

MGN-3/Biobran, modified arabinoxylan from rice bran, sensitizes human breast cancer cells to chemotherapeutic agent, daunorubicin[☆]

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Accepted 11 February 2008

Abstract

Background: MGN-3/Biobran, a modified form of arabinoxylan from rice bran, is a potent biological response modifier (BRM). Our previous studies demonstrated that MGN-3 sensitizes human leukemia cells to death receptor [CD95]-induced apoptosis [Ghoneum M, Gollapudi S. MGN-3 sensitizes human T cell leukemia cells to death receptor (CD95)-induced apoptosis. *Cancer Lett* 2003;201:41–9]. In this study, we evaluated the chemo-sensitizing activity of MGN-3 against human breast cancer cells (BCCs) *in vitro*. **Methods:** BCCs (MCF-7 and HCC70 cells) were cultured with different concentrations of daunorubicin (DNR) (from 1×10^{-9} to 1×10^{-6} M) in the presence or absence of selected concentrations of MGN-3 (100–1000 $\mu\text{g/ml}$) for 3 days. Cancer cell survival was determined by MTT assay and drug accumulation was determined by flow cytometry. **Results:** Treatment with MGN-3 increased susceptibility of BCCs to DNR (5.5-fold for MCF-7 and 2.5-fold for HCC70 cells) as compared to BCCs treated with DNR alone. The sensitizing effect of MGN-3 was associated with increased accumulation of DNR in cancer cells. **Conclusions:** Our data demonstrate that MGN-3 is an effective chemo-sensitizer and may represent a potential novel adjuvant for the treatment of breast cancer.

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Keywords: Daunorubicin; Flow cytometry; MCF-7; MGN-3; Biological response modifier; Chemosensitizer; Drug sensitivity; Cancer cell survival; Anticancer drugs

1. Introduction

Chemotherapy is considered the cornerstone of treatment for many types of cancer. However, many chemotherapeutic agents exhibit dose-limiting toxicities including: congestive heart failure [1], myelosuppression [2], neutropenia, alopecia, mucositis, diarrhea, myalgias [3,4], thrombocytopenia [5], neurotoxicity, immune-suppression, as well as mutagenic and carcinogenic effects [6–9]. In addition, a majority of cancer patients treated with chemotherapy relapse and die from their disease. It is therefore of particular interest to explore therapeutic approaches that reduce chemotherapeutic-

mediated toxicity. The use of chemotherapeutic agents in conjunction with safe, natural agents that act to incorporate them into less toxic, yet effective, combined-modality treatment may thus provide an answer to this urgent need.

MGN-3 is a natural product that is obtained by reacting rice bran hemicellulose with multiple carbohydrate hydrolyzing enzymes from Shiitake mushrooms. The main chemical structure of MGN-3 is arabinoxylan with a xylose in its main chain and an arabinose polymer in its side chain [10]. Previously, we presented evidence for the role of MGN-3 as a potent activator of human NK cells in healthy subjects [11,12] and in cancer patients [13]. Recently, we have demonstrated that MGN-3 sensitizes human leukemic HUT 78 cells to anti-CD95 antibody-induced apoptosis [14]. In this study, we examined the sensitizing ability of MGN-3 toward the chemotherapeutic agent, daunorubicin (DNR) in human breast cancer cells (BCCs) and examined the possible mechanism underlying its effect.

[☆] Data was partially presented at The Int. Res. Conf. on Food, Nutrition and Cancer, Washington, DC, 15–16 July 2004.

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2. Materials and methods

2.1. Tumor cell lines and culture conditions

Human breast cancer cell lines MCF-7 and HCC70 cells were used in the present study. Cells were purchased from American Tissue and Culture Collection (ATCC), Manassas, VA. Tumor cells were maintained in our laboratory in a complete medium (CM) that consisted of RPMI-1640, supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, and 100 µg/ml streptomycin and penicillin.

2.2. Drugs and chemicals

DNR and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma.

