Methyl-β-cyclodextrin induces programmed cell death in chronic myeloid leukemia cells and, combined with imatinib, produces a synergistic downregulation of ERK/SPK1 signaling

Jun Yan, Qing-Fang Li, Li-Sheng Wang, Hua Wang, Feng-Jun Xiao, Yue-Feng Yang and Chu-Tse Wu

Lipid rafts mediate several survival signals in the development of chronic myeloid leukemia (CML). Methyl-β-cyclodextrin (MβCD) is an inhibitor specifically designed to disrupt lipid rafts in cells by depleting the cholesterol component. We hypothesize that treatment of CML cells with MβCD and imatinib could reduce imatinib resistance. Apoptotic and autophagic cell death was assayed using annexin V–propidium iodide double staining, immunoblotting, and immunocytochemistry. We next investigated whether MβCD could enhance the cytotoxicity of imatinib in imatinib-sensitive and imatinib-resistant K562 cells. Extracellular signal-regulated kinase/sphingosine kinase 1 signaling downstream of lipid raft-activated signaling pathways was significantly inhibited by treatment of cells with a combination of MβCD and imatinib compared with treatment with either agent alone. MβCD induces programmed cell death in CML cells, and its antileukemia action is synergistic with that of imatinib. *Anti-Cancer Drugs* 23:22–31 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Lipid rafts, consisting of phospholipids, sphingolipids, and cholesterol, have various signaling molecules associated with them, which have the capacity to regulate cell function. Lipid rafts also play an important role in the development, turnover, and capacity to metastasize of cancer cells. As mediators, or inhibitors, of signaling between cells and the extracellular matrix, lipid rafts facilitate cell homeostasis. In addition, many studies have demonstrated that apoptotic signaling of tumor cells, induced by drugs or stress, is largely mediated by lipid rafts. Lipid rafts that recruit the Fas/CD95 complex, for example, have been shown to affect signaling mediated by antileukemia drugs [1–3]. The breakdown of cell membrane integrity by the disruption of lipid rafts can also disrupt the activation of prosurvival signals, including those mediated by p44/42 mitogen-activated protein kinase [4].

Methyl-β-cyclodextrin (MβCD) is often used to disrupt lipid rafts because of its ability to deplete cholesterol stores maintained in the cell membrane. A number of studies have also demonstrated that the disruption of lipid rafts by MβCD can damage cancer cells and cause cell death. In human epidermoid carcinoma cells, for example, cholesterol depletion by MβCD induced anoikis-like apoptosis and caveolae internalization [5]. Currently, the underlying mechanisms of MβCD-induced apoptosis remain unclear, although both intracellular calcium concentrations and mitochondrial signaling pathways are believed to play important roles [6–8]. Moreover, cytokine-induced activation of extracellular signal-regulated kinase/sphingosine kinase 1 (ERK/SPK1) signaling has been shown to be blocked by pretreatment with MβCD [9]. These results suggest that the ERK/SPK1 signaling pathway has a role in mediating the effects of MβCD.

In chronic myeloid leukemia (CML), the tyrosine kinase Bcr–Abl is constitutively activated. The Bcr–Abl fusion gene that produces this kinase is created from the reciprocal translocation of chromosomes 9 and 22, which is also referred to as the Philadelphia chromosome [10]. Imatinib mesilate (imatinib, Gleevec; Novartis, Basel, Switzerland), a targeted inhibitor of Abl, is a common treatment for Philadelphia chromosome-positive (Ph +) CML during the chronic phase of the disease. In addition, imatinib has been shown to be a Bcr–Abl inhibitor, and is currently the first-line treatment for CML. Imatinib treatment results in the death of CML cells and is associated with downregulation of Bcr–Abl-activated signaling pathways, including Ras/ERK, phosphoinositide 3-kinase (PI3K)/AKT, janus kinase/signal transducer and activatorof transcription, and nuclear factor-κB [11]. SPK1 has also been observed to be downregulated

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after treatment with imatinib, suggesting that SPK1 is a critical downstream target of ERK, AKT, and signal transducer and activator of transcription 3 after their activation by Bcr–Abl in CML cells [12]. However, subsets of patients have been observed to develop resistance to imatinib [13]. In particular, patients with CML in blast crisis have been shown to be less responsive to imatinib treatment [14]. Therefore, we hypothesize that the administration of imatinib in combination with other anticancer reagent(s) may overcome this resistance, thereby improving the efficacy of imatinib in the treatment of CML.

