Short Communication

Pharmacology

1-[2-(2-Methoxyphenylamino)ethylamino]-3-(naphthalene-1-yloxy)propan-2-ol as a Potential Anticancer Drug

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The results of the present study, thus, indicate that HUHS1015 induces apoptosis in a variety of cancer cells, possibly by activating caspase-4 and the effector caspase-3.

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Abstract

The aim of the present study was to assess the anticancer effect of several naftopidil analogues on human malignant mesothelioma cell lines NCI-H28, NCI-H2052, NCI-H2452, and MSTO-211H, human lung cancer cell lines A549, SBC-3, and Lu-65, human hepatoma cell lines HepG2 and HuH-7, human gastric cancer cell lines MKN-28 and MKN-45, and human bladder cancer cell lines 253J, 5637, KK-47, TCCSUP, T24, and UM-UC-3, human prostate cancer cell lines DU145, LNCap, and PC-3, and human renal cancer cell lines ACHN, RCC4-VHL, and 786-O. We newly synthesized 21 naftopidil analogues, and of them 1-[2-(2-methoxyphenylamino) ethylamino]-3-(naphthalene-1-yloxy)propan-2-ol (HUHS1015) most efficiently reduced cell viability for all the investigated malignant mesothelioma cell lines in a concentration (1–100 μmol/l)-dependent manner. HUHS1015 reduced cell viability for all other investigated cancer cell lines, to an extent similar to that for malignant mesothelioma cell lines. HUHS1015 activated caspase-3 and -4, without activating caspase-8 and -9, in malignant mesothelioma cell lines.

Introduction

Naftopidil, an antagonist for α_1-adrenoceptor, with high selectivity for α_{1A}- and α_{1D}-receptors, has been clinically used as a drug for treatment of benign prostate hyperplasia and hypertension [1]. A recent topic is that naftopidil might also exert an anticancer effect. Naftopidil has been shown to inhibit prostate cancer cell growth by arresting the G_1 phase of cell cycling [2, 3]. In our studies, naftopidil reduced cell viability for bladder, prostate, and renal cancer cell lines [4] or induced apoptosis in malignant mesothelioma cell lines [5]. These findings raise the possibility that naftopidil might be useful as an anticancer drug. The mechanism underlying the anticancer action of naftopidil remains to be explored. α_1-Adrenoceptor is divided into α_{1A}, α_{1B}, and α_{1D} subtypes and the receptor is linked to $G_{q/11}$ protein bearing phospholipase C activation followed by protein kinase C (PKC) activation [6–8]. Naftopidil, therefore, should suppress PKC activation by

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inhibiting α_1-adrenoceptor. Surprisingly, the PKC inhibitor GF109203X attenuated naftopidil-induced apoptosis in malignant mesothelioma cells [5]. Moreover, proliferation of malignant mesothelioma cells was prompted by knocking down α_{1D}-adrenoceptors [5]. It is suggested from these data that naftopidil induces apoptosis in malignant mesothelioma cells by a mechanism independent of α_1-adrenoceptor blocking.

For the present study, we newly synthesized naftopidil analogues and probed the anticancer effect of the analogues on a variety of human cancer cell lines originating from human malignant mesothelioma, lung cancer, hepatic cancer, gastric cancer, bladder cancer, prostate cancer, and renal cancer. The results of our study show that of 21 naftopidil analogues synthesized, 1-[2-(2-methoxyphenylamino)ethylamino]-3-(naphthalene-1-yloxy)propan-2-ol (HUHS1015) reduces cell viability and activates caspase-3 and -4 for all the investigated malignant mesothelioma cell lines with the most beneficial effect, and that a similar effect is found for other cancer cell lines.

Materials and Methods

Naftopidil Analogues and Cell Culture
We synthesized 21 different kinds of naftopidil analogues (fig. 1).

Cells of human malignant pleural mesothelioma cell lines NCI-H28, NCI-H2052, NCI-H2452, and MSTO-211H and a human nonmalignant mesothelial cell line, Met5A, were purchased from the American Type Culture Collection (Manassas, Va., USA). Cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 0.003% (w/v) L-glutamine.

Human lung cancer cell lines A549, SBC-3, and Lu-65 were purchased from Health Science Research Resources Bank (Osaka, Japan). A549 and SBC-3 cells were grown in minimum essential medium with 0.1 mmol/l nonessential amino acids and Lu-65 cells in RPMI-1640 medium.

