RESEARCH ARTICLE

Evaluation of the Cytotoxic Effect of the Epoxyconazole and Difenoconazole on Human Colorectal Cancer HCT116 Cell Line

Seher A. R. Almedeny[1], Zena H. Sahib[2]*, Entisar J. Al Mukhtar[3], Khalda K. Alkelaby[4]

[1]*Faculty of Pharmacy, Department of Pharmacology, University of Kufa, Kufa, Iraq.*
[2]*Department of Pharmacology, Hammurabi College of Medicine, University of Babylon, Hillah, Iraq.*
[3]*Department of Pharmacology, College of Medicine, University of Babylon, Hillah, Iraq.*
[4]*Faculty of Pharmacy, Department of Clinical and laboratory, University of Kufa, Kufa, Hillah, Iraq.*

Received: 24th February, 2022; Revised: 20th April, 2022; Accepted: 09th May, 2022; Available Online: 25th June, 2022

ABSTRACT

Since the anticancer role of itraconazole has been approved, interest in other triazole members has been increased. In this study, we try to investigate the anticancer effect of two triazole members, difenoconazole (DIF) and epoxyconazole (EPO) on the HCT116 human colorectal cancer cell line and compare their effect with the standard anticancer agent: methotrexate (MTX) by MTT cytotoxicity assay.

Aim: To assess the cytotoxic effect of epoxyconazole and difenoconazole on human colorectal cancer HCT116 cell line.

Results: The DIF, EPO, and MTX in all concentrations resulted in highly significant ($p < 0.001$) growth rate inhibition in the HCT-116 and Vero cells compared to the dose-dependent negative control group. All concentrations of DIF and EPO (except the concentration of 1000 µg/mL of EPO) show significant ($p < 0.01$) higher growth rate inhibition in the vero cell line in comparison to the HCT 116 cell line. The MTX group in all concentrations showed highly significant inhibition in the growth percentage of the HCT116cell line in comparison to the vero cell line except for the concentration of 1000 µg/ml in which no significant ($p > 0.05$) difference was seen between cell lines.

Conclusions: EPO and DIF are ineffective as anticancer drugs; EPO and DIF are toxic to the normal cells and nonselective in their action toward cancer cells.

Keywords: EPO, DIF, SHCT116, Veto.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.2.59

How to cite this article: Almedeny Seher AR, Sahib ZH, Al Mukhtar EJ, Alkelaby KK. Evaluation of the Cytotoxic Effect of the Epoxyconazole and Difenoconazole on Human Colorectal Cancer HCT116 Cell Line. International Journal of Drug Delivery Technology. 2022;12(2):805-808.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cancer chemotherapy is one of the great challenges that face the medical field nowadays regarding the therapeutic resistance, cost, and side effects.[1,2] Therefore, researchers' efforts are directed toward repurposing of the existing drugs in cancer management to overcome time and cost problems.[3,4]

Since the anticancer role of itraconazole has been approved, interest in other triazole members has been increased.[5] In this study we try to investigate the anticancer effect of two triazole members DIF and EPO on the HCT116 human colorectal cancer cell line and compare their effect with the standard anticancer agent: MTX by MTT cytotoxicity assay.

Difenoconazole is a wide-spectrum fungicide used to control disease in many fruits, vegetables, cereals, and other fields. It has a prophylactic and curative effect. It works by inactivation of demethylation in the ergosterol synthesis process.

No clinical trials were performed on difenoconazole, but experimental studies on rats revealed that it undergoes hepatic metabolism and its sulfate conjugates undergo urinary excretion; 31% of the oral dose is excreted in urine and 75% in feces.[6] It is also excreted in milk.[7] Its residues are concentrated in the liver more than in other tissues.

Epoxiconazole is an active fungicide from the azoles class used to protect crops.[8] Particularly, the drug inactivates fungal cells that can cause plant infection metabolism, thereby stopping mycelia growth. It can efficiently prevent the new fungal spores' production and inactivates the synthesis of the present mycelia. Epoxiconazole acts as an eradicant by encapsulating fungal haustoria, which are then deprived of their nutrient supply and so die. Some fungicide interactions

Author for Correspondence: zina.hasan@uobabylon.edu.iq

can actually lead to enhanced mycotoxins production, which is normal fungal metabolic products, and it has been seen that the use of triazoles, such as Epoxiconazole, in the fungicide mixture may be needed to decrease the levels of mycotoxin.[9]

MATERIALS AND METHODS

Cell Lines: HCT-116 colorectal carcinoma, Vero (kidney normal tissue from African green monkey)

Difenoconazole (DIF), Epoxiconazole (EPO), and MTX solutions: These solutions were set in (1000, 500, 100, 10, 1, and 0) µg/ml concentrations of each for MTT assay application.

