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The anthelmintic drug mebendazole inhibits growth, migration and invasion in gastric cancer cell model

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ABSTRACT

The present study aimed to investigate the effects of MBZ on a human malignant ascites cell line derived from a primary gastric cancer tumor. Our data reveal that MBZ showed high cytotoxicity in vitro, displaying an IC_{50} of 0.39 μM and 1.25 μM in ACP-02 and ACP-03, respectively. The association between MBZ and 5-FU increased slightly the cytotoxicity when compared to MBZ and 5-FU alone. Furthermore, MBZ disrupted the microtubule structure of AGP-01 cells and inhibited significantly the invasion and migration of these cells. Activity of active MMP-2 significantly decreased at all tested concentration of MBZ compared to negative control. These results support the indication of MBZ in combination with chemotherapeutic agents as a possible adjuvant therapy for the management/treatment of patients with advanced gastric cancer since MBZ is a drug of low cost with acceptable safety profile and reduced toxicity to normal cells. However, clinical trials must be performed in o to evaluate its efficacy in gastric cancer patients.

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1. Introduction

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer-related death in the world (Jemal et al., 2011). Two thirds of patients with gastric cancer have locally advanced or metastatic disease at diagnosis, generally owing to the absence of specific symptoms that renders early diagnosis of this disease difficulty (Catalano et al., 2005). Up to one half of patients have recurrent disease after curative surgery, and the outcomes for these patients are still very poor, with an estimated 5-year survival rate in only 20% of the patients (Hohenberger and Gretschel, 2003; Kamangar et al., 2006; Yoong et al., 2011).

Peritoneal carcinomatosis (PC) or malignant ascites (MA) is defined by the National Cancer Institute (NCI) from U.S. as "a condition in which fluid containing cancer cells collects in the abdomen" and represents an advanced state of several types of cancer, especially in gastric cancer (Chung and Kozuch, 2008). There is a lack of randomized controlled trials identifying the most favorable therapy, mainly because PC is highly resistant to the current therapeutics in clinics, and therefore evidence-based therapeutic guidelines have not been established yet (Sugiyama et al., 2011). Taking all together, there is an urgent need to introduce new therapeutic agents in the management of peritoneal carcinomatosis.

Mebendazole (MBZ), methyl N-[6-(benzoyl)-1H-benzimidazol-2-yl] carbamate, an approved drug by the US Food and Drug Administration (FDA), is available for treating roundworm (*Ascaris lumbricoides*), common hookworm (*Ancylostoma duodenale*), American hookworm (*Necator americanus*), pinworm (*Enterobius vermicularis*), and whipworm (*Trichuris trichiura*) in humans and animals, acting by depolymerizing tubulin and thus disrupting the functions of microtubules (El-On, 2003; Messaritakis et al., 1991; Munst et al., 1980; Vutova et al., 1999). Moreover, MBZ has been proved to inhibit the growth of different cancer cells in vitro and in vivo (Bai et al., 2011; Doudican et al., 2008; Martarelli et al., 2008; Mukhopadhyay et al., 2002). The mechanism of action for MBZ and other benzimidazoles is to bind to the tubulin subunits in the gut epithelium of the parasite, preventing polymerization of the tubulin, causing ultrastructural changes, and eventually preventing parasite growth (Kohler, 2001; MacDonald et al., 2004). Tubulin is vital to cell division and is also a cancer target for several chemotherapy drugs, including paclitaxel, colchicine, and vincristine (Bai et al., 2011).

Based upon these findings and the clinically proved safety of MBZ (Janssen Inc., 2014; Lacey, 1988; Sajid et al., 2006; Van der Westhuizen et al., 1984) this work analyzed the effects of MBZ on a human malignant ascites cell line derived from a primary gastric cancer tumor.

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2. Material and methods

2.1. Ethics statement

All samples were derived with written informed consent and approval from the University Hospital (Belém, Pará, Brazil) ethical review boards (protocol number: 142004).

2.2. Gastric cancer cells (GC)

Our group established and characterized cytogenetically three new GC cell lines obtained from primary gastric adenocarcinoma (ACP-02, diffuse type and ACP-03, intestinal type) from two different patients and an ascitic fluid (AGP-01, malignant ascites) from a patient with primary tumor on stomach (Intestinal-type adenocarcinoma), each of which exhibited a composite karyotype with several clonal chromosome alterations similar to the respective primary tumor from the stomach as described previously (Leal et al., 2009). All cell lines were maintained in DMEM medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C with 5% CO.