2.3. MGN-3

MGN-3 is a denatured hemicellulose obtained by reacting rice bran hemicellulose with multiple carbohydrate hydrolyzing enzymes from Shiitake mushrooms. The main chemical structure of MGN-3 is arabinoxylan with a xylose in its main chain and an arabinose polymer in its side chain [10]. MGN-3 was dissolved in CM at a concentration of 30 mg/ml. MGN-3 was provided by Daiwa Pharmaceutical Co. Ltd., Tokyo, Japan.

2.4. Drug sensitivity assay

Drug sensitivity was determined using a colorimetric MTT assay. This assay is based on the reduction of tetrazolium salt MTT [3-(4,5-dimethylthiazol-3-yl)-2,5-diphenyltetrazolium bromide] by a mitochondrial dehydrogenase in viable cells from a colorless to a blue-colored formazan product in viable cells that can be measured spectrometrically. The amount of formazan produced is proportional to the number of living cells. Cells (1×10^4 well⁻¹) were seeded in 96-well plates and cultured in triplicate in the presence or absence of various concentrations of MGN-3 (100–1000 µg/ml) and with or without selected concentrations of DNR (1×10^{-9} to 1×10^{-6} M). The final volume of medium in each well after addition of MGN-3 and/or DNR was 200 µl. The cultures were incubated at 37 °C for 3 days, after which 50 µg of MTT was added to each well and the cultures incubated for an additional 4 h. The plates were centrifuged, the medium carefully removed, the formazan crystals solubilized with acid alcohol, and the plates read at 590 nm using an ELISA plate reader (Molecular Devices, Menlo Park, CA). The 50% inhibitory concentration (IC₅₀) was determined as the drug concentration that resulted in a 50% reduction in cell viability. The IC₅₀ were determined by plotting the logarithm of the drug concentration versus the survival rate of the treated cells.

2.5. Daunorubicin accumulation

DNR is a fluorescent compound and its accumulation into cells was studied by flow cytometry as previously described [15]. Briefly, cells were incubated in the presence or absence of 500 µg/ml of MGN-3 at 37 °C for 15 min. DNR (2 µM) was then added to the cells, gently mixed, and incubated at 37 °C for 45 min. Accumulation of DNR was measured by flow cytometry using a FACScan (Becton Dickinson), the fluorescence intensity was recorded from histograms, and the data expressed as mean fluorescence channel (MFC) numbers.

Kinetics of drug uptake in the presence of MGN-3 was carried out. MCF-7 cells were cultured with DNR (2 µM) in the presence or absence of MGN-3 (500 µg/ml). The drug accumulation was examined at 15-min intervals (from 0 to 60 min) by flow cytometry.

2.6. Statistical analysis

Statistical significance was determined by Student's *t*-test. Differences were considered significant at the $p < 0.05$ level.

3. Results

3.1. Effects of MGN-3 on the BCC survival

BCCs were co-cultured with MGN-3 (100–1000 µg/ml) for 3 days. Treatment of MCF-7 cells with MGN-3 resulted in a significant decrease in cell survival that followed dose-dependent fashion. Data in Fig. 1A shows the percentages of MCF-7 cell survival were 75, 70 and 63% post-treatment with MGN-3 at concentrations of 100, 500 and 1000 µg/ml, respectively. On the other hand, response of HCC70 cells to the apoptotic effect of MGN-3 was less remarkable (Fig. 1B).

3.2. Effects of MGN-3 on the sensitivity of BCCs to DNR

BCCs were cultured with DNR at different concentrations (1×10^{-9} to 1×10^{-6} M) in the presence or absence of MGN-3 for 3 days, then cell survival and the IC₅₀ values were determined. DNR, as expected, inhibited the survival of MCF-7 cells in a dose-dependent manner. The IC₅₀ of DNR was 1 µM. However, when MCF-7 cells were co-cultured with MGN-3 and DNR, the IC₅₀ of DNR against MCF-7 cells was significantly reduced (IC₅₀ 0.2 µM). Data in Fig. 2A shows that MGN-3 at concentrations of 100, 500 and 1000 µg/ml decreased the DNR IC₅₀ of MCF-7 cells by 3-, 5- and 5.5-fold, respectively, as compared with DNR alone. MGN-3 also enhanced the sensitivity of HCC70 cells to DNR, but to a lesser extent as compared to MCF-7 cells. Data in Fig. 2B shows that MGN-3 decreased the DNR IC₅₀ of HCC70 cells by 2.5-fold.

