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ORIGINAL ARTICLE

Mebendazole Inexplicably Reducing the Breast Cancer Cells Viability Preclinically by Incitement Effects with Methotrexate

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ABSTRACT

Objective: To perform preclinical (*in vitro*) interventional trial of mebendazole.

Methods: This study carried out in Basic Medical Science Institute, JMPC in alliance with PCMD. The total study duration from March 2016 to February 2017. For assessment of cytotoxic incitement effects of mebendazole with methotrexate we used six different dilutions of mebendazole (1.5µM-100µM) both as alone and in combination with methotrexate (0.5µM-100µM). Cytotoxicity was assessed by MTT and trypan blue dye exclusion cytotoxicity assays. We used primarily two breast cancerous cell lines MCF-7 (representative of invasive ductal carcinoma) and MDA-MB-231 (representative of adenocarcinoma).

Results: Mebendazole more effectively reducing the % viability of studied cancerous cell lines as combination therapy with methotrexate. The average percentage decrease of % viability of MCF-7 and MDA-MB-231 were -81.3097 and -66.8711 respectively. This combination showed selectivity towards cancerous cell lines as indicated by non-significant (p=0.183) effects on normal breast epithelial cell line (MCF-10).

Conclusion: This study demonstrated that combination therapy of mebendazole with standard chemotherapeutic agent methotrexate unveil incitement effects.

Keywords: MCF-7, MDA-MB-231, MTT, mebendazole, tryptan blue dye exclusion assay.

INTRODUCTION

Microtubules are important intracellular cytoskeletons which are polymers of alpha and beta hetrodimers. These play a vital role in cellular division and survival by participating during cell development and division, motility, intracellular trafficking and the capacity to adjust to an assortment of shapes to connect with nature [1]. Therefore, microtubules are the important therapeutic target for chemotherapeutic drugs [2].

Mebendazole is benzimidazole derivative, safest and economical option for treatment of roundworms intestinal infections [3]. Mebendazole can exert antihelmintic effects by targeting the beta tubulin of microtubules and thus inhibiting their polymerization [4].

Recently it was found that mebendazole can exert anticancerous effects in human cancerous cells by arresting the cellular growth at G2/M phase by inhibiting the polymerization of microtubules [5].

Apart from that mebendazole inhibit the cancer progression by prompting effects on cellular apoptosis through declining the levels of X-linked inhibitors of apoptosis (XIAP) [6]. Furthermore, mebendazole can encourage cellular apoptosis by declines the activity of antiapoptotic protein Bcl-2 and increases the activity of caspases [7,8].

This trial was conducted to find combinatory effect of mebendazole with standard anticancerous drug (methotrexate) in a hope that mebendazole can complement the anticancerous effects of methotrexate.

METHODOLOGY

For assessment of preclinical (*in vitro*) cytotoxic activity of mebendazole on breast cancer cell lines we used MCF-7 which was symbolic of invasive ductal carcinoma and MDA-MB-231 cell line which was archetypal of Adenocarcinoma. Selectivity of mebendazole for cancerous cells was assessed by using MCF-10 which was representative of normal epithelial cells of mammary tissue [9,10].

Cytotoxicity activity of mebendazole both as a sole and in combination therapy with methotrexate evaluated through MTT assay known as 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, a calorimetric assay through which we calculate the % viability of studied cell cultured as *in vitro* by means of discerning the absorbance values of test (At) (mean reagent along with cell lines and studied drug) and compare with absorbance value of blank (Ab) and control (Ac). For this purpose, we incubated cellular cell lines with different dilutions (at least 6 dilutions) of studied drugs for at least 72 hours [11, 12].

Furthermore, for MCF-7 cell line (representative of invasive ductal carcinoma) we assessed the cytotoxicity activity of mebendazole combination therapy with methotrexate through trypan blue dye exclusion assay. For each assay readings should repeat quaternary for each dilution and for three separate days. In this assay viability of cellular culture incubated with studied drug combination was appraised by calculated the % viability through comparing the death and viable cell counts [13].