MTT Solution

This solution was set in an ultimate concentration of 5mg of MTT powder per ml of phosphate-buffered saline, which is then filtered and stored in a dark place at 4°C.[10]

Methods

MTT assay

The 96 microtiter plates were prepared after incubation in 5 ٪CO_2 at 37°C for 24 hours and brought to the safety cabinet to avoid any contaminated factor; media were launched away. The monolayers of HCT116 cells were then washed three times with PBS. Then all monolayers, including drug-treated, and negative control cells triplicate, were treated with maintenance media (100 µL) for each well. A 20 µL from MTT were added for every well. After 24 hours of incubation with 5٪ CO_2 at 37°C, dimethylsulfoxide DMSO isopropanol in (1:1) volume ratio for each well. Then the absorbance was read with an ELISA reader at 490 nm with a wavelength of 630 nm as a reference.[11] Then, IC50 for each agent was determined using the Dose-Response curve by plotting growth inhibition percentages against variable concentrations range.[12]

Measurements of Selectivity Index (SI)

It is calculated by the ratio between IC50 of each drug on a normal cell line and IC50 of the same drug on cancerous cell lines HCT116. Its value indicates the selectivity of the tested agents to the cancer cell lines. Agents with an SI of more than two were classified as having a high selectivity to the cancerous cells.[13]

$$\text{Selectivity index (SI)} = \frac{\text{VERO cell line IC50 of a drug}}{\text{HCT-116 cell line IC50 of drug}}$$

Statistical Analysis

By SPSS version 23, all the analyses were done. To compare the different groups mean analysis of variance (ANOVA) test was used. The p-value <0.05 was considered statistically significant. All variables were expressed in mean ± SD with a 95% confidence interval.

RESULTS

Cytotoxic Effect of Different Tested Groups on HCT116 Cells

The DIF, EPO, and MTX in all concentrations resulted in highly significant ($p<0.001$) growth rate inhibition in the HCT-116 and Vero cells compared to the dose-dependent negative control group. All concentrations of DIF and EPO (except the concentration of 1000 µg/mL of EPO) show significant ($p<0.01$) higher inhibition in the growth percentage of the Vero cell line in comparison to the HCT116 cell line. The MTX group in all concentrations shows highly significantly lower inhibition in the growth percentage of HCT116cell line compared to the Vero cell line except for the concentration of 1000 µg/ml in which no significant ($p>0.05$) difference was seen between cell lines (Tables 1-3).

The MTX group in all concentrations (except the 1 µg/mL) showed significant ($p<0.01$) higher GI% in the HCT116 cell line and significant ($p<0.01$) lower GI% in the Vero cell line from both DIF and EPO groups and DIF group show significant ($p<0.01$) higher GI% in vero cell line from both MTX and EPO groups. At the same time, the EPO group in all concentrations (except the 1-µg/mL) showed significant ($p<0.01$) higher GI% in the HCT116 cell line with significant ($p<0.01$) lower

Table 1: Effect of DIF on HCT116 and Vero cells growth presented by mean ± SEM.

		VERO cells GI%	*HCT116 cells GI%*
DIF	*N*	*Mean ± SEM*	*Mean ± SEM*
0	3	0	0
1	3	56.2*	12.5
10	3	58.6*	13.6
100	3	90.9*	15.8
500	3	97.8*	17.1
1000	3	99.6*	18.5

*($p<0.01$)

Table 2: Effect of EPO on HCT116 and Vero cells, presented by mean ± SEM.

		VERO cells GI%	*HCT116 cells GI%*
EPO	*N*	*Mean±SEM*	*Mean±SEM*
0	3	0	0
1	3	41.8*	17.6
10	3	38.2*	23.3
100	3	46.1*	29.5
500	3	46.1*	31.3
1000	3	49.7	45.1

*($p<0.01$)

Table 3: Effect of methotrexate on HCT116 and Vero cells, presented by mean ± SEM.

		VERO cells GI%	*HCT116 cells GI%*
Methotrexate	*N*	*Mean ± SEM*	*Mean ± SEM*
0	3	0	0
1	3	7.7	19.6*
10	3	13.1	35.2*
100	3	27.6	64.5*
500	3	48.6	88.6*
1000	3	79.6	96.0*

*($p<0.01$)

Table 4: The IC50 and selectivity index SI for each tested drug on both cell lines.