Cells from human hepatic cancer cell lines HepG2 and HuH-7, obtained from RIKEN cell bank (Ibaraki, Japan), were cultured in Dulbecco's Modified Eagle's Medium (DMEM).

Human gastric cancer cell line MKN-28 and MKN-45 cells, donated by Dr. Tatematsu (Nagoya University, Japan), were grown in RPMI-1640 medium.

Human bladder cancer cell lines used here were 253J, 5637, KK-47, TCCSUP, T24, and UM-UC-3. KK-47 was generously provided by Dr. Naito (Department of Urology, Kyushu University, Fukuoka, Japan), and 253J, 5637, TCCSUP, T24, and UM-UC-3 cells were purchased from the American Type Culture Collection. All of these cells were cultured in RPMI-1640 medium.

Human prostate cancer cell lines DU145, LNCap, and PC-3 cells were purchased from the American Type Culture Collection. DU145 cells were cultured in DMEM, and LNCaP and PC-3 cells in RPMI-1640 medium.

Human renal cancer cell lines ACHN, RCC4-VHL, and 786-O were purchased from the European Collection of Animal Cell Cultures (Salisbury, UK), and cultured in DMEM.

For all the cell cultures, culture medium was supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin (final concentration, 100 U/ml) and streptomycin (final concentration, 0.1 mg/ml), and cells were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Assay of Cell Viability
Cell viability was evaluated by the method using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) as previously described [9].

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was performed to detect in situ DNA fragmentation as a marker of apoptosis using an In Situ Apoptosis Detection Kit (Takara Bio, Otsu, Japan). Briefly, fixed and permeabilized cells were reacted with terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-deoxyuridine triphosphate for 90 min at 37°C. Fluorescein isothiocyanate signals were visualized with a confocal scanning laser microscope (LSM 510, Carl Zeiss Co., Ltd., Oberkochen, Germany).
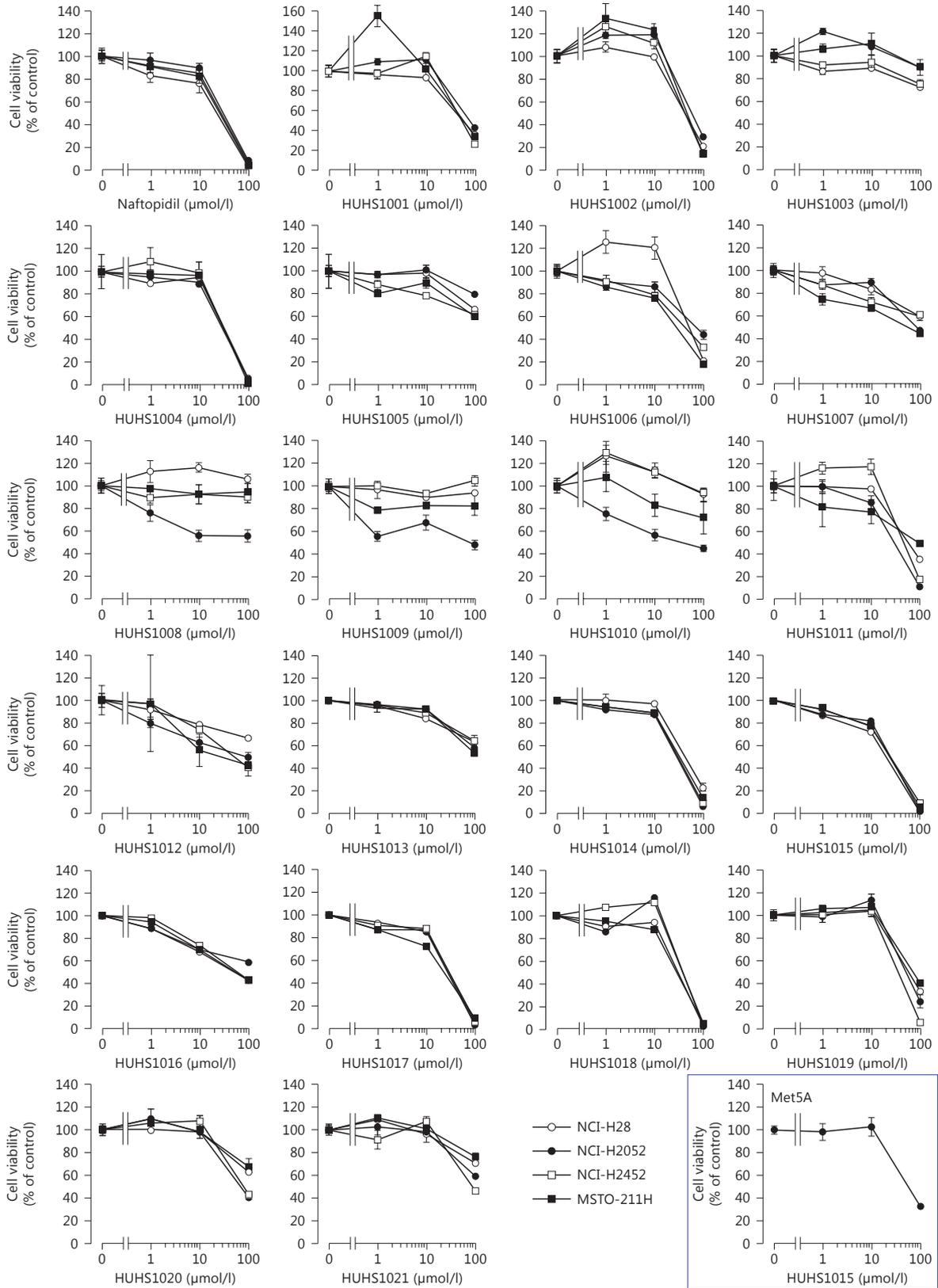
Enzymatic Assay of Caspase-3, -4, -8, and -9 Activities
Caspase activity was measured using a caspase fluorometric assay kit (Ac-Asp-Glu-Val-Asp-MCA for a caspase-3 substrate peptide; Ac-Leu-Glu-Val-Asp-AFC for a caspase-4 substrate peptide; Ac-Ile-Glu-Thr-Asp-MCA for a caspase-8 substrate peptide, and Ac-Leu-Glu-His-Asp-MCA for a caspase-9 substrate peptide) by a previously described method [9]. Briefly, cells were harvested before and after treatment with HUHS1015, and then centrifuged at 1,200 rpm for 5 min at 4°C. The pellet was incubated on ice in cell lysis buffer for 10 min, and reacted with the fluorescently labeled tetrapeptide at 37°C for 2 h. Fluorescence was measured at an excitation wavelength of 380 nm and an emission wavelength of 460 nm for caspase-3, -8, and -9 or an excitation wavelength of 400 nm and an emission wavelength of 505 nm for caspase-4 with a fluorometer (Fluorescence Spectrometer, F-4500, Hitachi, Japan).