2.3. Chemicals

MBZ (Medley® 500 mg, source 81G6I5V05I) was dissolved in DMSO to a concentration of 10 mg/mL and stored at −20 °C as a master stock solution. Paclitaxel (6 mg/mL) was purchased from Bristol-Myers Squibb Company (Princeton, NJ), 5-fluororacil (2.5 mg/mL), oxaliplatin (5 mg/mL), gencitabin (40 mg/mL) and irinotecan (20 mg/mL) were purchased from Genlibbs®, doxorubicin (100 μg/mL) and cisplatin (5 mg/mL) were purchased from Sigma®, and etoposide (20 mg/mL) was purchased from Pfizer®.

2.4. Cytotoxicity against cancer cell lines

MBZ (0.15–20 μM) were tested for cytotoxic activity against three cancer cell lines: ACP-02 (diffuse-type adenocarcinoma), ACP-03 (intestinal-type adenocarcinoma) and AGP-01 (malignant ascites). Each compound was dissolved with DMSO and diluted with media to obtain a concentration of 20 μM. They were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.005% in the highest concentration). Doxorubicin was used as a positive control of cytotoxicity in MTT assay. The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product after 72 h as described previously (Mosmann, 1983). Drug effect was quantified as the percentage of control absorbance of reduced dye at 550 nm.

The following experiments were performed in AGP-01 cell line and the concentrations used for MBZ (0.1 μM, 0.5 μM and 1.0 μM) were based on its IC_{50} after 72 h.

2.5. Immunofluorescence staining

Cells (5 × 10^3/well) were grown on a glass chamber slide and treated with 0.005% of DMSO, 0.1 μM of colchicine, 1.0 μM of paclitaxel, 0.1 μM, 0.5 μM and 1.0 μM of MBZ for 14 h and then fixed for 1 h at room temperature with 4% paraformaldehyde solution as previously described by (Doudican et al., 2008). Paclitaxel (1 μM) and colchicine (0.1 μM) were used as a positive control of tubulin inhibition in immunofluorescence staining. They were then washed with PBS and permeabilized with PBS and Triton 0.2% for 20 min. Cells were first blocked by 5% bovine serum albumin (BSA) in PBS for 30 min at room temperature and incubated with monoclonal mouse anti-α-tubulin antibody (Sigma) with 1% BSA overnight on 4 °C. This was followed by incubation with Alexa Fluor®488 for 1 h. They were then washed 3 times with PBS in between and after the process. After staining, the slides were covered with mounting medium containing DAPI (Roche Diagnostics) and examined on a fluorescence microscope (Olympus, Tokyo, Japan).

2.6. Trypan blue exclusion

Cell doubling time of AGP-01 was also determined by this assay (Louis and Siegel, 2011). Cells were seeded into 24-well plates at a density of 2 × 10^4 cells per well and were incubated for 12 h at 37 °C. After 12 h, aliquots from each well were removed from the cultures in 0 h and after 3 h, 6 h, 12 h, 24 h, 27 h and 30 h of incubation, stained with 0.4% trypan blue and counted in a Neubauer chamber. The staining was used to quantify the number of living cells in the samples. Experiments were performed in triplicate.

Cell viability was also determined by trypan blue assay (Louis and Siegel, 2011). Cells were seeded in a 12-well plates at a density of 4 × 10^4 cells per well. After 24 h, they were treated with MBZ at 0.1 μM, 0.5 μM and 1.0 μM. Aliquots from each well were removed from the cultures after 14 h of treatment to demonstrate that the exposure time of MBZ has no cytotoxicity for the cell invasion assay.

2.7. Wound healing assay

In vitro wound healing assay was performed to evaluate the migration of cells (Liang et al., 2007). Cells were seeded into 12-well plates at a density of 1 × 10^5 cells per well for 24 h at 37 °C. After the growing cell layers had reached confluence, wounds were prepared by a single scratch on the monolayer using a white pipette tip and washed with PBS to remove cell debris. Cells were treated with MBZ at concentrations (0.1 μM, 0.5 μM and 1.0 μM) in triplicate for 14 h. The areas between the edges of the scratches were quantified at different times (0 h, 6 h, 12 h and 24 h) using the Image J program.

2.8. Matrigel invasion assay

In vitro invasion assay was performed using modified Boyden chamber with filter inserts (8-μm pores) for 6-well plates (BD Biosciences, San Jose, CA, USA) (Albini et al., 1987). The upper surfaces of the membranes were coated with Matrigel (BD Biosciences, San Jose, CA, USA) and incubated at 37 °C overnight. Cells were treated with MBZ (0.1 μM, 0.5 μM and 1.0 μM) for 14 h. After, cells (2.5 × 10^5/mL) was added to each well with serum-free culture medium and lower chamber was filled with 2.8 mL of medium containing 10% FBS for 10 h. Cells were washed 3 times in PBS in between and then stained with giemsa. The non-invading cells were removed using a cotton swab and the numbers of invasive cells were then counted in seven random fields of the insert. Experiments were performed in triplicate.