For combination therapy either their effects will complementary to each other or antagonistic we calculated the combination drug index (CDI) value through Calcusyn system and according to CDI value we labeled that combination therapy of mebendazole was either show synergistic or antagonistic effects [14]. The different ranges of combination drug indices are shown in Table **1** [15].

Table 1. Different ranges of combination drug indices [15].

Range of Combination index	Treatment Effect
<0.1	Very strong synergism
0.1-0.3	Strong synergism
0.3-0.7	Synergism
0.7-0.85	Moderate synergism
0.85-0.9	Slight synergism
0.9-1.10	Nearly additive
1.10-1.20	Slight antagonism
1.20-1.45	Moderate antagonism
1.45-3.3	Antagonism
3.3-10	Strong antagonism
>10	Very strong antagonism

The data was analyzed by using SPSS ver.24 and comparison of dose dependent cytotoxic effects of combination therapy of mebendazole and methotrexate on all cell lines were done by using non-parametric ANOVA "*Kruskull-wallis test*" (an ideal statistical analytic method for biological cell cultures based trials). A p-value of 0.05 or less was considered as statistically significant and highly significant at 0.01 or less.

RESULTS

Dose related effects of combination therapy of mebendazole with methotrexate showed statistically highly significant effects on % viability of MCF-7 (χ^2 (2)= 26.483, p<0.001) as evaluate by MTT assay with mean % viability decreases to 18.639±1.95 for dose 6 as compare to 99.726±0.373 at dose 0 with average percentage decrease was about -81.3097. As depicted in Table **2**. The mean CDI was about 0.8072±0.06.

On MDA-MB-231 cell line appraisal of cytotoxic effects of different amounts of amalgamation therapy of mebendazole with methotrexate on % viability revealed statistically highly significant (p<0.001, χ^2 (2) = 26.483), as % viability decreases to 33.097 ± 3.017 at dose 6 from 99.907±0.031 at dose 0. As depicted in Table **3**. Average

percentage decrease of % viability was about -66.8711 with mean CDI value was about 0.7240±0.037.

Table 2. Comparison of dose dependent effects of mebendazole combination therapy on MCF-7 cell line viability assessed by MTT assay.

Doses (µM)		N = 28	Variables				
MBZ	Meth		Ab' Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	0	4	3.8 ± 0.59	0.268 ± 0.013	0.267 ± 0.012	99.726 ± 0.373	0.0012 ± 0.0006
			(3.0- 4.2)	(0.252 - 0.280)	(0.251-0.278)	(99.167-99.913)	(0.0008-0.0021)
1.5	0.5	4	4.1 ± 0.37	0.237 ± 0.015	0.268 ± 0.0121	88.227 ± 1.638	0.117 ± 0.016
			(3.7- 4.5)	(0.218-0.252)	(0.252-0.278)	(86.72-90.135)	(0.098-0.133)
3.5	1	4	4.4 ± 0.32	0.202 ± 0.014	0.267 ± 0.013	74.545 ± 1.795	0.250 ± 0.0179
			(4.0- 4.7)	(0.185-0.218)	(0.250-0.279)	(73.545-77.322)	(0.226-0.264)
4.5	1.5	4	3.9 ± 0.7	0.164 ± 0.016	0.266 ± 0.012	60.968 ± 3.606	0.390 ± 0.036
			(3.0 - 4.7)	(0.148-0.181)	(0.249-0.276)	(57.281-64.879)	(0.351-0.427)
6.5	2	4	4.2 ± 0.37	0.125 ± 0.011	0.265 ± 0.012	45.945 ± 2.625	0.540 ± 0.026
			(3.7- 4.5)	(0.115-0.137)	(0.249-0.275)	(42.669-48.777)	(0.512-0.573)
7.5	2.5	4	4.2 ± 0.41	0.090 ± 0.009	0.264 ± 0.012	32.954 ± 2.345	0.67 ± 0.023
			(3.7- 4.7)	(0.081-0.099)	(0.248-0.275)	(30.836-35.328)	(0.646-0.692)
9.5	3	4	4.4 ± 0.42	0.0534 ± 0.007	0.263 ± 0.012	18.639 ± 1.95	0.816 ± 0.014
			(4.0-5.0)	(0.046-0.063)	(0.247-0.275)	(17.316-21.53)	(0.795-0.827)
p-value			0.677	<0.001**	0.880	<0.001**	< 0.001**