Previously, the combination of imatinib with proteasome inhibitors, histone deacetylase inhibitors [15], or the Src kinase inhibitor [16], CGP76030 [17], was found to provide better results compared with imatinib alone for the treatment of Ph + CML cells, including resistant cells. Knowing that lipid rafts can serve as signal platforms for tyrosine kinase receptors in the plasma membrane, we hypothesized that the disruption of lipid rafts by MβCD could mediate an antileukemia action in Ph + CML cells and could augment the proapoptotic signaling induced by imatinib when it is administered in combination with MβCD. Therefore, this study describes the role of MβCD in regulating chronic leukemia cell function, as well as the synergistic effects of killing CML cells with the coadministration of MβCD and the tyrosine kinase inhibitor, imatinib.

Materials and methods

Reagents

Reagents used in the study included Roswell Park Memorial Institute-1640 and Iscove's Modified Dulbecco's Media (IMDM) (Gibco BRL, UK), fetal bovine serum (FBS) (Thermo Scientific, Massachusetts, USA), MβCD, and chloroquine (CQ) (Sigma, USA), Imatinib (Novartis Pharma, Switzerland), and Z-Val-Ala-Asp-CH_2F (z-VAD-FMK; Beyotime Institute of Biotechnology, China). An annexin V–fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit was purchased from Zhongkangzhiheng Biotechnology (Beijing, China) and a phycoerythrin (PE) Active Caspase-3 Apoptosis Kit was purchased from BD Pharmingen (BD Biosciences, Maryland, USA).

Cell culture

K562 and K562 imatinib-resistant (K562R) cells were incubated in Roswell Park Memorial Institute-1640 supplemented with 10% FBS and penicillin/streptomycin at 37°C and 5% CO_2. K562R cells were derived from resistant clones that had been established by adding increasing concentrations of imatinib to K562 culture media [18]. Primary CML cells were cultured in IMDM supplemented with 20% FBS and penicillin/streptomycin.

Cell death assay

K562 and K562R cells were incubated with MβCD with or without imatinib for 24 h. Cell death was examined by double staining cells with annexin V–FITC and PI, with cell fluorescence detected by flow cytometry. Each assay was performed in triplicate.

Caspase-3 activation measurement

Cleaved caspase-3 expression was detected using a PE Active Caspase-3 Apoptosis Kit. After stimulation with MβCD, cells were washed using 1 × washing buffer and permeabilized with Cytofix/Cytoperm (BD Biosciences, Erembodegem, Belgium) on ice for 20 min. Cells were then incubated with PE-conjugated caspase-3 antibody for 1 h. After the cells had been washed twice, they were then resuspended with washing buffer and analyzed by fluorescence activated cell sorting.

Isolation of primary chronic myeloid leukemia samples

Primary CML samples were obtained, after written informed consent, from newly diagnosed patients ($n = 3$). CD34 + cells were isolated using a previously described method [19]. In brief, mononuclear cells were isolated from blood samples by density centrifugation (Ficoll-Paque Plus; GE Healthcare Life Sciences, New Jersey, USA), washed with PBS and EDTA (2 mmol/l), then resuspended in cell culture medium (IMDM + 20% FBS). Isolated cells were labeled with CD34 microbeads isolated by magnetic positive selection (BD Biosciences), and the purity of the CD34 + CML population obtained was determined to be more than 85% by fluorescence activated cell sorting analysis.

Immunocytochemistry

Immunocytochemistry was performed using previously described methodology [20]. In brief, cells were cytospun on microscope slides using a cytocentrifuge (Bio-Rad, Berkeley, California, USA). Slides were then dried, fixed with 4% paraformaldehyde, and permeabilized with 0.5% Triton-X 100 for 15 min. Cells were incubated initially with a primary anti-LC3 antibody (PM036, MBL, Japan) for 1 h, followed by FITC-conjugated secondary anti-rabbit antibody (MBL) for 30 min. Nuclei were stained with 4′,6-diamidino-2-phenylindole (Sigma) before slides were analyzed using a laser scanning confocal microscope (LSM 510 Zeiss; Carl Zeiss, Inc., Jena, Germany).

Sphingosine kinase 1 activity

Protein levels were quantified using a BCA protein assay reagent (Pierce Biotechnology, Illinois, USA), and SPK1 activity was detected using a Sphingosine Kinase Activity Assay Kit (K-3500; Echolon, Utah, USA), according to the manufacturer's directions. Relative luminescence unit (RLU) was recorded using a Varioscan Flash (Thermo Scientific). An ATP standard curve was initially determined, and sphingosine kinase activity was reported as the inverse of the RLU detected.

Adenoviral-mediated SPK1 gene transfer

As previously described, an adenoviral-null empty vector (control) and an adenoviral-SPK1 vector containing the

human SPK1 sequence were used [21]. Using these recombinant replication-defective adenoviruses, K562 cells were infected with a multiplicity of infection of 150; infection efficiency was evaluated by western blot.