Results

For all the investigated malignant mesothelioma cell lines, naftopidil reduced cell viability in a concentration (1–100 μmol/l)-dependent manner, the viability reaching almost 0% of control at 100 μmol/l (fig. 2). Likewise, all the investigated naftopidil analogues reduced cell viability for malignant mesothelioma cell lines in a concentration (1–100 μmol/l)-dependent manner, with different potentials (fig. 2). Of 21 naftopidil analogues HUHS1002, HUHS1004, HUHS1014, HUHS1015, HUHS1017, HUHS1018, and HUHS1019 had the highest potential to reduce cell viability as compared with that for other naftopidil analogues (fig. 2). HUHS1015 also reduced the viability of Met5A cells, a human mesothelial cell line, in a

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Fig. 1. Chemical structures for naftopidil and newly synthesized naftopidil analogues.

concentration (1–100 μmol/l)-dependent manner, but to a lesser extent than on the investigated malignant mesothelioma cell lines (fig. 2). This implies that HUHS1015 kills nonmalignant mesothelial cells with a weaker potential as compared with that on malignant mesothelioma cells. HUHS1015 (100 μmol/l) markedly increased TUNEL-positive cells after 12-hour treatment for NCI-H28

(fig. 3a), NCI-H2052 (fig. 3b), NCI-H2452 (fig. 3c), and MSTO-211H cells (fig. 3d), the extent reaching 61, 69, 37, and 58% relative to total cells. This indicates that HUHS1015 induces apoptosis in malignant mesothelioma cells.