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug

'Mean ± SD in $x10^{-3}$ '(Min - Max) in $x10^{-3}$ **Significant at1%

Table 3. Comparison of dose dependent effects of mebendazole combination therapy on MDA-MB-231 cell line viability evaluate by MTT assay.

Doses (µM)		N = 28	Variables				
MBZ	Meth		Ab' Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	0	4	4.2 ± 0.87	0.339 ± 0.013	0.338 ± 0.013	99.907± 0.031	0.0009 ± 0.0003
			(3.2 - 5.2)	(0.322-0.354)	(0.321-0.354)	(99.861-99.927)	(0.0007-0.0014)
1.5	0.5	4	4.7 ± 0.4	0.312 ± 0.011	0.339 ± 0.013	91.631± 0.607	0.0837 ± 0.006
			(4.2 - 5.2)	(0.297-0.323)	(0.323-0.354)	(90.959-92.170)	(0.078-0.090)
3.5	1	4	4.2 ± 0.3	0.276 ± 0.012	0.339 ± 0.013	80.915± 0.631	0.191 ± 0.006
			(3.7- 4.5)	(0.259-0.289)	(0.323-0.355)	(80.008-81.439)	(0.185-0.199)
4.5	1.5	4	4.1 ± 0.48	0.236 ± 0.012	0.338 ± 0.012	69.375 ± 1.526	0.309 ± 0.015
			(3.7- 4.7)	(0.220-0.246)	(0.323-0.352)	(67.375-70.865)	(0.291-0.326)
6.5	2	4	4.7± 0.45	0.195 ± 0.015	0.337± 0.011	56.922 ± 2.806	0.431±0.028
			(4.2-5.2)	(0.175-0.206)	(0.322-0.351)	(53.099-59.582)	(0.404-0.469)
7.5	2.5	4	4.4 ± 0.72	0.155 ± 0.013	0.336 ± 0.012	45.046 ± 2.649	0.549±0.026
			(4.0-5.5)	(0.138-0.167)	(0.320-0.351)	(41.575-47.310)	(0.526-0.584)
9.5	3	4	4.0 ± 0.97	0.115 ± 0.013	0.336 ± 0.012	33.097± 3.017	0.669±0.030
			(2.7-5.0)	(0.099-0.128)	(0.320-0.35)	(29.58-36.606)	(0.634-0.704)
p-value			0.591	0.001**	0.897	< 0.001**	< 0.001**

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug 'Mean ±

'Mean ± SD in $x10^{-3}$ '(Min - Max) in $x10^{-3}$ **Significant at1%

Table 4. Evaluation of effects of mebendazole and methotrexate combination therapy on MCF-10 cell line viability evaluates by MTT assay.