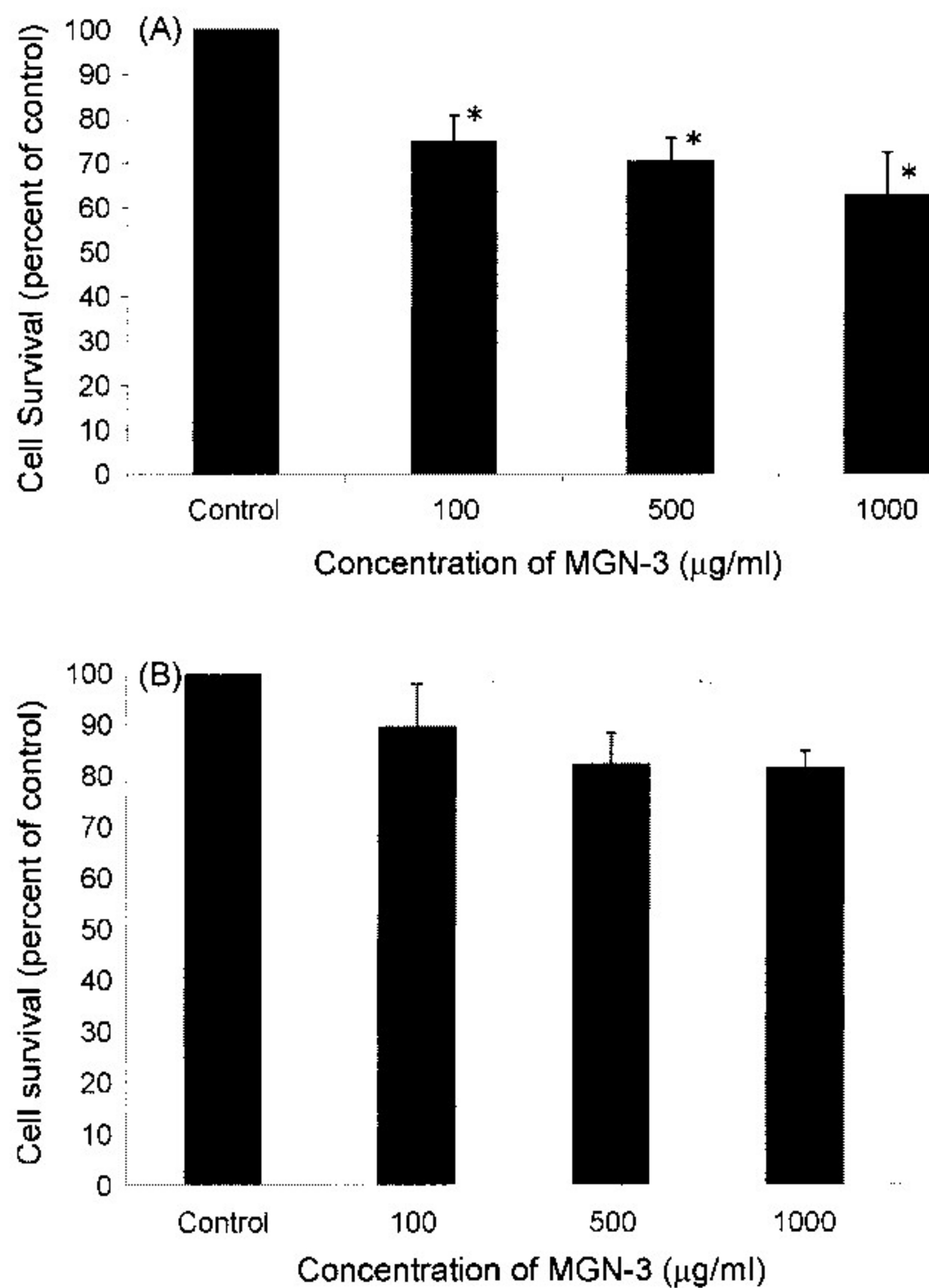


Fig. 1. Effect of MGN alone on survival of breast cancer cells. MCF-7 or HCC70 cells (1×10^4 well $^{-1}$) were seeded in 96-well plates and cultured in triplicate in the presence or absence of various concentrations of MGN-3 (100–1000 mg/ml) for 3 days and survival of cells was determined by MTT assay. Cell survival is shown as a percent of control, untreated cells. (A) *MCF-7 cells*: the data represents the mean \pm S.D. of six experiments, each experiment in triplicate. (B) *HCC70 cells*: the data represents the mean \pm S.D. of three experiments, each experiment in triplicate. * $p < 0.01$ as compared with the control untreated cells.

3.3. Effects of MGN-3 on accumulation of DNR in BCCs

To determine if the observed enhancement of DNR cytotoxicity by MGN-3 is related to alteration in drug transport, we studied accumulation of DNR by flow cytometry. Results show that MGN-3, at a concentration of 500 µg/ml, significantly enhanced the accumulation of DNR in MCF-7 cells (Fig. 3A) and HCC70 cells (Fig. 3B) as compared to control, unlabelled cells. The figures include graphs of unlabelled cells as a control.

3.4. Kinetics of drug uptake in the presence of MGN-3

DNR accumulation in MCF-7 cells was examined in the presence or absence of MGN-3 at 15-min intervals. Fig. 4 shows that a difference in DNR accumulation was detected at 45 min with further increased accumulation at 60 min. The presence of MGN-3 enhanced drug accumulation from a mean fluorescence channel number of 103 to 130.

