue –3, May - 2018, Page No. : 152 - 158

In-vitro study on drugs causing bone marrow depression to evaluate their effects on leukaemia cell lines

Dr. Jaisen Lokhande[1], Dr. Sudhir Pawar[2]

[1]Assistant Professor, Department of Pharmacology, LTMMC and GH, Sion, Mumbai

[2] Professor and Head of Department, Department of Pharmacology, LTMMC and GH, Sion, Mumbai

Correspondence Author: Dr. Jaisen Lokhande, Assistant Professor, Department of Pharmacology, LTMMC and GH, Sion, Dr B R Ambedkar Road, Mumbai 400022

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract: Development of new drugs is a time consuming and costly endeavour with apparently low success ratio. One of the alternate ways is to evaluate the known drugs, with their known actions and adverse effect, for new targets.

Objective: The present study was conducted to screen drugs like chloramphenicol, linezolid, vancomycin and zidovudine, known to cause bone marrow depression, to find out if these have any potential anti-leukaemic effect.

Methods: in-vitro method was utilised for screening the above drugs using sulforhodamine B (SRB) assay to evaluate the growth inhibition effects on MOLT-4 and K-562 leukaemic cell lines.

Results: the study showed that chloramphenicol and linezolid lead to growth inhibition (GI_{50}) in 50% of MOLT-4 leukaemia cell line. Chloramphenicol also showed total growth inhibition (TGI) within the concentrations studied.

Conclusions: Certain adverse effects of known older drugs may be further evaluated in special conditions like cancers and this can offer alternate low cost and less time consuming method for drug development.

Keywords: bone marrow depression, sulforhodamine B assay, chloramphenicol, linezolid, leukaemia cell lines.

Introduction

In India, leukaemia continues to be one of the major contributors to cancer-related mortality especially in children, followed by lymphomas and central nervous system (CNS) tumors.[1] Of all the leukaemias reported in children in India, 60% to 85% are acute lymphoblastic leukaemia (ALL).[1]

Compared to the developed world, the biology of ALL appears to be different here, with a higher proportion of T-Cell ALL (20-50% as compared to 10-20% in the developed world), hypodiploidy and translocations t(1;19), t(9;22), and t(4;11), all of which contribute to a poorer prognosis of this leukaemia.[1] On the other hand, in adults in India, chronic myeloid leukaemia (CML) is one of the commonest adult leukaemias accounting for 30% to 60% of all leukaemias.[2]

Extensive array of drugs are available for the treatment of such cancers, however the search for new classes of anticancer agents with higher efficacy, lesser systemic toxicity and selectivity for tumour cells is continuously ongoing.[3]

The process for development of new drugs has its own issues. It has been estimated that it takes about 15 years to bring a drug to the market with a cost of about £400 million. Moreover, only about 20–30 of all drugs investigated are approved by the Food and Drug

Administration in the USA each year.[4] Whereas, only 5% of the oncology drugs entering Phase I clinical trials are finally approved for clinical use. The progressively increasing failure rates, high cost, lengthy testing process have necessitated undertaking alternative approaches for the discovery of new treatment.[5] One such strategy is the exploration of known drugs and investigate these for other conditions. We propose that apart from the mechanisms of actions, even the adverse reactions can be used in certain conditions like cancers.

In relation to this in our previous study, we investigated a known drug chloramphenicol with its known adverse reaction of bone marrow depression, for possible beneficial effect in severe conditions like leukaemia, using in-vitro model.[6] Apart from chloramphenicol, there are a lot of other drugs which also have bone marrow depression as their known adverse reaction.

The current study was initiated to confirm the previous results of chloramphenicol and also to do the screening of other known agents having similar adverse reaction of bone marrow depression, using in-vitro model. The evidence supporting the selection of other drugs in this study was as follows:

Chloramphenicol – previous study[6] showed chloramphenicol producing GI_{50} in HL-60 cell lines and hence this study was conducted for confirmation of previous results of chloramphenicol using different cancer cell lines.