2.9. Gelatin zymography

Cells were seeded into 6-well plates at a density of 2 × 10^5 per well for 24 h at 37 °C. Cells were treated with MBZ (0.1 μM, 0.5 μM and 1.0 μM) with serum-free culture medium for 14 h. HT1080 cells were used as a positive control. Protein concentration was determined by using Bradford Reagent (Serva).

Proteins (25 μg/mL) were separated in gel SDS-PAGE 10% copolymerized with gelatin 10% (Sigma-Aldrich) (Troeberg and Nagase, 2004). Electrophoresis was carried out using the minigel slab apparatus Mini Protean 3 (Biorad) at initial voltage of 90 V in stacking gel, after constant voltage of 100 V, until the dye reached the bottom of the gel. Following electrophoresis, gels were washed 4 times in renaturation buffer (2.5% Triton X-100) for 15 min in an orbital shaker. Then the zymograms were incubated for 18 h at 37 °C in incubation buffer (50 mM M Tris, 10 mM $CaCl_2$, 50 mM ZnCl). Gels were then stained with Coomassie blue and destained with methanol, acetic acid and water (4.5:1:4.5). Areas of enzymatic activity appeared as clear bands

over the dark background. The calculation of inhibition was performed using the area of the band selection and analyzed using Image J program that is capable of quantifying the optical density of each pixel, using the bottom of the gel as a blank (Xueyou and Christine, 2010). Experiments were carried out in triplicates.

2.10. Statistical analyses

Data obtained from different experiments are presented as mean ± SEM from at least three independent experiments in triplicate and evaluated by analysis of variance (ANOVA) followed by Tukey test and Bonferroni test using a significance level of 5%.

3. Results

3.1. MBZ inhibits the proliferation of gastric cancer cells

All gastric cancer cell lines were treated with increasing concentrations of MBZ (0.15–20 μM) and well-known clinical chemotherapeutics for 72 h and analyzed by MTT assay. A significant suppression of cell growth was observed in the presence of MBZ (Table 1).

MBZ was more potent against diffuse type gastric cancer cell line (ACP-02) (IC_{50} 0.39 μM) than the other well-known chemotherapeutic drugs used in clinics as 5-FU (IC_{50} 19.71 μM), oxaliplatin (IC_{50} 8.85 μM), gemcitabine (IC_{50} 7.47 μM), irinotecan (IC_{50} 29.83 μM), paclitaxel (IC_{50} 2.43 μM) cisplatin (IC_{50} 15.82 μM) and doxorubicin (IC_{50} 0.82 μM).

As for intestinal type gastric cancer cell line (ACP-03) the IC_{50} was 1.25 μM, being 3 times more resistant to MBZ than diffuse type gastric cancer cell line (ACP-02). MBZ is still more potent than the current chemotherapeutic drugs used in clinics such as oxaliplatin, irinotecan, etoposide and cisplatin (Table 1).

Malignant ascites cell line (AGP-01) derived from a primary intestinal type gastric cancer, showed IC_{50} of 0.59 μM for MBZ, which is mainly half of the IC_{50} observed for ACP-03 (IC_{50} 1.25 μM) which is a primary intestinal-type gastric cancer cell line. MBZ also presented similar activity to 5-FU (IC_{50} 0.67 μM) and paclitaxel (IC_{50} 0.62 μM) and showed higher activity in AGP-01 cells when compared with oxaliplatin (IC_{50} 4.77 μM), irinotecan (IC_{50} 41.10 μM), etoposide (IC_{50} 10.31 μM) and cisplatin (IC_{50} 17.23 μM). The association between MBZ and 5FU increased slightly the cytotoxicity when compared to MBZ and 5-FU alone. However, they are not statistically different.

The association between MBZ and 5-FU demonstrates a decrease in toxicity (IC_{50} 0.38 μM) (Table 1). Our results showed MBZ as a potential candidate in the chemotherapy regiments for malignant ascites.

3.2. MBZ disrupts the microtubule structure of AGP-01 cells

The effects of MBZ on microtubule formation in AGP-01 cells were directly assessed through immunofluorescence microscopy (Fig. 1). After treatment with MBZ 0.1, 0.5 and 1.0 μM for 14 h, we observed overall microtubular network disarray characterized by diffuse staining (Fig. 1D, E and F). Paclitaxel and colchicine were used as a positive control, showing a similar effect (Fig. 1B and C). In contrast, Fig. 1A shows that vehicle-treated malignant ascites cells display discrete networks of microtubule structure.

