The Pharma Innovation Journal 2021; 10(11): 907-909

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 907-909

www.thepharmajournal.com
Received: 13-08-2021
Accepted: 20-10-2021

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Evaluation of cytotoxicity potential of 6-thioguanine on Hela cancer cell line

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Abstract
Cancer is the second leading cause of death globally, despite good advances in tumor biology research and chemotherapy. Major obstacles in cancer treatment arise from tumor heterogeneity, drug resistance, and systemic toxicities. 6-thioguanine (6-TG), a thiopurine been used extensively in the treatment of acute leukemias since the 1940s. In this study, we investigated the cytotoxicity potential of 6-thioguanine (6-TG) on the Human cervical carcinoma cell line (HeLa). The percent cell viability was determined by conducting MTT [3-(4, 5- dimethylthiazolyl- 2)-2, 5- diphenyltetrazolium bromide] assay at 48h incubation. IC_{50} values of 6-TG for HeLa was 28.79µM. This study suggested that the cytotoxicity potential in terms of percent cell viability for 6-TG is dose-dependent.

Keywords: 6-thioguanine, cytotoxicity, HeLa cancer cell lines, MTT assay

1. Introduction
Cancer is one among the most difficult global healthcare problems. It is a complex and multistep process driven by both genetic and epigenetic modifications that affect major cellular processes and pathways such as proliferation, differentiation, invasion, and survival [1-3]. The hallmarks of cancer comprise sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis, reprogramming of energy metabolism, and evading immune destruction. Recognition of the widespread applicability of these concepts will increasingly help the development of new means to treat cancer [4-5].
6-thioguanine (6-TG), an antimetabolite used as an anticancer, anti-inflammatory, and immunosuppressive agent in the treatment of a variety of diseases [6-9]. The thiopurines 6-TG and 6- mercaptopurine (6-MP) were co-developed by Gertrude Elion and George Hitchings, who received the Nobel Prize for the development of these and other anticancer agents. 6-TG and 6-MP were produced by substitution of oxygen by sulfur at carbon 6 of guanine and hypoxanthine, respectively [10-11]. The cytotoxicity of 6-TG on proliferating cells is due to DNA *via* the purine salvage pathway, and resistance to these agents can be conferred by lack of the salvage pathway enzyme hypoxanthine-guanine phosphoribosyltransferase [12]. Thiopurines are prodrugs, and to achieve their efficacy and cytotoxicity, they need to be converted to active metabolites, i.e., ^{S}G nucleotides, and subsequently incorporated into DNA [13-16]. 6-TG acts as a DNA methylation regulator in acute lymphoblastic leukemia cells. It reactivates epigenetically silenced genes by facilitating proteasome-mediated degradation of DNMT1 [17-19]. Besides, the cytotoxicity of 6-TG on HeLa cell lines is currently unclear and requires further research. Hence, the present evaluation was undertaken to determine the cytotoxicity of 6-TG on HeLa cell lines.

2. Materials and Methods
2.1 Materials
6-Thioguanine (≥98.0%, #A4882), Dimethyl sulfoxide (Sigma, #D8418) were purchased from Sigma-Aldrich Chemical Co. Ltd. Dulbecco's phosphate-buffered saline (GE Healthcare Life Sciences, #SH30028.02), Dulbecco's Modified Eagle's Medium (GE Healthcare Life Sciences, #SH30243.01). Trypan blue, MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide], trypsin, streptomycin, penicillin, amphotericin, fetal bovine serum, absolute ethanol (HiMedia Labs, Mumbai, India), and other reagents of analytical grade were also used in the study.

2.2 Cell lines and culture conditions

Human cervical carcinoma cell line (HeLa) (National Centre for Cell Science, Pune, India) was used for *in vitro* cytotoxicity assay. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (4.5g/L Glucose), supplemented with 10% heat-inactivated fetal bovine serum (FBS), L-glutamine (4mM), penicillin 10,000 units/mL, streptomycin 1000µg/mL and Amphotericin-B solution 25µg/mL. Cells were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. After 24h of incubation, the adherent cells were detached using trypsin-EDTA solution 1X/0.25%. Cell count was carried out using the Luna automated cell counter (Logos Biosystems, USA) based on the trypan blue dye exclusion method.