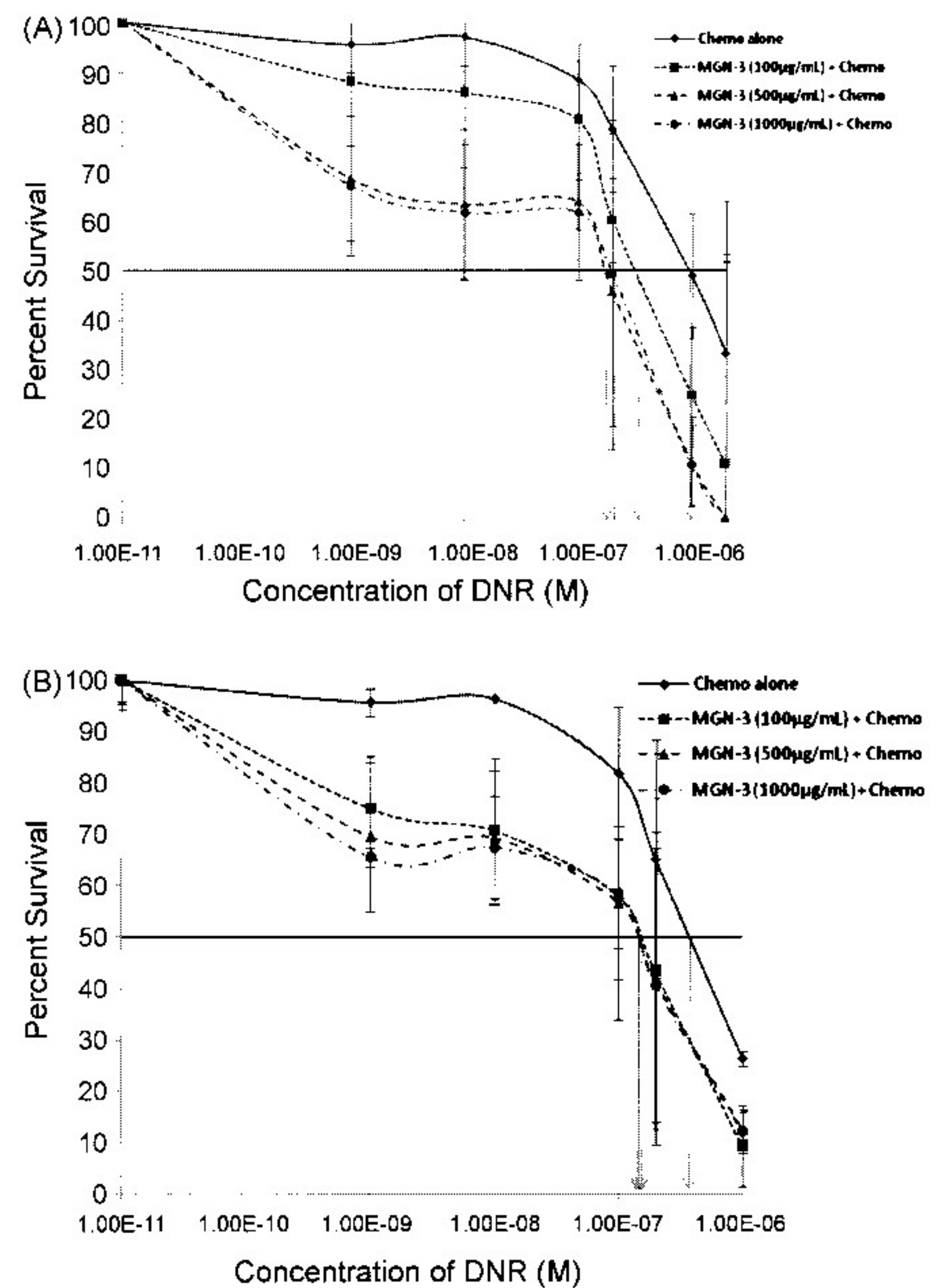


Fig. 2. Effect of MGN-3 on DNR cytotoxicity in BCCs. MCF-7 or HCC70 cells (1×10^4 well $^{-1}$) were seeded in 96-well plates with DNR (1×10^{-9} to 1×10^{-6} M) in the presence or absence of MGN-3 (100–1000 mg/ml) for 3 days. Cell survival was determined using an MTT assay. (A) *MCF-7 cells*: the data represents the mean \pm S.D. of six experiments, each experiment in triplicate. (B) *HCC70 cells*: the data represents the mean \pm S.D. of three experiments, each experiment in triplicate.

4. Discussion

In this study, we examined the sensitizing effects of MGN-3 on human breast cancer cells to the chemotherapeutic agent, daunorubicin. Treatment with MGN-3 significantly increased susceptibility of MCF-7 and HCC70 cells to DNR 5.5- and 2.5-fold, respectively, as compared to cells treated with DNR alone. Earlier studies have shown a potential for MGN-3 in reducing chemo-toxic effects in murine and cancer patients. A beneficial effect of MGN-3 on some adverse actions of anticancer drugs was reported, including protection against severe weight loss in mice due to cisplatin (CIS) [16] and in rats due to CIS and adriamycin (ADR), as demonstrated in some of the gross gastrointestinal pathological changes and in the prevention of death induced by CIS [17]. In addition, results of clinical trials on progressive cancer patients treated with chemotherapy in the presence or absence of MGN-3 have shown that treatment with MGN-3 resulted in a higher survival rate and a marked improvement in the appetite in patients receiving