3.3. MBZ suppresses the migration and invasion of AGP-01 cells

We first ensured that the inhibition of invasion was not due to cell death. Thus, the viability of AGP-01 cells was assessed after 14 h of treatment with 0.1, 0.5 and 1.0 μM of MBZ. The results indicated that MBZ did not alter the viability of AGP-01 cells in all tested concentrations after 14 h of treatment (Fig. 2E). Thus, MBZ significantly inhibited invasion at concentration of 0.1 μM ($p < 0.0005$), 0.5 μM and 1.0 μM ($p < 0.0001$) (Fig. 2F), as shown in cells untreated (Fig. 2A) or treated with MBZ 0.1 μM (Fig. 2B), 0.5 μM (Fig. 2C), 1.0 μM (Fig. 2D).

These findings were further confirmed by the wound healing assay. For this, we previously established cell-doubling time to avoid proliferation interference. In this assay, viable cells were quantified with trypan blue dye at the times 0 h, 3 h, 6 h, 12 h, 24 h, 27 h and 30 h and observed that the cells doubled approximately after 24 h (Fig. 3A). Thus, MBZ significantly decreased migration at 0.5 μM ($p < 0.01$) and 1.0 μM ($p < 0.05$) after 12 h of treatment and at 0.1 μM ($p < 0.01$), 0.5 μM ($p < 0.001$) and 1.0 μM ($p < 0.001$) after 24 h of treatment as shown in Fig. 3B and C.

3.4. MBZ decreases metalloproteinase 2 activity in AGP-01

Zymography was performed to determine MMP-2 and MMP-9 activities in all tested concentrations of MBZ after 14 h of treatment (Fig. 4A). As shown in Fig. 4B and C, no inhibition was observed for MMP-9 inactive and MMP-9 active in all tested concentrations of MBZ compared

Table 1
In vitro cytotoxic activity of MBZ. IC_{50} values (μM) and confidence interval of 95% obtained by the MTT assay in gastric cancer cell lines after 72 of exposure. Experiments were performed in three independent experiments in triplicate.

IC_{50} (μM)			
	ACP-02	ACP-03	AGP-01
Mebendazole	0.39 (0.26–0.59)	1.25 (1.04–1.51)	0.59 (0.44–0.79)
5-Fluororacil	19.71 (15.68–24.78)	0.38 (0.30–0.49)	0.67 (0.53–0.86)
Oxaliplatin	8.85 (6.40–12.22)	1.88 (1.58–2.23)	4.77 (4.18–5.44)
Gemcitabine	7.47 (5.05–11.04)	0.21 (0.19–0.22)	0.02 (0.003–0.16)
Irinotecan	29.83 (25.26–35.23)	32.07 (26.73–38.47)	41.10 (31.06–54.38)
Etoposide	0.20 (0.10–0.38)	18.47 (14.29–23.87)	10.31 (8.01–13.27)
Paclitaxel	2.43 (2.24–2.64)	0.99 (0.64–1.55)	0.62 (0.51–0.76)
Cisplatin	15.82 (12.78–19.58)	28.62 (23.43–34.99)	17.23 (12.69–23.38)
Doxorubicin	0.82 (0.63–1.07)	0.23 (0.18–0.29)	0.25 (0.19–0.33)
Mebendazole + 5FU	ND	ND	0.38 (0.24–0.62)

ND: not determined; 5-FU: 5-fluororacil.

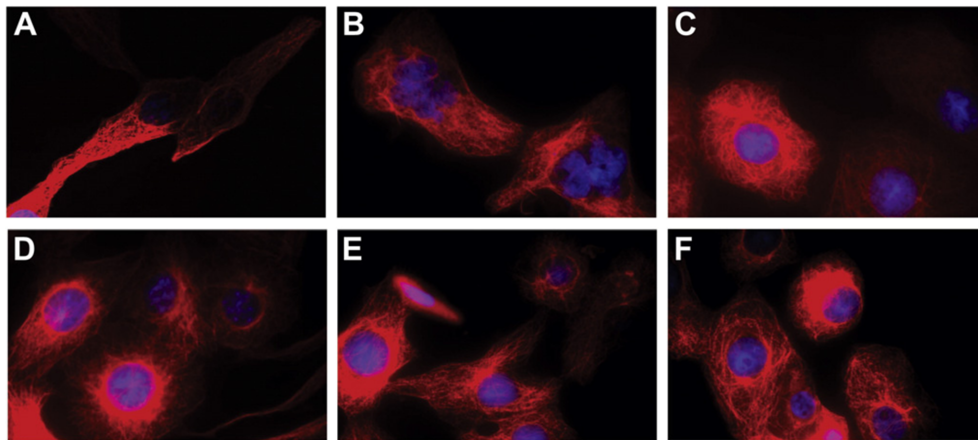

Fig. 1. Mebendazole (MBZ) induces disruption of microtubules in AGP-01 cells. Cells were untreated (A) or treated with paclitaxel 1 μM (B), colchicine 0.1 μM (C) and MBZ 0.1 μM (D), 0.5 μM (E), and 1.0 μM (F) after 14 h of treatment and examined by fluorescent microscopy (40×). Analyses were performed in three independent experiments with two biological replicates.