Immunoblotting

Cell lysis and immunoblotting were performed using a previously described method [12]. Anti-poly ADP-ribose polymerase (PARP), antiphospho-ERK, anti-ERK, anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH), anti-β-actin, and horseradish peroxidase-conjugated anti-rabbit antibodies, as well as anti-Bcr–Abl, were purchased from Santa Cruz Biotechnology (California, USA). Anti-Mcl-1 (BioVision, USA), anti-SPK1, and anti-BECN1 (Abcam, Hong Kong), anti-P-CrkL and anti-c-Myc (Cell Signaling Technology, Massachusetts, USA), and anti-LC3 (MBL, Japan) antibodies were also purchased. Detection of β-actin or GAPDH was used as a loading control.

Results

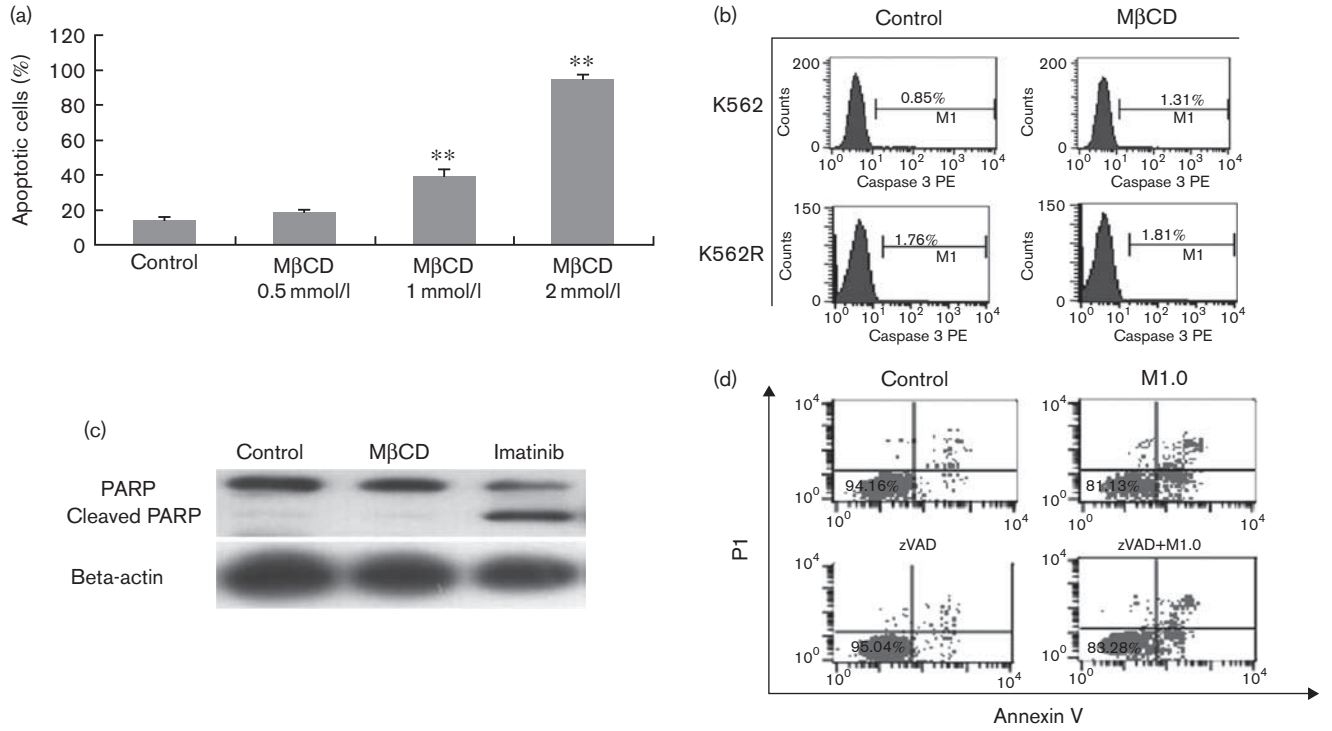
MβCD induces apoptosis but does not activate caspase-3

In a previous study, apoptosis of keratinocytes was induced by MβCD in a caspase-dependent manner [22]. Levels of apoptosis in K562 cells were assayed, as well as the expression of effectors of the caspase cascade as they have been shown to mediate apoptosis. For K562 cells treated with 1 mmol/l or 2 mmol/l MβCD, the percentage of apoptotic cells detected was approximately 40% and 90%, respectively (Fig. 1a). For the same treatment groups, cleavage of caspase-3 was not detected for K562 or K562R cells treated with MβCD (Fig. 1b). Similarly, no change in the levels of PARP fragment was detected in treated and untreated K562 cells (Fig. 1c). In contrast, where treatment of K562 cells with imatinib was used as a positive control, PARP cleavage was detected in approximately 66% of the cells assayed (Fig. 1b and c). In addition, as shown in Fig. 1d, K562 cell apoptosis induced by MβCD was not attenuated by z-VAD-FMK (a pan-caspase inhibitor). These results, therefore, indicate that while MβCD can induce apoptosis, it does not activate caspase-3, suggesting that the mechanism may be independent of caspase pathways.