Linezolid – linezolid causes bone marrow suppression in 1-10% patients. The mechanism is hypothesized to be similar to chloramphenicol-induced myelosuppression.[7,8]

Vancomycin - vancomycin induced bone marrow suppression is an important adverse effect which appears to be dose dependent.[9,10] The present study intended to investigate a possible effect of vancomycin on leukaemia cell lines.

Zidovudine (ZDV) – anaemia has been reported in 5.4 - 34.5% of patients on ZDV-containing regimen. The mechanism of ZDV-induced anaemia is mainly attributable to inhibition of proliferation of blood cell progenitor cells in a time-and dose-dependent fashion.[11] This study was intended to evaluate any potential effect on leukaemia cell lines.

Doxorubicin – was used as standard control drug.

The study was initiated after obtaining a written approval from institutional ethics committee.

Materials and methods

A. Drugs and concentrations for evaluation

The doses of the above mentioned drugs were based on the peak plasma concentration (Cmax) which these drugs achieved in adult patients on usual therapeutic dose. Four incremental doses were selected for investigation in reference to the peak plasma concentration (Cmax) achieved as given in Table 1.

Drugs	peak plasma concentration(mcg/mL)	Conc. 1 (mcg/ml)	Conc. 2 (mcg/ml)	Conc. 3 (mcg/ml)	Conc. 4 (mcg/ml)
Chloramphenicol	14	5	10	20	40
Linezolid	20	5	10	20	40
Vancomycin	63	10	20	40	80
Zidovudine	1.1	0.5	1	2	4
Doxorubicin	1.1	1	5	10	20

Table 1: Four incremental doses of the drugs selected for evaluation

B. Source of drugs

drugs chloramphenicol, linezolid, vancomycin and zidovudine were purchased from local pharmacy and injection doxorubicin was a kind donation from the pharmaceutical company Cipla Ltd.

C. Cancer cell lines

The cell lines selected were MOLT-4 and K562 leukaemia cell lines. MOLT-4 represents acute

lymphoblastic leukaemia (T lymphoblast type)[12] whereas K-562 represents chronic myelogenous leukaemia. K562 cells are of the erythroleukaemia type and are positive for the bcr-abl fusion gene which represent more resistant type of leukaemia.[13] The cell lines were used from the Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Mumbai where the study was conducted.

D. Sulforhodamine B (SRB) Assay

The SRB assay was performed to assess growth inhibition. This assay estimates the cell number indirectly by staining the 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.[14]

E. Endpoint Measurement

Percentage growth inhibition (values below 100%) where the growth of the cancer cells is inhibited and the final number of cells after drug treatment are less than the reading for the control drug. 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

The results showed that all drugs except vancomycin produced GI_{50} on the MOLT-4 leukaemia cell lines within the concentration ranges tested (table 2 and figure 1). The cell viability was reduced in dose dependent manner with chloramphenicol. In this study, chloramphenicol showed particularly good results as it showed GI_{50} and also TGI within the concentration range (within the Cmax). Similar result was seen with the control drug doxorubicin with TGI within the range of concentrations evaluated.

Drugs	Drug concentrations (mcg/ml) calculated from graph		
	LC50	**TGI**	**GI50**
Chloramphenicol	>40	<5	<5
Doxorubicin	>20	<1	<1
Linezolid	>40	28.0	<5
Vancomycin	>80	>80	53.2
Zidovudine	>4	>4	3.1

Table 2: Effect of drugs on MOLT 4 cell line (concentration are extrapolated from figure 1 as per the methodology)

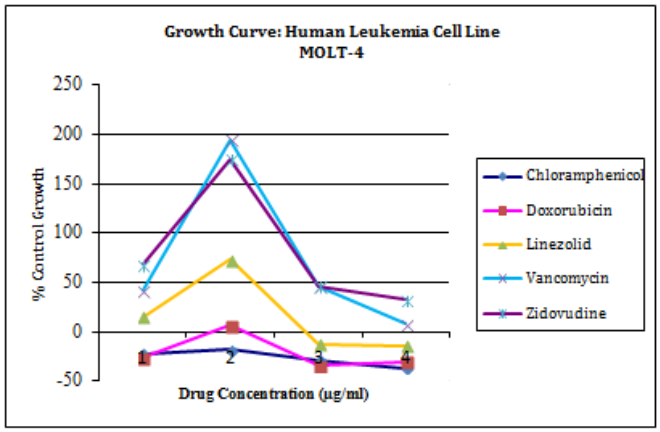

Figure 1: Effect of drugs on MOLT 4 cell line. The four points for each drugs (1, 2, 3, and 4) corresponds to the 4 incremental concentrations as mentioned in table 1.