to negative control. On the contrary, MBZ significantly decreased activity of MMP-2 active in all tested concentrations compared to negative control ($p < 0.0001$) (Fig. 4D). HT1080 was used as a positive control for metalloproteinase activity.

4. Discussion

Herein, we described MBZ as a potentially effective agent in the treatment/management of malignant ascites tumors derived from gastric cancer. In recent years, gastric cancer patients have been treated with 5-FU/cisplatin, however additional drugs were introduced in chemotherapy regimens such as oxaliplatin, irinotecan and taxanes. The results of phase II studies showed a promising efficacy and manageable toxicity profile for combination chemotherapy, including these new substances (Al-Batran et al., 2004; Bouche et al., 2004; Chao et al., 2004).

In phase III study, Kim et al. (1993) reported an overall response rate of 51% and an overall survival with a median of 9.0 months for regimen 5-FU/cisplatin in gastric in previously untreated patients with advanced gastric cancer. First-line chemotherapy with paclitaxel/cisplatin also showed response rates of 44–46% and a median overall survival of 11.2 to 13.8 months (Kornek et al., 2002; Park et al., 2004; Lee et al., 2005). Efforts to improve the treatment of cancer have been a major problem being a challenge to search for new chemotherapeutic agents and combinations in an attempt to reduce acute toxicity and treatment failure.

Our study demonstrated an increase in activity when MBZ is associated to 5-FU in malignant ascites cell lines (AGP-01 cells). The toxicity of 5-FU is a serious and common problem for many cancer patients mainly