HUHS1015 also reduced cell viability in a concentration (1–100 μmol/l)-dependent manner for human lung

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Fig. 3. TUNEL staining. NCI-H28 (**a**), NCI-H2052 (**b**), NCI-H2452 (**c**), and MS-TO-211H cells (**d**) were untreated (Cont) or treated with HUHS1015 (100 µmol/l, HUHS) for 12 h, and then TUNEL staining was carried out. DIC = Differential interference contrast. Bars = 100 µm.
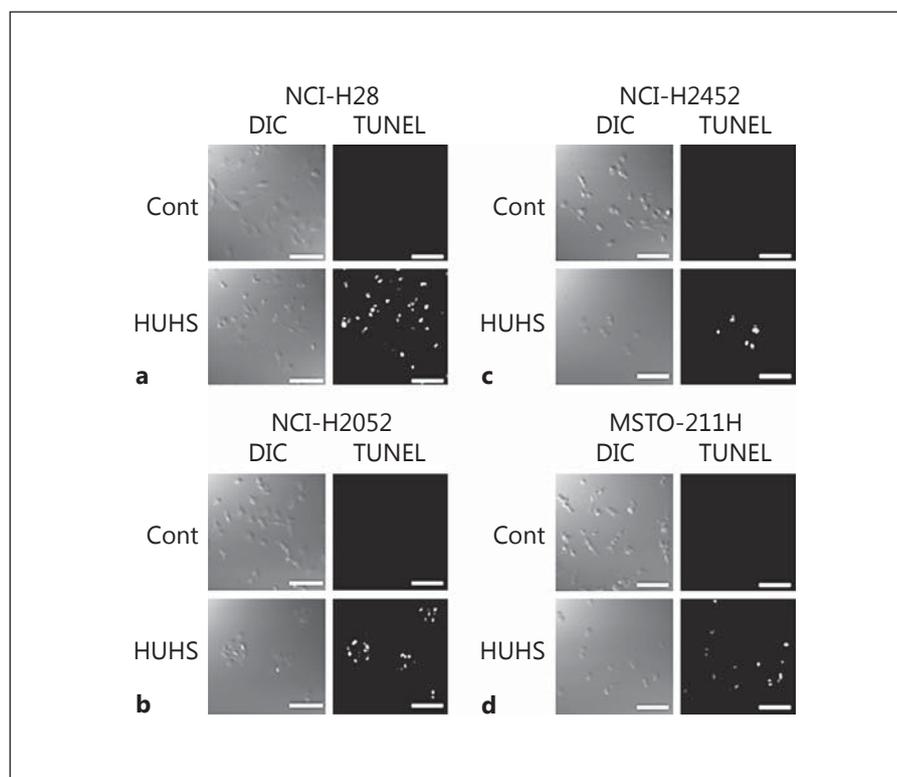
cancer cell lines A549, SBC-3, and Lu-65 (fig. 4a), human hepatic cancer cell lines HepG2 and HuH-7 (fig. 4b), human gastric cancer cell lines MKN-28 and MKN-45 (fig. 4c), human bladder cancer cell lines 253J, 5637, KK-47, TCCSUP, T24, and UM-UC-3 (fig. 4d), human prostate cancer cell lines DU145, LNCap, and PC-3 (fig. 4e), and human renal cancer cell lines ACHN, RCC4-VHL, and 786-O (fig. 4f).

In the enzymatic assay of caspase activities, HUHS1015 significantly activated caspase-3 for all malignant mesothelioma cell lines, NCI-H28, NCI-H2052, NCI-H2452, and MSTO-211H, but caspase-8 and -9 were not activated (fig. 5a–d). This indicates that HUHS1015-induced

Fig. 2. Effects of naftopidil and its analogues on cell viability for malignant mesothelioma cells. MTT assay was carried out in NCI-H28, NCI-H2052, NCI-H2452, and MSTO-211H cells untreated and treated with naftopidil or its analogues at concentrations indicated for 24 h. In a different set of experiments Met5A cells were treated with HUHS1015 at concentrations indicated for 24 h followed by MTT assay. In the graphs, each point represents the mean (±SEM) percentage of control (MTT intensities for cells untreated with naftopidil or its analogues; n = 4 independent experiments).

caspase-3 activation is not mediated through death receptors relevant to caspase-8 activation or mitochondrial damage followed by caspase-9 activation. Intriguingly, HUHS1015 still activated caspase-4 for all the investigated malignant mesothelioma cell lines (fig. 5a–d). Taken together, these results indicate that HUHS1015 activates caspase-4 and in turn, the effector caspase-3, leading to apoptosis in malignant mesothelioma cells.