Doses (µM)		N = 28	Variables				
MBZ	Meth		Ab' Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	0	4	4.2 ± 0.2	0.485 ± 0.013	0.485±0.013	99.759±0.091	0.0024±0.0009
			(4.0-4.5)	(0.468-0.0498)	(0.468-0.0498)	(99.646-99.858)	(0.0014-0.0035)
1.5	0.5	4	4.0 ± 0.6	0.483 ± 0.014	0.486±0.0127	99.746±0.087	0.0025±0.0008
			(3.2-4.7)	(0.466-0.497)	(0.470-0.499)	(99.643-99.841)	(0.0016-0.0035)
3.5	1	4	4.4 ± 0.6	0.482±0.012	0.486±0.0138	99.775±0.068	0.0022±0.0007
			(3.7-5.2)	(0.467-0.496)	(0.469-0.501)	(99.69-99.85)	(0.0014-0.0031)
4.5	1.5	4	4.7± 0.5	0.481±0.013	0.485±0.014	99.723±0.072	0.0027±0.007
			(4.2-5.2)	(0.466-0.494)	(0.468-0.501)	(99.641-99.796)	(0.0.002-0.0036)
6.5	2	4	4.0 ± 0.4	0.478±0.013	0.483±0.0144	99.683±0.080	0.0032±0.0008
			(3.5-4.5)	(0.463-0.494)	(0.466-0.499)	(99.583-99.764)	(0.0024-0.0042)
7.5	2.5	4	4.2 ± 0.4	0.476±0.0124	0.482±0.013	99.653±0.079	0.0034±0.0007
			(3.7-4.7)	(0.462-0.491)	(0.465-0.496	(99.557-99.737)	(0.0026-0.0004)
9.5	3	4	4.2 ± 0.4	0.476±0.012	0.481±0.014	99.634±0.082	0.0036±0.0008
			(3.7-4.7)	(0.461-0.490)	(0.464-0.495)	(99.538-99.72)	(0.0028-0.0046)
p-value			0.585	0.889	0.961	0.183	0.183

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug

'Mean ± SD in $x10^{-3}$ '(Min - Max) in $x10^{-3}$

Table 5. Association of CI values of mebendazole combination therapy among all treated cells.

Cell lines N=5	CDI Mean ± SD	p-value
MCF-7	0.8072±0.06	0.019*
	(0.73-0.87)	
MDA-MB-231	0.7240±0.037	
	(0.68-0.76)	
HT-29 human colorectal adenocarcinoma cell line	0.9565±0.023	
	(0.92-0.97)	
Hela cell line	1.0197±0.049	
	(0.98-1.08)	
MCF-10	0.8895±0.479	
	(0.26-1.42)	

Mean ± SD
(Min - Max)
*Significant at 1%

However, combination therapy of mebendazole and methotrexate revealed statistically non-significant effects on % viability of MCF-10 cell line (χ^2 (2) = 8.830, p=0.183) with average percentage decrease was about -0.126. The mean CDI index for mebendazole and methotrexate showed slight synergism as their values lies between 0.7-0.9 with mean CDI of MCF-7 was 0.8072±0.06 and MDA-MB-231 was 0.7240±0.037 as depicted in Table **4-6**.

Table 6. Evaluation of TBDEA among different doses of combination therapy of mebendazole.

Doses(µM)		N	Viable Cells Mean ± SD	Total Cells Mean ± SD	Viability (%) Mean ± SD	Death cells Mean ± SD
MBZ	Methx					
0	0	3	258.268±2.259	263.273±1.609	98.66±0.513	5.005±0.758
			(255.68-259.85)	(261.42-264.325)	(98.1-99.1)	(4.225-5.74)
1.5	0.5	3	240.91±2.478	262.686±1.586	91.695±0.763	21.776±2.101
			(239.18-243.75)	(260.86-263.725)	(90.95-92.475)	(19.725-23.925)
3.5	1	3	222.06±3.462	262.126±1.604	84.701±1.264	40.066±3.399
			(219.5-226.0)	(260.28-263.175)	(83.4-85.925)	(36.925-43.675)
4.5	1.5	3	201.342±2.95	261.578±1.58	76.936±1.23	60.236±3.331
			(198.575-204.452)	(259.76-262-625)	(75.55-77.9)	(57.897-64.05)
6.5	2	3	179.495±1.16	261.001±1.581	68.768±0.715	81.506±2.429
			(178.15-180.17)	(259.18-262.025)	(68.05-69.48)	(79.02-83.875)
7.5	2.5	3	157.163±1.167	260.46±1.637	60.335±0.823	103.296±2.741
			(156.15-158.44)	(258.58-261.57)	(59.775-61.28)	(100.14-105.075)
9.3	3	3	135.65±0.878	259.873±1.61	52.166±0.639	124.216±2.476
			(134.9-136.62)	(258.02-260.95)	(51.725-52.9)	(121.40-126.05)
P-value			0.003**	0.228	0.003**	0.003**

N=4 samples per day for each dose for 3 days so N=12 but Data analysis were done after entering mean value for each dose for each day so N became 3 for each dose and Total N=21 for individual drug
'Mean ± SD in x 105 '(Min - Max) in x 105
**Significant at 1% ; *Significant at 5%

DISCUSSION

Breast tumor is one of the most common public health problems universally especially in term of diagnosis and treatment. The main drawback of conventionally available anticancerous therapy against breast cancer was poor compliance of patients mostly due to their side effects and economic burden to patient [16].