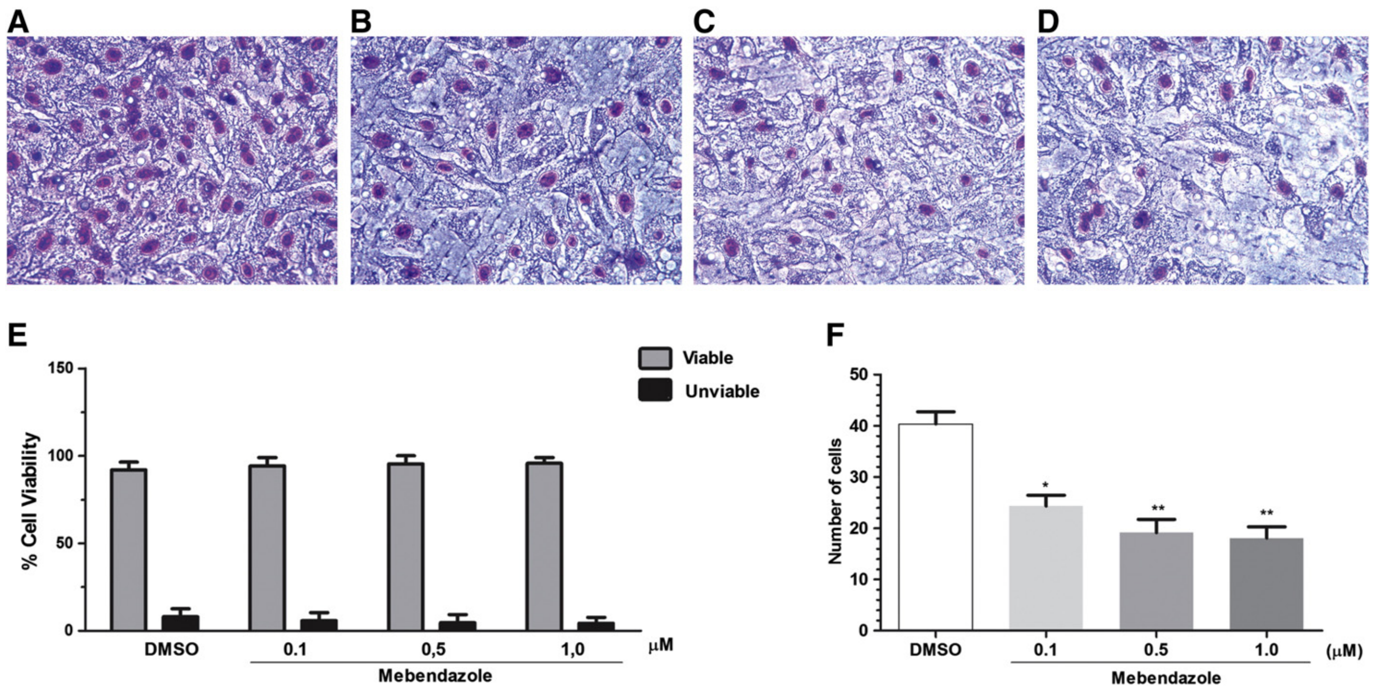

Fig. 2. Mebendazole (MBZ) significantly inhibited invasion in AGP-01 cells. Cells were untreated (A) or treated with MBZ 0.1 μM (B), 0.5 μM (C), and 1.0 μM (D) in matrigel invasion assay. AGP-01 cells viability after 14 h of treatment with MBZ in trypan blue assay. The bars represent the mean ± standard error of mean of three independent experiments in triplicate (E). Cell invasion assay after 14 h of treatment with MBZ in three concentrations. The bars represent the mean ± standard error of mean of three independent experiments in triplicate (F). *$p < 0.0005$, **$p < 0.0001$ compared with the negative control by ANOVA followed by Tukey test.

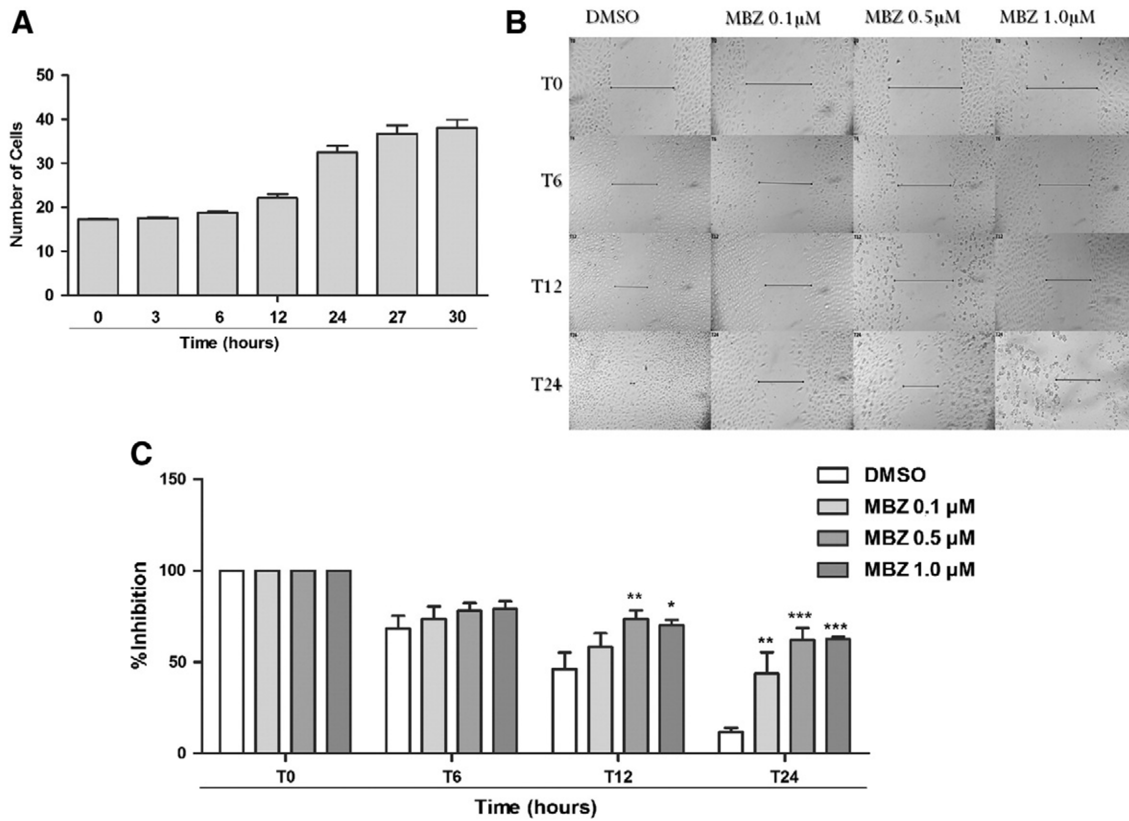

Fig. 3. Mebendazole (MBZ) significantly decreased migration in AGP-01 cells. Cell doubling time of AGP-01 cells monitored for 30 h. The bars represent the mean ± standard error of mean of three independent experiments (A). Microphotograph of cell migration assay after 24 h of treatment in three concentrations studied showing the areas of scratches (B). Values calculated of the areas of scratches cell migration assay after treatment with different concentrations of the MBZ. The bars represent the mean ± standard error of mean of three independent experiments in triplicate (C) *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ compared to negative control by 2-way ANOVA followed by Bonferroni test.