Table 3 and figure 2 shows the effects of drugs on K-562 cell lines. Here none of the drugs showed any favourable effects including the standard doxorubicin.

Drugs	Drug concentrations (mcg/ml) calculated from graph		
	LC50	**TGI**	**GI50**
Chloramphenicol	>40	>40	>40
Doxorubicin	>20	>20	15.2
Linezolid	>40	>40	>40
Vancomycin	>80	>80	>80
Zidovudine	>4	>4	>4

Table 3: Effect of drugs on K-562 cell line (concentration are extrapolated from figure 2 as per the methodology)

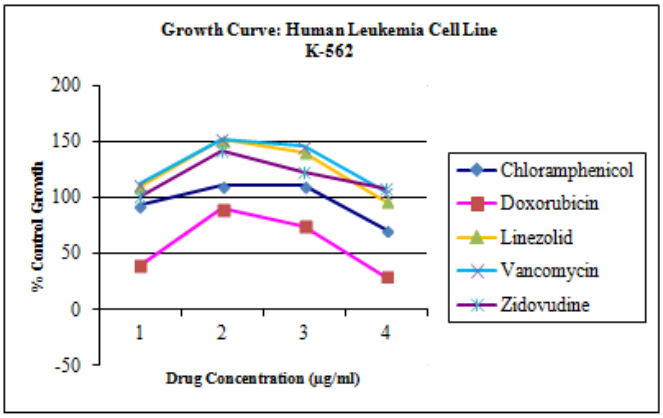

Figure 2: Effect of drugs on K-562 cell line. The four points for each drugs (1, 2, 3, and 4) corresponds to the 4 incremental concentrations as mentioned in table 1.

Discussion

It is known that most of the anti-cancer agents causes severe side effects in patients resulting in postponing the chemotherapy cycle or providing suboptimal levels of chemotherapy all of which can contribute in treatment failure.[15] Hence there is always a need for newer, specific and safer drugs,[16] which by itself is a major challenge due to the high cost, long waiting period and failure rates of new drugs. In this context we conducted the present screening study to explore newer use of old drugs by investigating the effect of adverse reactions on leukaemic cell lines of MOLT-4 and K-562.

The result of the study appeared to be encouraging when we consider the MOLT-4 cell lines. It was seen that all the drugs except vancomycin showed GI_{50}. In fact, chloramphenicol, also resulted in TGI at concentration (<5mcg/ml) which was within the peak plasma levels (14mcg/ml) in adults. The other drug to be mentioned is linezolid which also showed GI_{50} within the defined concentration. As seen in table 2, even though zidovudine shown GI_{50}, the concentration is much higher than the peak plasma level of 1.1mcg/ml. Hence the results of this study are more relevant for chloramphenicol and linezolid.

The mechanism for the bone marrow damage due to chloramphenicol has been described as either dose dependant[17] or as idiosyncratic reaction.[18] The irreversible and fatal aplastic anemia due to chloramphenicol is probably due to the nitrobenzene metabolites that act on DNA whereas the mild and reversible bone marrow suppression is ascribed either to mitochondrial protein synthesis inhibition[19] or to the antimetabolic effects of chloramphenicol.[17] The mechanism of linezolid-induced hematological adverse events is unclear but resembles that of chloramphenicol. Laboratory evidence also suggested that linezolid-induced thrombocytopenia may be immune-mediated.[20]