Discussion

The results of the present study clearly demonstrate that naftopidil and its analogues exhibit an anticancer effect on malignant mesothelioma cells, with the highest potential for HUHS1015, and that HUHS1015 also reduced cell viability for lung cancer cells, hepatic cancer cells, gastric cancer cells, and urological cancer cells such as bladder, prostate, and renal cancer cells. Naftopidil serves as an inhibitor of α_{1A}- and α_{1D}-adrenoceptors [1]. α_1-Adrenoceptors are linked to $G_{q/11}$ protein to activate PKC [6–8]. Thus, one could speculate that the anticancer action of naftopidil might be due to PKC inhibition in association with α_1-adrenoceptor blocking. However, this
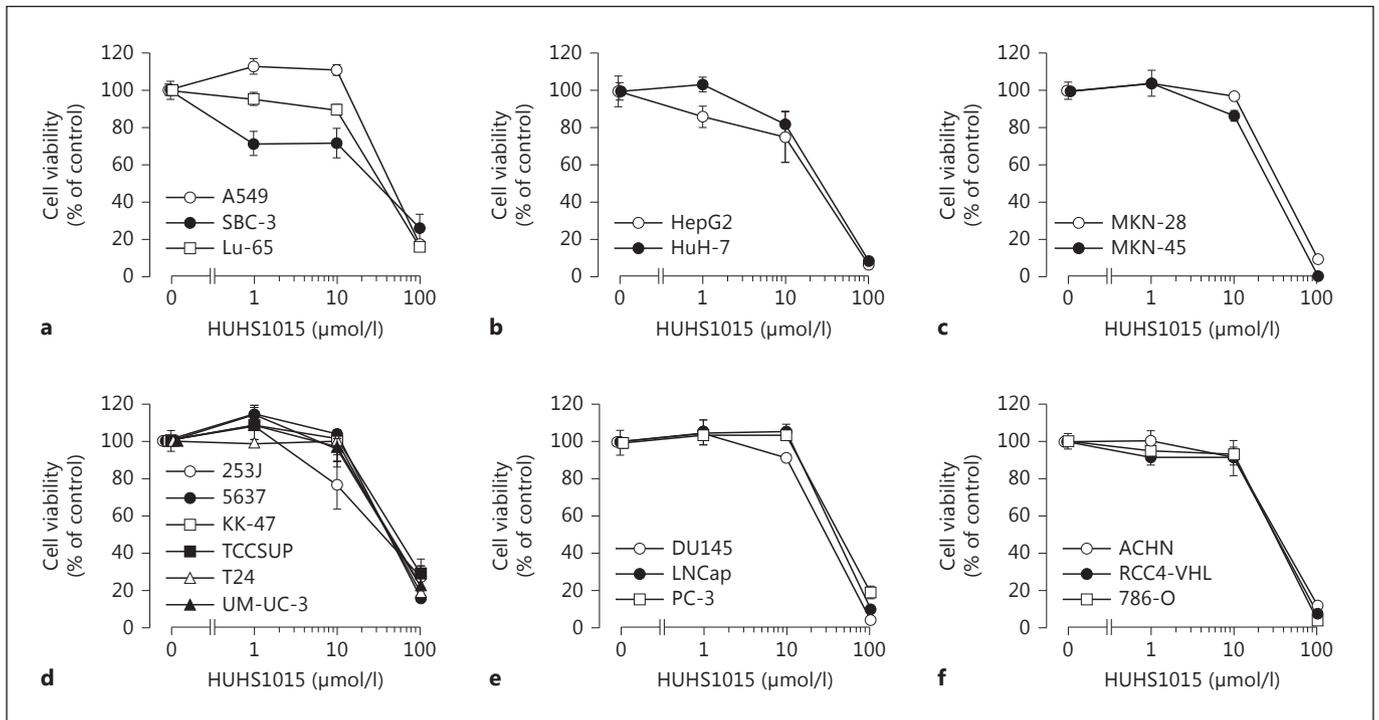
Fig. 4. Effect of HUHS1015 on cell viability for a variety of human cancer cell lines. MTT assay was carried out in lung cancer cell lines (**a**), hepatic cancer cell lines (**b**), gastric cancer cell lines (**c**), bladder cancer cell lines (**d**), prostate cancer cell lines (**e**), and renal cancer cell lines (**f**) untreated and treated with HUHS1015 at concentrations as indicated for 24 h. In the graphs, each point represents the mean (±SEM) percentage of control (MTT intensities for cells untreated with HUHS1015, n = 4 independent experiments).
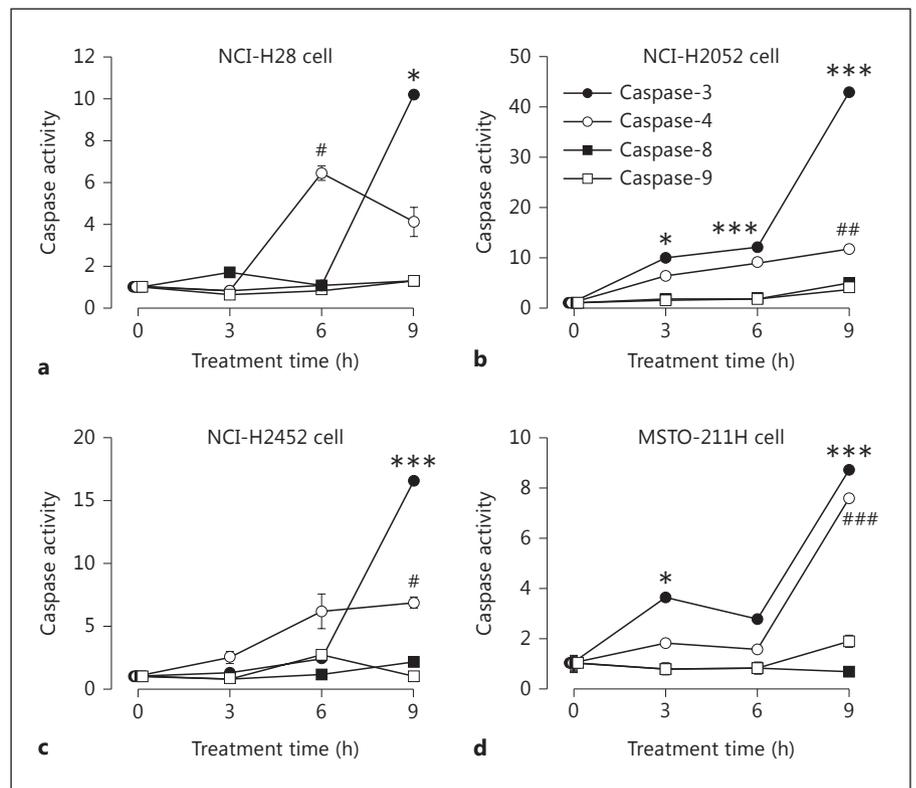
Fig. 5. HUHS1015-induced caspase activation in malignant mesothelioma cell lines. NCI-H28 (**a**), NCI-H2052 (**b**), NCI-H2452 (**c**), and MSTO-211H cells (**d**) were treated with HUHS1015 (100 μmol/l) for 3–9 h, and then activities of caspase-3, -4, -8, and -9 were enzymatically assayed. In the graphs, each point represents the mean (±SEM) ratio against basal caspase activities (before treatment with HUHS1015, n = 4 independent experiments). *, # $p < 0.01$, **, ## $p < 0.001$, ***, ### $p < 0.0001$ as compared with basal caspase activities (0 h), Dunnett's test.