Mebendazole was a popular anthelmintic specialist yet now look into demonstrated that it can likewise diminish the feasibility of tumor cells by exasperating the microtubule or tubulin polymerization and inhibits the growth cycle at G2/M. It can likewise boast up cellular apoptosis by phosphorylation or constraining the apoptosis inhibiting protein, subsequently repressing their associations with apoptosis promoting protein Bax. Along these lines causing continuous impacts of proapoptotic proteins and advancing cell apoptosis [17].

In our study combination therapy of mebendazole and methotrexate showed synergistic effects against breast cancer cell lines as indicated by CDI values which were 0.807±0.06 and 0.724±0.037 for MCF-7 and MDA-MB-231 cell lines in that order. These outcomes were in line with the study conducted by Coyne et al. (2014) [18]. As they demonstrated that combination therapy of mebendazole with Gemcitabine (option for resistant breast carcinoma) more effectively reducing the viabilities of chemoresistant breast carcinoma (HER2/neu bearing tumor) as compare to alone therapy of either.

This was further supported by Spagnuolo et al. (2010) [19], as they revealed that flubendazole (benimidazole derivative just like mebendazole) in addition to direct cytotoxic effects on cancerous cells can also diminish the resistance of adjuvant chemotherapeutic agent by declining the expression of P-glycoprotein (which is responsible for resistance to most of the chemotherapeutic drugs by acting as an efflux pump).

However, mebendazole both as alone and in combination inept to hindering the viability of cells line model of normal breast epithelial cells MCF-10 (χ^2 (2) = 8.830, p=0.183). This shows that mebendazole combination therapy with methotrexate unable to constrain the growth of normal epithelial cells, this finding was coherent with the study directed by Bai et al. (2011) [20] in xenograft model of Glioblastoma Multiforme.

Thus combination therapy of benzimidazole derivatives can enhance the cytotoxic effects of conventional chemotherapeutic agent for both chemosensitive and chemoresistance mammary carcinomas. For this purpose nowadays the most debatable combination therapies of benzimidazoles are vinblastine/benzimidazole and mebendazole/gemcitabine [21].

CONCLUSION

In search of effective economical and safer cytotoxic agent against breast cancer mebendazole would be a better addition as an adjuvant therapy with conventional chemotherapeutic agent. After this trial mebendazole came in limelight or hold a strong place in field of chemotherapy.

REFERENCES

1. Mukhtar E, Adhami VM, Mukhtar H. Targeting microtubules by natural agents for cancer therapy. Mol Cancer Ther. 2014; 13(2):275-84.
2. Chandrasekaran G, Tatrai P, Gergely F. Hitting the brakes: targeting microtubule motors in cancer. Br J Cancer. 2015; 113(5):693-8.
3. Panic G, Duthaler U, Speich B, Keiser J. Repurposing drugs for the treatment and control of helminth infections. Int J Parasitol Drugs Drug Resist. 2014; 4(3):185-200.
4. Stanton RA, Gernert KM, Nettles JH and Aneja R. Drugs that target dynamic microtubules: a new molecular perspective. Med Res Rev. 2011; 31(3):443-81.
5. Aleyasin H, Karuppagounder SS, Kumar A, Sleiman S, Basso M, Ma T et al. Antihelminthic Benzimidazoles Are Novel HIF Activators That Prevent Oxidative Neuronal Death via Binding to Tubulin. Antioxid Redox Signal. 2015; 22(2): 121–34.
6. Doudican NA, Byron SA, Pollock PM and Orlow SJ. XIAP downregulation accompanies mebendazole growth inhibition in melanoma xenografts. Anticancer Drugs. 2013;24 (2):181-8.
7. Tan SK, Jermakowicz A, Mookhtiar AK, Nemeroff CB, Schürer SC, Ayad NG. Drug Repositioning in Glioblastoma: A Pathway Perspective. Front Pharmacol. 2018; 9:218-37
8. Bai RY, Staedtke V, Aprhys CM, Gallia GL, Riggins GJ. Antiparasitic mebendazole shows survival benefit in 2 preclinical models of glioblastoma multiforme. Neuro Oncol. 2011; 13(9):974-82.
9. Grigoriadis A, Mackay A, Noel E, Wu PJ, Natrajan R, Frankum J, Reis-Filho JS, Tutt A. Molecular characterisation of cell line models for triple-