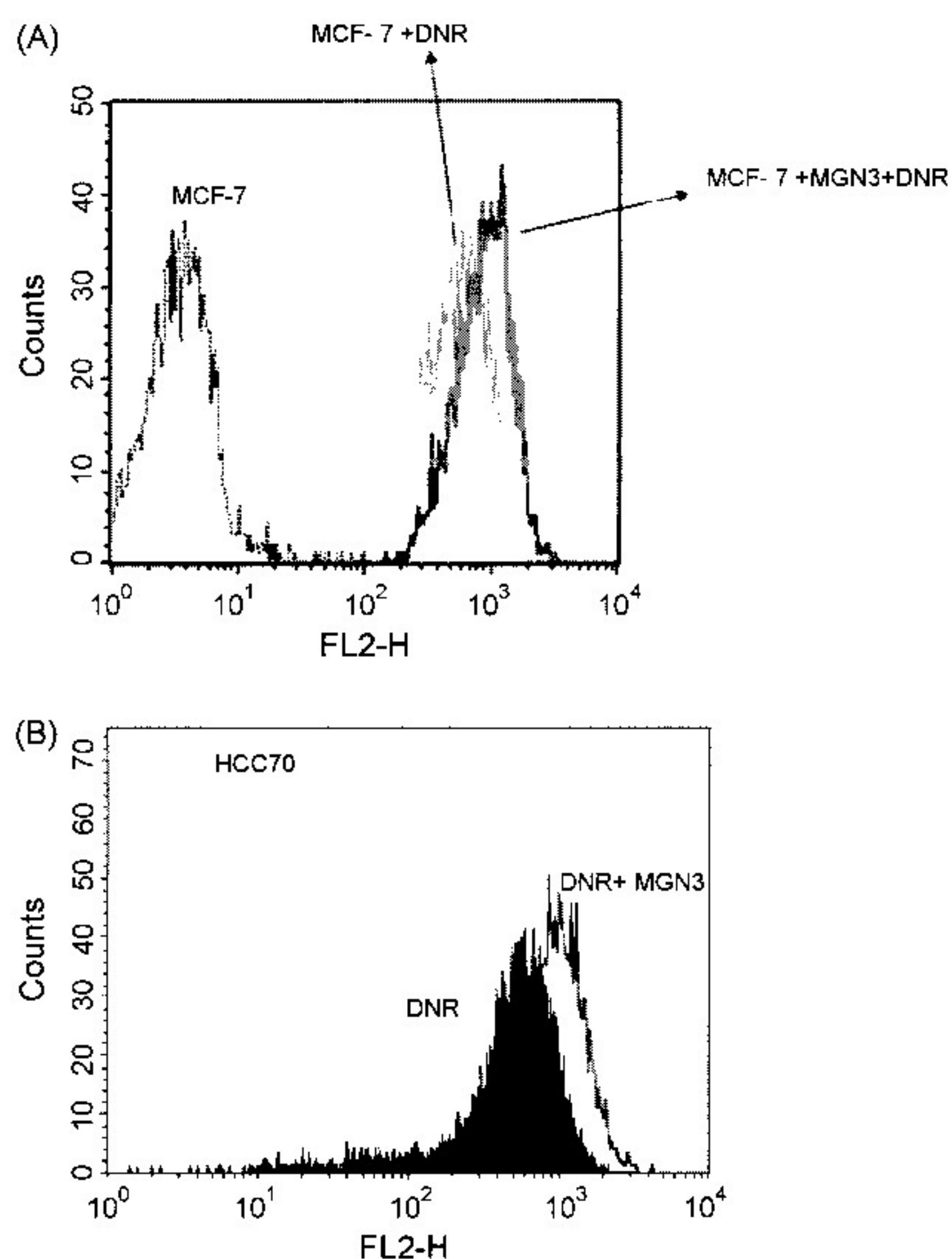


Fig. 3. Effect of MGN-3 on the accumulation of DNR in BCCs. MCF-7 or HCC70 cells (1×10^4) were incubated with DNR ($2 \mu\text{M}$) with or without MGN-3 ($500 \mu\text{g/ml}$) and drug accumulation was assessed using flow cytometry. (A) MCF-7 cells. (B) HCC70 cells.

chemo as compared with patients treated with chemo alone [18].