Drug	*IC50 on Vero cells*	*IC50 on HCT116 cells*	*Selectivity index SI*
EPO	153.817	281.843*	0.54
DIF	75.23	6316.75*	0.01
MTX	748.169*	345.176	2.2

*($p < 0.01$)

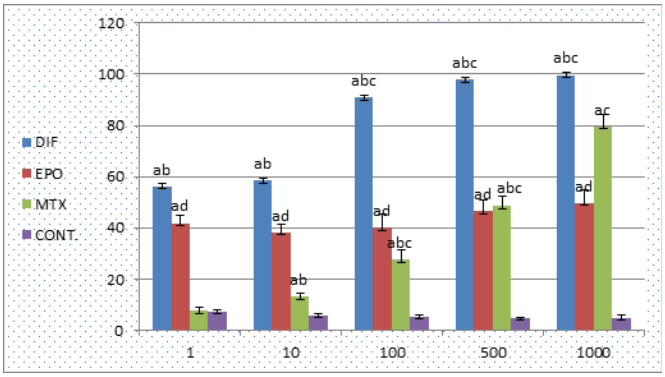

Figure 1: Cytotoxic effect of different tested groups on HCT116 cells a = significant difference from the negative control group. b = significant difference from other treated groups. c = significant difference from other concentrations within the same treated group. d= significant difference from DIF treated group.
*($p < 0.01$)

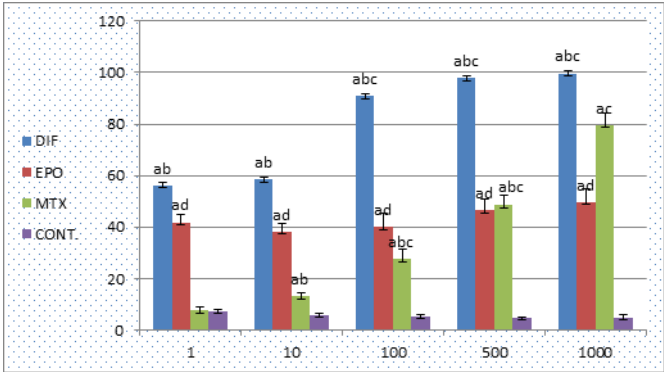

Figure 2: Cytotoxic effect of different tested groups on Vero cells
*($p < 0.01$)
a= significant difference from the negative control group. b= significant difference from other treated groups. c= significant difference from other concentrations within the same treated group. d= significant difference from MTX treated group.

GI% in Vero cell line from DIF treated group. As shown in Figures 1 and 2.

IC50 and SI

The IC50 of both DIF and EPO was highly significant ($p < 0.001$) higher in HCT116 cell line than in the Vero cell line. In the MTX group, the IC50 was significantly higher ($P < 0.001$) in the Vero cell line than in the HCT116 cell line. The selectivity index SI for DIF and EPO was less than two, so the two drugs show no selectivity toward cancerous cells. The SI for MTX was more than two, so it has selectivity toward cancerous cells Table 4.

DISCUSSION

Effect on HCT116 and Vero cells

All drugs used in this study showed highly significant inhibition of growth in HCT 116 and Vero cell line as compared to the control group and showed a dose-dependent inhibition pattern. This means that all drugs are cytotoxic for both malignant and normal cells, and this effect is dose-dependent. Regarding MTX, it is documented by other researchers, such as Karami et al., to have a dose-dependent cytotoxic effect. 14 No comparative data are found regarding EPO and DIF.

When we compare the cytotoxic effect of drugs on the two cell lines, we found that all concentrations of DIF and EPO (except the concentration of 1000 µg/mL of EPO) showed a significant ($p < 0.01$) higher inhibition in the growth percentage of Vero cell line in comparison to HCT116 cell line. This result indicates that DIF and EPO are more cytotoxic to the normal cell than the malignant cells.

The MTX group in all concentrations showed higher inhibition in the growth percentage of HCT116 cell line compared to the Vero cell line except for the concentration of 1000 µg/mL in which no significant ($p > 0.05$) difference was seen between cell lines. This reflects that the Mtx is more toxic to the malignant cells than normal cells. Benz and Cadman found that the pretreatment of HCT 8 colorectal cancer cell line before administration of 5FU increases the concentration of 5FU in the malignant cells rather than the control cells; this effect of MTX supports our result regarding the selectivity of MTX to the malignant cells.[15]

Om comparison to the cytotoxic effect among drug groups, the MTX group in all concentrations (except the 1-µg/mL) showed significant ($p < 0.01$) higher GI% in HCT116 cell line and significant ($P < 0.01$) lower GI% in vero cell line from both DIF and EPO groups this indicates that MTX has more anticancer activity than the other 2 drugs. It is safer than them to normal cells. In contrast, the DIF group showed significant ($P < 0.01$) higher GI% in vero cell line from both MTX and EPO groups which means that it is more cytotoxic o the normal cells than MTX and EPO. on the other hand EPO group in all concentration (except the 1 µg/mL) showed significant ($P < 0.01$) higher GI% in HCT116 cell line with significant ($p < 0.01$) lower GI% in vero cell line from DIF treated group, i.e.it is more effective on cancer cell and less toxic on normal cells than DIF.