The results for the other cell line K-562 were not favourable for any of the investigational drugs. One of the reasons could be that these cells are positive for the bcr:abl fusion gene,[13] which are inherently resistant to drugs.[21]

In summary, chloramphenicol and linezolid appears to have growth inhibiting effect on the MOLT-4 leukaemia cell line. Even though further clinical studies will clarify the utility of these drugs in leukaemia patients, some advantages can be clearly mentioned. These drugs which are used in neutropenic fever in various cancers can provide an advantage of anti-leukaemic effects along with the needed antibiotic coverage. Moreover, other systemic adverse effects of these drugs appear to be much lesser compared to the anti-cancer drugs. Effects of combination of these drugs with other anti-cancer drugs may be evaluated in refractory cancers when other options are less.

There also may be a possibility to bring about structural changes in these drugs to make them more potent and

reliable anti-cancer agents. Some investigators have already synthesized a series of chloramphenicol-dimers and have performed studies using in-vitro model. Their results showed that the chloramphenicol-dimers were almost twice as active in inhibiting the growth of T-leukaemic cells, without affecting the viability of normal human lymphocytes.[22]

Apart from the favourable results and potential role in leukaemia, there are some concerns for the use of these agents in leukaemia treatment. (1) Chloramphenicol has been believed to be a carcinogen based on the nitrobenzene moiety present and some evidence from in-vitro[23] and few clinical studies. Three case reports have shown the development of leukaemia after chloramphenicol therapy. In a case control study, chloramphenicol was found to increase the risk of childhood leukaemia, depending on the duration of chloramphenicol administration, whereas two case-control studies revealed increases in the risk of aplastic anemia. However, other studies found no association between the use of chloramphenicol and the development of adult leukaemia.[23]

In this perspective, one also has to keep in mind that many of the anti-cancer agent are themselves carcinogenic and are still in use to treat various cancers.[24] (2) It may be needed to investigate further the traits of patients who will respond better to these drugs and hence more extensive studies will be needed. (3) There may not be commercial viability for the development of such drugs as these drugs or new indications cannot be patented and hence pharmaceutical companies may be reluctant to invest further for their research. Hence the onus is on all the vigilant clinicians and researchers to note their observations on many such known drugs and to study and publish these data so as to generate enough evidence on old drugs for the important new indications.

Conclusion

Extensive information exists on the pharmacodyanamics, uses and adverse reactions of available drugs. Some of these can be utilised for screening and evaluation of old drugs for different indications. In the current screening study, chloramphenicol and linezolid, which inherently has the adverse reactions of bone marrow suppression, showed good results in suppression the growth of MOLT-4 cancer lines. Further clinical studies needs to be undertaken to evaluate the clinical utility of this results.

References

1. Arora R S, Eden T, Kapoor G. Epidemiology of childhood cancer in India. Indian J Cancer 2009;46:264-73.
2. Bhutani M, Kochupillai V. Hematological malignancies in India. In: Kumar L, editor. New York: Progress in Hematologic Oncology. Pub. The Advanced Research Foundation New York; 2003: p10.
3. Senthilraja P, KathiresanK. In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF -7 cell line study of Marine Yeast. Journal of Applied Pharmaceutical Science. 2015;5(03): 80-84.
4. Aronson JK. Old drugs – new uses. British Journal of Clinical Pharmacology. 2007;64(5):563-565.
5. Zamboni W, Torchilin V, Patri A. Best practices in cancer nanotechnology: perspective from NCI nanotechnology alliance. Clin. Cancer Res. 2012;18:3229–3241.
6. Lokhande J, Juvekar AS, Kulkarni KP. Chloramphenicol: screening and review to evaluate its potential beneficial effects in leukaemia. J Indian Med Assoc 2007;105(4):224, 226-8.
7. Lakhani N, Thompson W, Bombassaro A M. Probable linezolid-induced pancytopenia. Can J Infect Dis Med Microbiol. 2005;16(5):286-8.

