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# Quercetin, a bioflavonoid, inhibits the increase of human multidrug resistance gene (*MDR1*) expression caused by arsenite

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Expression of the *MDR1* gene, which encodes P-glycoprotein, is increased under some stress conditions. We have reported that quercetin, a bioflavonoid, inhibits the expression of heat-shock proteins. We have identified the effects of quercetin on the *MDR1* gene expression in the human hepatocarcinoma cells line, HepG2. The increase of P-glycoprotein synthesis and *MDR1* mRNA accumulation caused by exposure to arsenite were inhibited by quercetin. The CAT assay suggested that quercetin suppressed the transcriptional activation of the *MDR1* gene after exposure to arsenite. Although many drugs that prevent the P-glycoprotein function have been reported, this is the first report to describe the inhibition of *MDR1* expression by a reagent.

Heat shock; Stress protein; *MDR1*; Multidrug resistance; P-Glycoprotein

## 1. INTRODUCTION

The development of low levels (two- to several-fold) of multidrug resistance in cultured cells is initially accompanied by elevated expression of the *MDR1* gene without gene amplification [1]. The expression of *MDR1* is induced in human renal adenocarcinoma cell lines (HTB-44 and HTB-46) [2] and a human hepatocarcinoma cell line (HepG2) [3] after exposure to some stresses. Because the emergence of cancer cells that have acquired 2- to 3-fold resistance should be a serious problem in chemotherapy, reagents that inhibit the increase of *MDR1* expression might be useful to suppress the emergence of resistant cells.

Quercetin, a bioflavonoid widely distributed in plants, has many biological effects [4–6] and inhibits the synthesis of heat-shock proteins induced by heat shock and other stresses [7]. Quercetin inhibited the induction of *hsp70* at the level of mRNA accumulation through the inhibition of the activation of a heat-shock factor (HSF). In this report we showed that quercetin inhibited the increase of P-glycoprotein synthesis and *MDR1* mRNA in HepG2 cells after exposure to sodium arsenite. The CAT assay analysis indicated that quercetin affected the transcriptional activity of *MDR1*.

*Abbreviations:* HSE, heat shock responsive element; HSF, heat shock factor; CAT, chloramphenicol acetyltransferase.

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## 2. MATERIALS AND METHODS

### 2.1. *Drug addition, metabolic labeling, and immunoprecipitation*

Cells were treated with 100 μM quercetin for 4 h, and then treated with 100 μM sodium arsenite for 4 h or heat shock at 42°C for 2 h in the presence of 100 μM quercetin. After recovering for 2 h in fresh medium containing quercetin, cells were labeled with [$^{35}$S]methionine for 1 h and harvested by adding lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris–HCl, pH 8.0, 5 mM EDTA, 2 mM *N*-ethylmaleimide, 2 mM (*p*-aminophenyl)methanesulfonyl fluoride hydrochloride, 1 μg/ml leupeptin, and 1 μg/ml pepstatin). Cell lysates with the same amount of radioactivity were reacted with the anti-P-glycoprotein monoclonal antibody C219 (Centocor) for 1 h at 4°C. The P-glycoprotein–C219 complexes were precipitated using protein-A-Sepharose. Electrophoresis was done with 7% PAGE as described by Ling et al. [8].

### 2.2. *Slot blot hydridization, and ribonuclease protection*

Slot blot hybridization and ribonuclease protection assay were done as described [3]. Comparable RNA loading was confirmed using a human β-actin probe [9].

### 2.3. *CAT assay*

Cells were exposed to 100 μM quercetin and 100 μM sodium arsenite 4 days after the transfection because DNA transfection and glycerol shock may cause some stresses. Cell extracts that showed the same β-galactosidase activity were used for the CAT assay as described previously [3].

## 3. RESULTS

### 3.1. *Quercetin inhibits the increase of P-glycoprotein synthesis induced by arsenite*

To analyze the effect of quercetin on P-glycoprotein synthesis after exposure to arsenite, HepG2 cells were treated with arsenite and quercetin and cell extracts were immunoprecipitated using the anti-P-glycoprotein

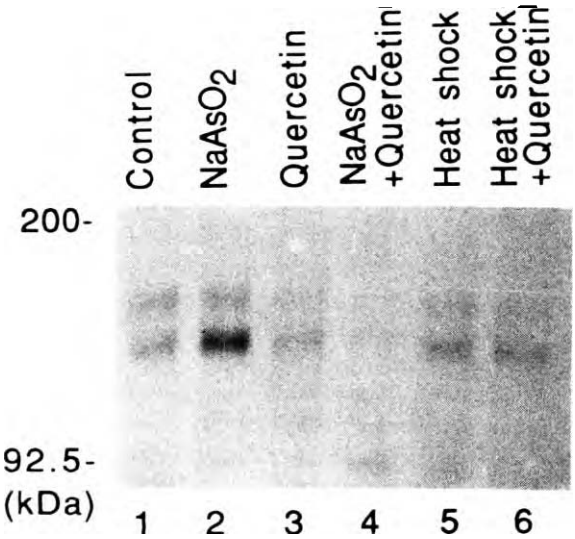


Fig. 1. Inhibition of the increase of P-glyoprotein synthesis caused by exposure to arsenite. HepG2 cells treated with or without 100 $\mu$M sodium arsenite or heat shocked (42°C, 2 h) in the presence or absence of 100 $\mu$M quercetin were labeled with [$^{35}$S]methionine and analyzed as described in Materials and Methods. The positions of size markers in kilodaltons are on the left.