2.3 Determination of Cytotoxicity

MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay [20] is a colorimetric assay was carried out to evaluate the cytotoxicity of 6-TG on HeLa cell lines. The cell suspension of 200µL was seeded in 96-well micro plates (Corning®, USA) at a density of 20,000 cells/well and incubated in a 5% CO_2 incubator at 37°C for 24h. Then the cells were exposed to a graded concentration (3.125, 6.25, 12.5, 25, 50 µM) of 6-TG and incubated for 48h. Later, media was removed from all treated and vehicle control (1% DMSO) cells and replaced with fresh media containing MTT (0.5 mg/ml) and were re-incubated for 3 hours at 37°C. MTT solution was removed and treated with 100µL of DMSO to dissolve insoluble formazan crystals formed within mitochondria of viable cells. The plate was incubated with DMSO for 5min with gentle shaking. Cell viability was determined by measuring the absorbance on a micro plate reader (ELx800, BioTek, USA) at λ max 570 nm. The concentration of 6-TG that resulted in 50% inhibition of cell growth was calculated as the half-maximal inhibitory concentration (IC_{50}) by constructing a dose-response curve. The IC_{50} was determined by a linear curve fit generated after obtaining dose-response to five different concentrations of 6-TG. Cell viability percentages (y-axis) were plotted against increasing concentrations of 6-TG on the x-axis. The linear regression equation y=mx+c was used to estimate IC50 values, where 50 is substituted for y, yielding x as the IC_{50} value.

$$\text{Cell Viability (\%)} = \frac{\text{Number of cells treated with the test item}}{\text{Number of untreated cells}} \text{ X } 100$$

2.4 Statistical Analysis

The *in vitro* cytotoxicity study data was subjected to linear regression analysis to obtain dose-response curves and IC_{50} values. The student *t*-test was carried out, and all the values were expressed as Mean±SD (Graphpad Prism, Version 5).

3. Results and discussion

3.1 *In vitro* cytotoxicity assay

Cytotoxicity assay was performed to evaluate the sensitivity and selectivity of the 6-TG on HeLa cancer cell line [21-23]. The MTT assay was used to measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. The cytotoxicity of 6-TG in terms of percent cell viability on HeLa cell lines was dose-dependent (Fig.1a and 1b, respectively). IC_{50} values of 6-TG for HeLa was 28.79 µM (Fig.2). The cytotoxicity of 6-TG towards HeLa cell line depends on the classification and degree of malignancy of cancer cells [24] and also the level of expression of thiopurine methyltransferase (TPMT) [9, 25-26]. In conclusion, the sensitivity of 6-TG towards HeLa cell lines may depend on the extent of thiopurine methyltransferase expression. Further, an additional set of investigations on underlying molecular mechanisms is recommended to improve efficacy and encounter drug resistance.

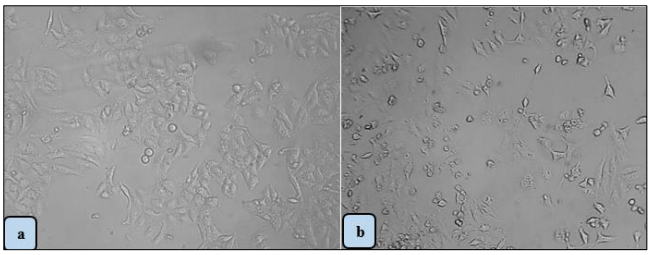

Fig 1: Representative images of cytotoxic effect of a) vehicle control b) 6-TG on HeLa cell lines at 48h incubation (10X)

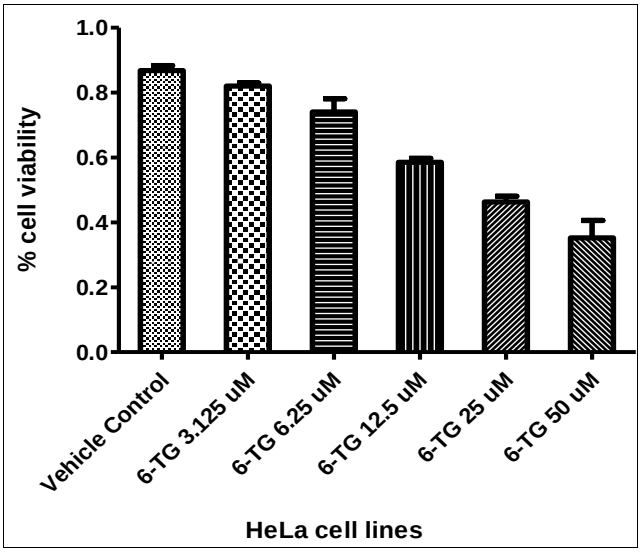

Fig 2: Bar graph depiction of dose-dependent cytotoxicity data of 6-TG as determined by MTT assay on HeLa cell lines. Values are Mean ± SD, n=3, (***p< 0.0001)

4. Conflict of interests

The authors declare that they have no conflict of interest.

5. Acknowledgment

The authors are grateful to SCSP/TSP grants from, Government of Karnataka, India, for funding the research work.