negative breast cancers. BMC genomics. 2012;13(1):619-33.

10. Holliday DL and Speirs V. Choosing the right cell line for breast cancer research. Breast Cancer Res.2011; 13(4):215-22.
11. Florento L, Matias R, Tuaño E, Santiago K, Dela Cruz F and Tuazon A. Comparison of Cytotoxic Activity of Anticancer Drugs against Various Human Tumor Cell Lines Using In Vitro Cell-Based Approach. Int J Biomed Sci. 2012; 8(1):76-80.
12. Sliwka L, Wiktorska K, Suchocki P, Milczarek M, Mielczarek S, Lubelska K et al. The Comparison of MTT and CVS Assays for the Assessment of Anticancer Agent Interactions. PLoS One. 2016; 11(5): e0155772.
13. Paramee S, Sookkhee S, Sakonwasun C, Na Takuathung M, Mungkornasawakul P, Nimlamool W, Potikanond S. Anti-cancer effects of Kaempferia parviflora on ovarian cancer SKOV3 cells. BMC Complement Altern Med. 2018; 18(1):178-91.
14. Foucquier J and Guedj M. Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect. 2015;3(3): e00149.
15. Reynolds CP and Maurer BJ. Evaluating response to antineoplastic drug combinations in tissue culture models.Methods Mol Med. 2005; 110:173-83.
16. Badisa RB, Darling-Reed SF, Joseph P, Cooperwood JS, Latinwo LM and Goodman CB. Selective Cytotoxic Activities of Two Novel Synthetic Drugs on Human Breast Carcinoma MCF-7 Cells. Anticancer Res. 2009; 29(8):2993-6.
17. Nygren P, Fryknas M, Agerup B and Larsson R. Repositioning of the anthelmintic drug mebendazole for the treatment for colon cancer. J Cancer Res Clin Oncol. 2013; 139(12):2133-40.
18. Coyne CP, Jones T and Bear R. Gemcitabine-(C4-amide)-[anti-HER2/neu] Anti-Neoplastic Cytotoxicity in Dual Combination with mebendazole against Chemotherapeutic-Resistant Mammary Adeno-carcinoma. J Clin Exp Oncol. 2013; 2(2): 100-9.
19. Spagnuolo PA, Hu J, Hurren R, Wang X, Gronda M, Sukhai MA et al. The antihelmintic flubendazole inhibits microtubule function through a mechanism distinct from Vinca alkaloids and displays preclinical activity in leukemia and myeloma. Blood. 2010; 115(23):4824-33.
20. Pantziarka P, Bouche G, Meheus L, Sukhatme V and Sukhatme VP. Repurposing Drugs in Oncology (ReDO)-mebendazole as an anti-cancer agent. Ecancermedicalscience. 2014; 8(1):443-59.
21. Zhang F, Li Y, Zhang H, Huang E, Gao L, Luo W et al. Anthelmintic mebendazole enhances cisplatin's effect on suppressing cell proliferation and promotes differentiation of head and neck squamous cell carcinoma (HNSCC). Oncotarget. 2017; 8(8): 12968-82.