Previous studies have also shown that other food supplements, such as soy isoflavones and genistein, increase induction of apoptosis of cancer cell lines [19,20] and inhibit the growth of breast tumors in mice when administered in

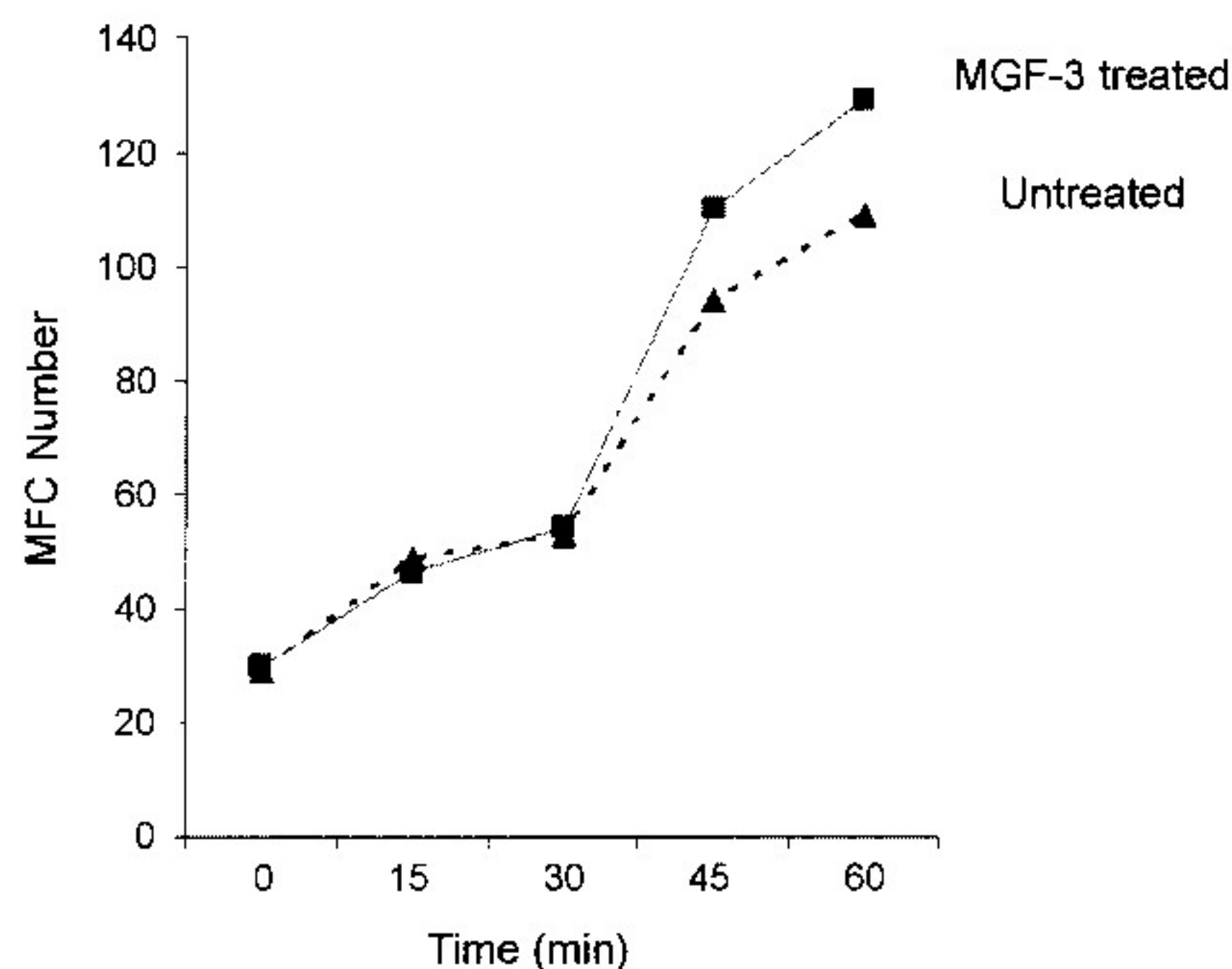


Fig. 4. Kinetics of DNR uptake in the presence of MGN-3. The accumulation of DNR ($2 \mu\text{M}$) by MCF-7 cells was examined in the presence or absence of MGN-3 ($500 \mu\text{g/ml}$) at 15-min intervals (0–60 min) by flow cytometry, and is expressed as mean fluorescence channel (MFC) number.