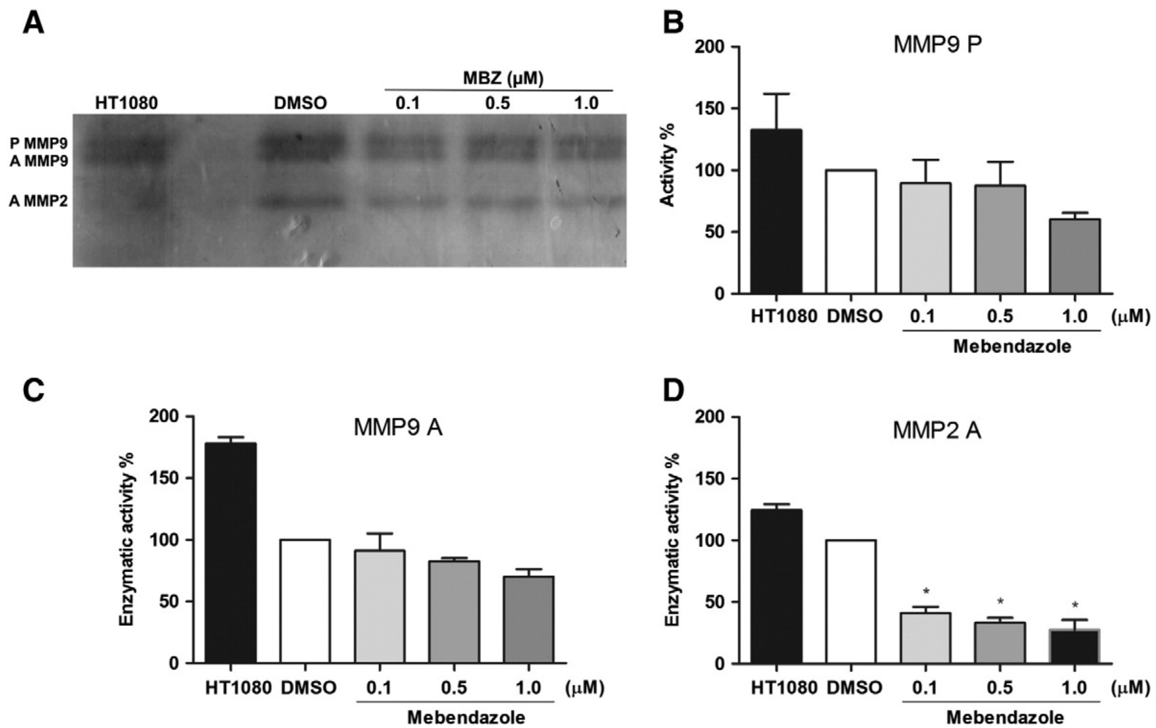

Fig. 4. Mebendazole (MBZ) significantly decreased activity of MMP-2 active. Gel zymography of AGP-01 cells treated with MBZ concentrations 0.1 μM, 0.5 μM, and 1.0 μM (A). Inactive MMP9 (B). Active MMP9 activity (C). Active MMP2 activity (D). Data was calculated from densitometry of the bands in the program Image J. Bars represent the mean ± standard error of three independent experiments in triplicate. *$p < 0.0001$ compared with the negative control by ANOVA followed by Tukey test. HT1080 is used as a positive control. P: inactive. A: active.

due to myelosuppression and gastrointestinal toxicity being the most commonly observed side effects (Saif et al., 2009). The clinical activity of 5-FU is modest at normal used doses and, in general, treatment is limited by safety profile. Various strategies have been developed to increase clinical efficacy/safety of 5-FU, such as the biochemical modulation (Leichman et al., 2002), changes in management schemes (Levi et al., 1994) and the use of combination therapy (Aquino et al., 1998; Melen-Mucha et al., 2004; Obi et al., 2006). In contrast, MBZ is orally available and exhibits favorable pharmacokinetics, suggesting that this drug deserves further investigation as anticancer agents because of their potential in improving the efficacy and remarkable safety profile to other drugs (Doudican et al., 2008).