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is unlikely, since the PKC inhibitor GF109203X prevented naftopidil-induced apoptosis in malignant mesothelioma cells, and malignant mesothelioma cell growth was not suppressed by knocking down α_{1D}-adrenoceptor [5]. Naftopidil, accordingly, appears to induce apoptosis in malignant mesothelioma cells in a PKC- and α_1-adrenoceptor-independent manner. This raises the possibility that the $\alpha_{q/11}$ subunit of $G_{q/11}$ protein may protect cells from apoptosis by activating an as yet unidentified effector other than phospholipase C and that naftopidil may induce cell death by inhibiting $\alpha_{q/11}$ subunit signal transduction.

In the present study, HUHS1015 activated caspase-3 and -4 in malignant mesothelioma cells. This, taken together with the finding that HUHS1015 increased TUNEL-positive cells in malignant mesothelioma cells, indicates that HUHS1015 induces apoptosis by activating caspase-4 and the effector caspase-3. This also suggests that HUHS1015 induces apoptosis in other cancer cell types by a similar mechanism. Emerging evidence shows that caspase-4 is activated in association with endoplasmic reticulum stress [10–12]. It is presently unknown whether HUHS1015 induces endoplasmic reticulum stress or not and how HUHS1015 activates caspase-4. An explanation may be that the $\alpha_{q/11}$ subunit of $G_{q/11}$ protein is implicated in the activation of caspase-4. To address this question, we are currently carrying out further experiments.

In conclusion, the results of the present study show that the naftopidil analogue HUHS1015 reduces cell viability in a variety of human cancer cell lines including malignant mesothelioma cell lines, possibly by activating caspase-4 and the effector caspase-3. HUHS1015 thus could develop into a promising new anticancer drug.

Acknowledgments

Disclosure Statement

The authors have no conflict of interest.

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