monoclonal antibody, C219. The exposure to arsenite caused a 2.2-fold increase in the P-glycoprotein synthesis (Fig. 1, lane 2) Quercetin slightly inhibited the constitutive synthesis of P-glycoprotein (lane 3). Quercetin, however, completely inhibited the increase of the P-glycoprotein synthesis caused by exposure to arsenite (lane 4). Heat shock at 42°C for 2 h did not increase the synthesis of P-glycoprotein in HepG2 significantly (lane 5) nor did quercetin affect P-glycoprotein synthesis after heat shock (lane 6).

### 3.2. *Quercetin inhibits the increase of* MDR1 *mRNA exposure to arsenite*

The increase in the P-glycoprotein synthesis after exposure to arsenite was due to the mRNA accumulation of *MDR1* [3]. To examine whether quercetin inhibits the increase of *MDR1* mRNA after exposure to arsenite, slot blot analysis of total RNA and ribonuclease protection assay were done (Fig. 2). *MDR1* mRNA transcribed from the downstream promoter was increased after exposure to arsenite (Fig. 2A and B, lane 2). Densitometric scanning of ribonuclease protection indicated arsenite treatment increased *MDR1* mRNA 2.6-fold. Quercetin completely inhibited the increase of *MDR1* mRNA after exposure to sodium arsenite (lane 4), while it did not affect the level of *MDR1* mRNA before the exposure (lane 3). Quercetin reduced the increase of *hsp70* mRNA induced by sodium arsenite as described previously [7] (Fig. 2A). The mRNA level of $\beta$-actin gene did not change after exposure to these agents (Fig. 2A).