This study demonstrates the anticancer effects of MBZ in malignant ascites and agrees with previously reported studies in other types of cancer cell lines (Bai et al., 2011; Doudican et al., 2008; Martarelli et al., 2008; Mukhopadhyay et al., 2002). MBZ has shown preclinical anticancer activity in vivo and in vitro in glioblastoma multiforme (IC_{50} ~ 0.1 μM) (Bai et al., 2011), melanoma (IC_{50} = 0.32 μM) (Doudican et al., 2008), adrenocortical carcinoma (IC_{50} = 0.23 μM) (Martarelli et al., 2008), and lung cancer (IC_{50} = 0.16 μM) (Mukhopadhyay et al., 2002). However, this is the first study showing the cytotoxic effects of MBZ in gastric cancer cell lines and in malignant ascites cells derived from a primary gastric adenocarcinoma showing an IC_{50} lower than 1 μM.

Microtubules are widely recognized as effective targets for anticancer therapy. As key components of the cytoskeleton, microtubules are essential for cellular structure, intracellular transport, mitosis, and cell division (Nogales, 2001). Several studies demonstrated cytoskeletal components are involved in adhesive interactions during formation of distant tumor metastasis (Haier et al., 1999; Kadi et al., 1998; Korb et al., 2004).

Microtubule-targeted drugs such as paclitaxel and Vinca alkaloids exert their inhibitory effect on cancer cell proliferation primarily by disrupting the mitotic spindle, causing cell cycle arrest at the metaphase–anaphase transition and inducing apoptosis (Jordan et al., 1996; Yvon et al., 1999). The disruption of the balance between the monomers/dimers and polymers of tubulin microtubules caused by microtubule stabilizing agents (such as paclitaxel, docetaxel), or microtubule destabilizing agents (e.g., vinblastine, vincristine, nocodazole, colchicine) activates signaling cascades of stress-activated protein kinases. Such disruption of microtubules is associated with the phase lock G2-M (Bhalla et al., 1993; Horwitz, 1992; Long and Fairchild, 1994).

Our study demonstrated a disruption of microtubule structure in AGP-01 cells treated with MBZ and the same results were observed in glioblastoma multiforme cells (Bai et al., 2011) and in melanoma cells (Doudican et al., 2008).

The progression of malignant cells results in the invasion and metastasis processes, which are directly involved in poor prognosis. The development of peritoneal metastasis is a multistep process, beginning with the detachment of cancer cells from the primary tumors, their attachment to peritoneal mesothelial cells, retraction of the mesothelial cells, and exposure of the basement membrane. After attachment to the basement membrane, the cancer cells degrade the extracellular matrix and proliferate (Yonemura et al., 1996). Finally, the cancer cells induce angiogenesis and lymphangiogenesis. Many cytokines, adhesive factors, growth factors, matrix metalloproteinases (MMPs), and angiogenic factors play important roles in these steps. During this process, several stages are involved as critical event such as cell motility and invasiveness, which involves the interaction of tumor cells with the extracellular matrix (Akiyama et al., 1995).

Degradation of the extracellular matrix is considered to be a prerequisite for metastasis, specially, for peritoneal metastasis, and MMPs are thought to play an important role in this process (Nagase and Woossner, 1999; Mizutani et al., 2000). There are many reports showing that highly invasive cancer cells with a high potential for metastasis stimulate the production of MMPs (Nagase and Woossner, 1999), and MMP-2 is significantly correlated with depth of invasion, lymph node metastasis, and distant metastasis of gastric cancer (Monig et al., 2001). Moreover, cadherin and MMPs are related to the detachment of cancer cells from the gastric wall (Shimoyama and Hirohashi, 1991; McDonnell et al., 1991). MBZ was effective in reducing the activity of MMP-2 in the AGP-01 cells, suggesting its action in inhibiting the invasion of cancer cells in vitro. This is the first study demonstrating the inhibition of matrix metalloproteinases activity after treatment with MBZ.

Agents that can change any of the steps of the metastatic cascade has become potential candidates for anticancer therapy due mainly in metastatic tumors where therapy is still ineffective (Da-Yong et al., 2014). Our study showed that MBZ inhibited migration and invasion in malignant ascites cells, and these results corroborate with Martarelli et al. (2008) that shown that MBZ inhibited adrenocortical carcinoma cells migration and invasion in vitro and metastasis formation in vivo (Martarelli et al., 2008).

In summary, these initial results support the finding that MBZ is effective alone or in combination with chemotherapeutic agents in the management of advanced gastric cancer in vitro. Furthermore, this is a drug of low cost with acceptable safety profile, reduced toxicity to normal cells, and able to reduce the growth and the migration and invasion of cancer cells in vitro and in vivo, however clinical trials must be performed in order to evaluate its efficacy in gastric cancer patients.

Conflict of interest

The authors declare that there are no conflicts of interest.

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