8. Bernstein WB, Trotta RF, Rector JT, Tjaden JA, Barile AJ. Mechanisms for linezolid-induced anemia and thrombocytopenia. Ann Pharmacother. 2003;37(4):517-20.
9. Meehan KR, Verma UN, Esteva-Lorenzo F, Mazumder A. Suppression of progenitor cell growth by Vancomycin following autologous stem cell transplantation. Bone Marrow Transplant. 1997;19(10): 1029-32.
10. Schwartz MD. Vancomycin-induced neutropenia in a patient positive for an antineutrophil antibody. Pharmacotherapy. 2002;22(6):783-8.
11. Ejeliogu EU, Oguche S, Ebonyi AO, Okpe SE, Yiltok ES, Ige O, et al. Zidovudine-Induced Anaemia in Human Immunodeficiency Virus Infected Children on Highly Active Anti-Retroviral Therapy in Jos, Nigeria. Journal of Advances in Medical and Pharmaceutical Sciences.2014;1(1):1-10
12. Greenberg JM, Gonzalez-Sarmiento R, Arthur DC, Wilkowski CW, Streifel BJ, Kersey JH. Immunophenotypic and cytogenetic analysis of Molt-3 and Molt-4: human T-lymphoid cell lines with rearrangement of chromosome 7. Blood. 1988;72(5):1755-60
13. Klein E, Ben-Bassat H, Neumann H, Ralph P, Zeuthen J, Polliack A, Vánky F. Properties of the K562 cell line, derived from a patient with chronic myeloid leukaemia. Int J Cancer. 1976;18(4):421-31
14. 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.
15. Jemal A, Siegel R, Xu J, Ward E., Cancer statistics, 2010. Cancer Journal for Clinicians. 2010:60(5):277–300.
16. Akindele AJ, Wani ZA, Sharma S, Mahajan G, Satti NK, Adeyemi OO, et al. In Vitro and In Vivo Anticancer Activity of Root Extracts of Sansevierialiberica Gerome and Labroy (Agavaceae). Evid Based Complement Alternat Med. 2015;2015:560404.
17. McCubdy PR. Plasma Concentration of Chloramphenicol and Bone Marrow Suppression. Blood. 1963;21(3):363-372.
18. Malkin D, Koren G, Saunders EF. Drug-induced aplastic anemia: pathogenesis and clinical aspects. Am J Pediatr Hematol Oncol. 1990;12(4):402-10.
19. Dinos GP, Athanassopoulos CM, Missiri DA, Giannopoulou PC, Vlachogiannis IA, Papadopoulos GE, Papaioannou D, Kalpaxis DL. Chloramphenicol Derivatives as Antibacterial and Anticancer Agents: Historic Problems and Current Solutions. Antibiotics (Basel). 2016;5(2)
20. Lakhani N, Thompson W, Bombassaro AM. Probable linezolid-induced pancytopenia. Can J Infect Dis Med Microbiol. 2005;16(5):286-8.
21. Hochhaus A. Chronic myelogenous leukaemia (CML): resistance to tyrosine kinase inhibitors. Annals of Oncology. 2006;17(Supplement 10):274–279.
22. Kostopoulou ON, Magoulas GE, Papadopoulos GE, Mouzaki A, Dinos GP, Papaioannou D, et al. Synthesis and evaluation of chloramphenicol homodimers: molecular target, antimicrobial activity, and toxicity against human cells. PLoS One. 2015;10(8):e0134526
23. Yuan ZR, Shi Y. Chloramphenicol induces abnormal differentiation and inhibits apoptosis in activated T cells. Cancer Res. 2008;68(12):4875-81

24. Blagosklonny MV. Carcinogenesis, cancer therapy and chemoprevention. Cell Death Differ. 2005;12(6):592-602.

Acknowledgement: We would like to acknowledge Dr. Jyoti Kode of Anti-Cancer Drug Screening Facility (ACDSF) at ACTREC, Tata Memorial Centre, Navi Mumbai for conducting the study and Dr Aarti Juvekar for reviewing the article and giving valuable suggestions.

Conflict of Interest: There is no conflict of interest.

Funding: the study was funded by Staff and Research Society, LTMMC and GH, Sion Mumbai.