### 3.3. *Quercetin inhibits the transcription of* MDR1

Our previous study suggested that transcriptional ac-

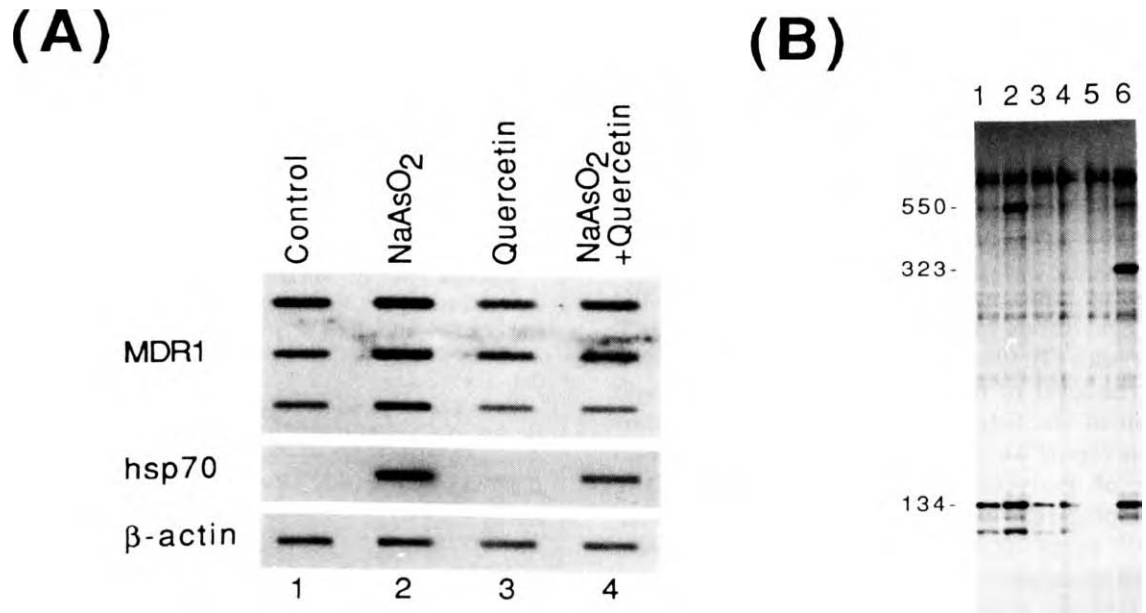


Fig. 2. Inhibition of the increase of *MDR1* mRNA accumulation. (A) Slot blot hybridization. HepG2 cells treated with or without sodium arsenite in the presence or absence of quercetin. Twelve micrograms of total RNA and two-fold serial dilutions were applied to GeneScreen plus and hybridized with a *MDR1* probe. Two micrograms of RNA were hybridized with a hsp70 probe or human $\beta$-actin probe as a control. (B) Ribonuclease protection analysis. Total RNA (10 $\mu$g) was extracted from untreated HepG2 cells (lane 1), treated with sodium arsenite (lane 2), quercetin (lane 3), and both reagents (lane 4), from drug-sensitive KB3-1 cells (lane 5), and from drug-resistant KB8-5 cells (lane 6). Nucleotide numbers of the protected fragments are indicated on the left. The 134-nucleotide fragment is the transcript from the major initiation site of the downstream promoter. The 550-nucleotide fragment in lane 2 in considered to be the nascent transcript (splicing intermediate) from the downstream promoter. The 323-nucleotide fragment in lane 6 is the transcript from the upstream promoter [11].

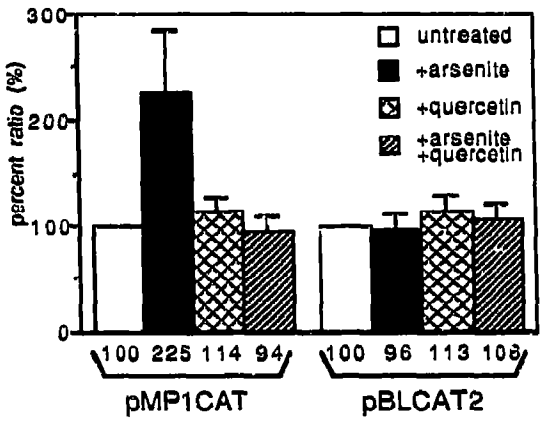


Fig. 3. CAT assay analysis. Plasmid DNA pMP1CAT or pBLCAT2 was transfected to HepG2. Cells were treated with or without arsenite in the presence or absence of quercetin as described in Materials and Methods. The CAT activity is shown by the percent ratio relative to the CAT activity of cell extracts of untreated cells. Each value is the average (shown under each bar) and standard deviation of five experiments.

tivation is involved in the increase of *MDR1* mRNA caused by sodium arsenite and depends on a 60-bp region containing two heat-shock responsive elements (HSEs) [3]. To discover whether quercetin inhibits the transcriptional activation of the *MDR1* gene after exposure to arsenite, pMP1CAT [3], which contains the CAT gene under the control of the downstream promoter of *MDR1*, was transfected into HepG2 cells, and the CAT assay was done. Four hours after addition of 100 $\mu$M of quercetin, cells were treated with or without sodium arsenite for an additional 4 h in the presence of quercetin. After 2 h of incubation with fresh medium containing quercetin, the CAT activity in cell extracts was assayed. The CAT activity of pMP1CAT was increased 2.3-fold after arsenite treatment while the CAT activity of pBLCAT2 [10], which contains the *tk* promoter, did not change (Fig. 3). Quercetin did not affect the promoter activity of either pMP1CAT or pBLCAT2 in the absence of arsenite. In the presence of arsenite, however, quercetin suppressed the CAT activity of pMP1CAT to the level without arsenite treatment. The CAT activity of pBLCAT2 was not changed in the presence of these agents. These results suggested that quercetin specifically inhibited the increase of transciptional activity of the *MDR1* gene induced by arsenite.

## 4. DISCUSSION

Transcriptional activation of heat-shock protein genes by heat shock or other stresses is regulated by the activation of HSF. Activated HSF post-transcriptionally acquires DNA binding ability. We have reported that quercetin inhibited the induction of heat-shock proteins [7]. Treatment with quercetin inhibited the binding of HSF to HSEs in cell extracts activated in vivo and in vitro by heat shock (Hosokawa, N., unpublished). Thus quercetin interacts with HSF, and inhibits the induction of heat-shock proteins after heat shock through the inhibition of HSF activation.

The *MDR1* gene has two HSEs on the promoter and its expression is increased by heat shock [2] or arsenite treatment [3]. Deletion analysis of the *MDR1* promoter indicated that the transcriptional activation after exposure to arsenite depended on a 60-bp region containing two HSEs [3]. Quercetin inhibited the increase of P-glycoprotein synthesis and *MDR1* gene expression after exposure to sodium arsenite, probably through the inhibition of HSF activation.

Many reagents have been screened for reversing multidrug resistance and some calcium channel blockers, such as verapamil and quinidine and other chemicals, have been reported to have this property. These reversing agents interact with P-glycoprotein and inhibit its function competitively. However, the most promising way to conquer multidrug resistance should be to suppress the emergence of cancer cells with acquired resistance. This is the first report to describe a reagent that inhibits the increase of the *MDR1* gene expression.

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