6. References

1. Schnekenburger M, Dicato M, Diederich M. Plant derived epigenetic modulators for cancer treatment and prevention. Biotechnol. Adv 2014;32(6):1123-1132.
2. Shinjo K, Kondo Y. Targeting cancer epigenetics: Linking basic biology to clinical medicine. Adv. Drug Deliv. Rev 2015;95:56-64.
3. Valencia-Gonzalez HA, Ruiz G, Ortiz-Sanchez E, Garcia-Carranca A. Cancer Stem Cells from Tumor Cell Lines Activate the DNA Damage Response Pathway after Ionizing Radiation More Efficiently Than Non-cancer Stem Cells. Stem Cells Int 2019, 1-11.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646-674.
5. Nogales-Cadenas R, Cai Y, Lin J, Zhang Q, Zhang W,

Montagna C *et al*. Micro RNA expression and gene regulation drive breast cancer progression and metastasis in PyMT mice. Breast Cancer Res 2016;18:75.
6. Krynetskaia NF, Cai X, Nitiss JL, Krynetski EY, Relling MV. Thioguanine substitution alters DNA cleavage mediated by topoisomerase II, FASEB J 2000;14:2339-2344.
7. Estlin EJ. Continuing therapy for childhood acute lymphoblastic leukaemia: clinical and cellular pharmacology of methotrexate, 6-mercaptopurine and 6-thioguanine. Cancer Treat Rev 2001;27:351-363.
8. Ravindranath Y, Chang M, Steuber CP, Becton D, Dahl G, Civin C *et al*. Pediatric Oncology Group (POG) studies of acute myeloid leukemia (AML): a review of four consecutive childhood AML trials conducted between 1981 and 2000. Leukemia 2005;19:2101-2116.
9. Hogarth LA, Redfern CPF, Teodoridis JM, Hall AG, Anderson H, Case MC *et al*. The effect of thiopurine drugs on DNA methylation in relation to TPMT expression. Biochem. Pharmacol 2008;76(8):1024-1035.
10. Hitchings GH, Elion GB. The chemistry and biochemistry of purine analogs. Ann. N. Y. Acad. Sci 1954;60(2):195-199.
11. Munshi PN, Lubin M, Bertino JR. 6-thioguanine: a drug with unrealized potential for cancer therapy. Oncologist. 2014;19(7):760-765.
12. Morgan CJ, Chawdry RN, Smith AR, Siravo-Sagraves G, Trewyn RW. 6-Thioguanine-induced growth arrest in 6-mercaptopurine-resistant human leukemia cells. Cancer Res 1994;54(20):5387-5393.
13. Elion GB. The purine path to chemotherapy. Science 1989;244:41-47.
14. Karran P, Attard N. Thiopurines in current medical practice. Molecular mechanisms and contributions to therapy-related cancer. Nat. Rev. Cancer 2008;8:24-36.
15. LePage GA. Basic biochemical effects and mechanism of action of 6-thioguanine. Cancer Res 1963;23:1202-1206.
16. You C, Dai X, Yuan B, Wang Y. Effects of 6-Thioguanine and S^6 - Methylthioguanine on transcription *in vitro* and in human cells. J Biol. Chem 2012;287(49):40915-40923.
17. Yuan B, Zhang J, Wang H, Xiong L, Cai Q, Wang T *et al*. 6-Thioguanine reactivates epigenetically silenced genes in acute lymphoblastic leukemia cells by facilitating proteasome-mediated degradation of DNMT1. Cancer Res 2011;71:1904-1911.
18. Zhang D, An X, Yu H, Li Z. The regulatory effect of 6-TG on lncRNA–miRNA–mRNA ceRNA network in triple-negative breast cancer cell line. Biosci. Rep 2021;41:1213.
19. Flesner BK, Kumar SR, Bryan JN. 6-Thioguanine and zebularine down regulate DNMT1 and globally demethylate canine malignant lymphoid cells. BMC Vet Res 2014;10:290.
20. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol methods 1983;65(1-2):55-63.
21. Vistica VT, Skehan P, Scudiero D, Monks A, Pittman A, Boyd MR. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. Cancer Res 1991;51(10):2515-2520.
22. Maehara Y, Anai H, Tamada R, Sugimachi K. The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. Eur. J Cancer Clin. Oncol 1987;23(3):273-276.
23. Slater T, Sawyer B, Strauli U. Studies on succinate-tetrazolium reductase systems. Biochim et Biophys Acta 1963, 77383-393.
24. Alsemari A, Alkhodairy F, Aldakan A, Al-Mohanna M, Bahoush E, Shinwari Z *et al*. The selective cytotoxic anti-cancer properties and proteomic analysis of *Trigonella Foenum-Graecum*. BMC Complement Altern Med 2014;14(1):114.
25. Dervieux T, Blanco JG, Krynetski EY, Vanin EF, Roussel MF, Relling MV. Differing contribution of thiopurine methyl transferase to mercaptopurine versus thioguanine effects in human leukemic cells. Cancer Res 2001;61:5810-6.
26. Coulthard SA, Hogarth LA, Little M, Matheson EC, Redfern CP, Minto L *et al*. The effect of thiopurine methyl-transferase expression on sensitivity to thiopurine drugs. Mol. Pharmacol 2002;62:102-109.