combination with tamoxifen [21]. In addition *Chlorella vulgaris*, a unicellular green algae belonging to the Phylum Chlorophyta, alleviated some of the side effects of 5-fluorouracil treatment in animal experiments [22], and Vitamin E supplementation has a neuroprotective effect in chemotherapy-induced peripheral nerve damage [23].

In this report, we examined the mechanism by which MGN-3 increases the sensitivity of BCC to DNR in human breast cancer cells. Our results show that treatment with MGN-3 increased accumulation of DNR in both MCF-7 and HCC70 cells. Several agents enhance the cytotoxic effect of chemotherapeutics in cancer cells via increasing intracellular drug accumulation and reverse multidrug resistance (MDR) in cancer cells, including the calcium channel blocker diltiazem and the bisoclaurine alkaloid cepharanthine [24–26], antiarrhythmic agent quinidine [27,28], and synthetic isothiocyanate E-4IB [29]. In addition, nutritional intervention toward tumor responsiveness to chemotherapy has recently been examined. Pardini [30] reported enhancement of tumor responsiveness to chemotherapy by omega-3 polyunsaturated fatty acids and also increases in intracellular drug accumulation. Finally, we showed that MGN-3 reverses multidrug resistance in HL60/Adriamycin-resistant (AR) cells [31].

Research over the last two decades has revealed that many anticancer drugs function by inducing apoptosis [32–35]. We have recently examined the role of MGN-3 in caspase activation. Results showed that treatment with MGN-3 resulted in an increased number of cancer cells having active caspases 8, and 9 (MCF-7) and 3, 8, and 9 (HCC70) [36]. In addition, the sensitizing effect of MGN-3 against human leukemic HUT 78 cells to anti-CD95 antibody-induced apoptosis was also correlated with an increased number of cells having active caspases 3, 8, and 9 [14]. This suggests that MGN-3 sensitizes cancer cells to DNR by a mechanism that involves caspase cascades. Similar findings were recently reported by Bodo et al. [29] whereby increased intracellular platinum accumulation post-treatment with a synthetic isothiocyanate derivative, ethyl 4-isothiocyanatobutanoate, was accompanied by the stimulation of caspase-3 activity [29].

The promise of anti-cancer activity by rice and rice bran derivatives has recently been the focus of much research. MGN-3 is an arabinoxylan extracted from rice bran [10] that has proven to be a potent biological response modifier (BRM) that has the ability to boost the function of different arms of the immune system, such as NK cells [11–13], T and B cells [10], macrophages [37], and the production of TNF- α and IFN- γ [12]. In addition, MGN-3 has demonstrated an additional characteristic as a novel anti-tumor agent able to sensitize: (1) human leukemia cells to death receptor [CD95]-induced apoptosis [14], (2) cancer cells to yeast-induced apoptosis [36], (3) human AR myeloid leukemia cells to ADR treatment [31], and (4) human breast cancer cells to DNR treatment, as shown in the current study. Other rice bran products have demonstrated anti-tumor activity

including polysaccharide RBS [38], lipoprotein fraction [39], and agglutinin (RBA) [40]. In addition, a recent study showed that rice (*Oryza sativa* L.) inhibits the growth of human leukemic U937 cells through the activation of peripheral blood mononuclear cells [41]. We conclude that treatment of human breast cancer cells with MGN-3 significantly sensitizes these cells to the chemotherapeutic agent daunorubicin. These data may suggest that the food supplement MGN-3 in conjunction with chemotherapy may be useful for the treatment of breast cancer.

Acknowledgement

The authors would like to acknowledge Daiwa Pharmaceutical Co. Ltd., Tokyo, Japan, for the financial support of this project.

Conflict of interest

None declared.

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