The IC50 of both DIF and EPO was highly significant ($P < 0.001$) higher in the HCT116 cell line than in the Vero cell line i.e., both EPO and DIF are less potent on cancerous cells and more toxic on Vero cells. In the MTX group the IC50 was highly significant ($p < 0.001$) higher in the Vero cell line than in HCT116 cell line. The selectivity index SI for DIF and EPO was less than two, so the two drugs show no selectivity toward cancerous cells, while the SI for MTX was more than two. So it has selectivity toward cancerous cells. This indicates

that MTX is safer on normal cells and more selective to cancer cells than EPO and DIF.

CONCLUSIONS

- EPO and DIF are not effective as anticancer drugs
- EPO and DIF are toxic to the normal cells and are nonselective in their action toward cancer cells.

REFERENCES

1. Elkins C. How Much Cancer Costs, drug watch, 2015. https://www.drugwatch.com
2. Phipps C. 'Combination of Chemotherapy and Antiangiogenic Therapies : A Mathematical Modelling Approach ; 2009; Thesis.
3. Pantziarka P, Bouche G, Meheus L, Sukhatme V, Sukhatme VP, Vikas P. The Repurposing Drugs in Oncology (ReDO) Project. E cancer medical science. 2014; (8):442-445).
4. Nosengo, N. Can you teach old drugs new tricks? Nature (2016); 534: 314–316
5. Jennifer R. Pace, Albert M. DeBerardinis, Vibhavari Sail, Silvia K. Tacheva-Grigorova, Kelly A. Chan, Raymond Tran, Daniel S. Raccuia, Robert J. Wechsler-Reya, and M. Kyle Hadden. Repurposing the Clinically Efficacious Antifungal Agent Itraconazole as an Anticancer Chemotherapeutic (2016); 59, 8, 3579-4032.
6. Capps TM and Anderson W. Supplemental report on the metabolism of 14C-phenyl-CGA 169374 in rats –identification of the major urinary metabolites. Ciba-Geigy Corp., Greensboro,
7. Maynard MS [14C]-CGA 169374 phenyl and triazole label distribution, elimination and metabolism in goats. Ciba-Geigy Corp., Greensboro, United States. Report ABR-89100, 1990.
8. Zhang XY, Loyce C, Meynard JM, Savary S: Characterization of multiple disease systems and cultivar susceptibilities for the analysis of yield losses in winter wheat. In: Crop Protection. Nr. 2006: 25, 1013-1023.
9. Xu X, Nicholson P and Ritieni A.: Effects of fungal interactions among Fusarium head blight pathogens on disease development and mycotoxin accumulation.In: International Journal of Food Microbiology Nr. 2007; 119 (1-2),: 67-71
10. Nikzad, S.; Baradaran-Ghahfarokhi, M. and Nasri, P. Dose-response modeling using MTT assay: a short review. Life Sci J, 2014:11(9):432-437.
11. Wang H, Cheng H, Wang F, Wei D, Wang X. An improved 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of Escherichia coli cells. J Microbiol Methods. 2010; 82(3):330-333.
12. Kuppusamy P, Ichwan SJA., Al-Zikri PNH., Suriyah WH, Soundharrajan, I., Govindan, N., et al 'In Vitro Anticancer Activity of Au, Ag Nanoparticles Synthesized Using Commelina nudiflora L. Aqueous Extract Against HCT-116 Colon Cancer Cells', Biological Trace Element Research, (2016) 173(2), 297–305.
13. Badisa, RB.; Darling-Reed, SF.; Joseph, P.; Cooperwood, JS.; Latinwo, LM. and Goodman, C.B. Selective cytotoxic activities of two novel synthetic drugs on human breast carcinoma MCF-7 cells. Anticancer res, (2009). ; 29(8): 2993-2996.
14. Karami F, Ranjbar S, Ghasemi Y, Negahdaripour M . Analytical methodologies for determination of methotrexate and its metabolites in pharmaceutical, biological and environmental samples. J Pharm Anal. 2019; 9(6):373-391.
15. Benz C, Cadman E.,.Modulation of 5-fluorouracil metabolism and cytotoxicity by antimetabolite pretreatment in human colorectal adenocarcinoma HCT-8. Cancer Res. 1981;41(3):994-999.