MβCD induces K562 cells autophagic cell death

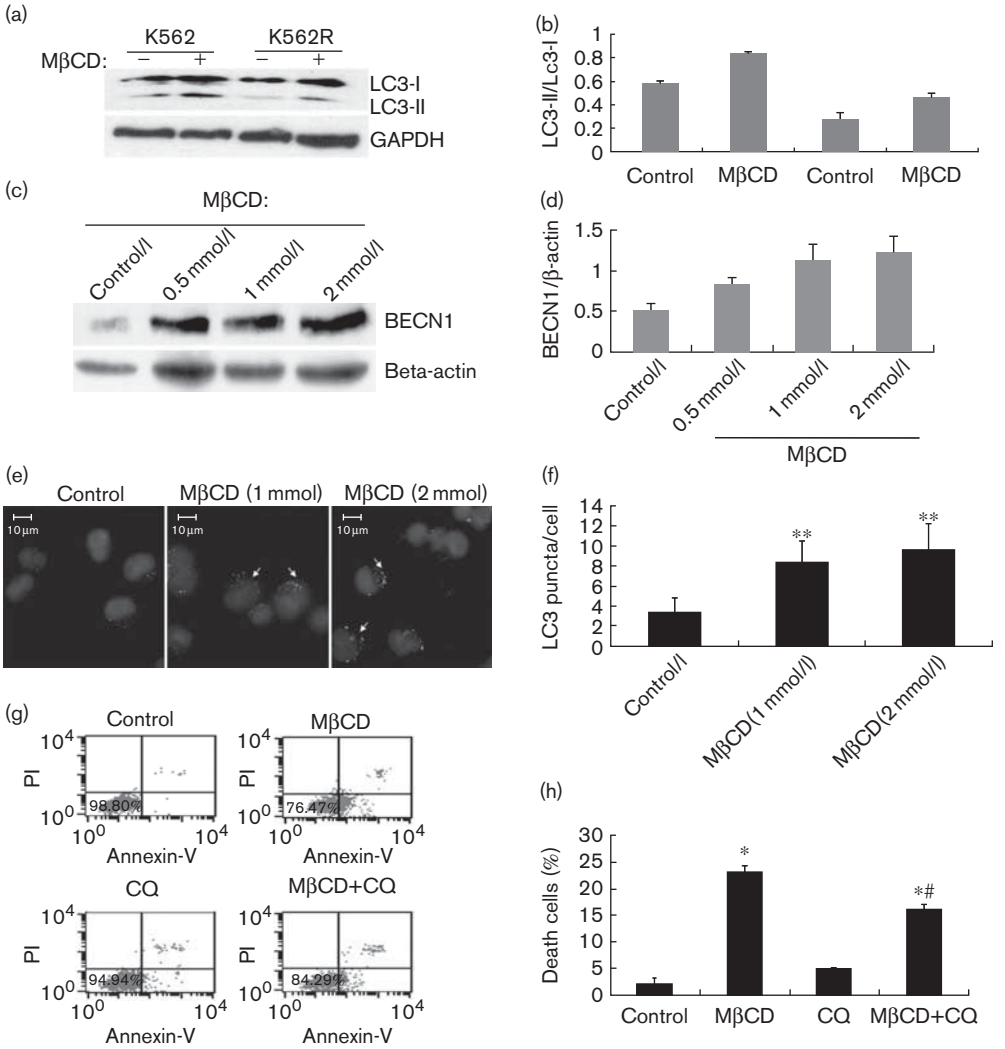
Autophagic cell death, also known as programmed cell death type II, is distinct from apoptosis. Recently, the

Fig. 1



(a) K562 cells were exposed to 0, 0.5, 1, and 2 mmol/l methyl-β-cyclodextrin (MβCD) for 24 h and stained with annexin V and propidium iodide (PI). The percentage of annexin V+/PI+ cells was analyzed using Student's *t*-test for each sample in triple experiments and the mean ± standard error of the mean is shown.**$P<0.01$. (b) Cleavage of caspase-3 was analyzed by flow cytometry for K562 and K562R cells treated with MβCD (1 mmol/l) versus untreated cells (control) for 24 h. (c) Western blot analysis of these same treatment groups was used to detect PARP expression. Imatinib (2 μmol/l) was used as a positive control and detection of β-actin was used as a loading control. (d) K562 cells pretreated with z-VAD-FMK (z-VAD) were incubated with or without (control) MβCD (1 mmol/l) (M1.0) for 24 h, then stained with annexin V and PI, and analyzed by flow cytometry.

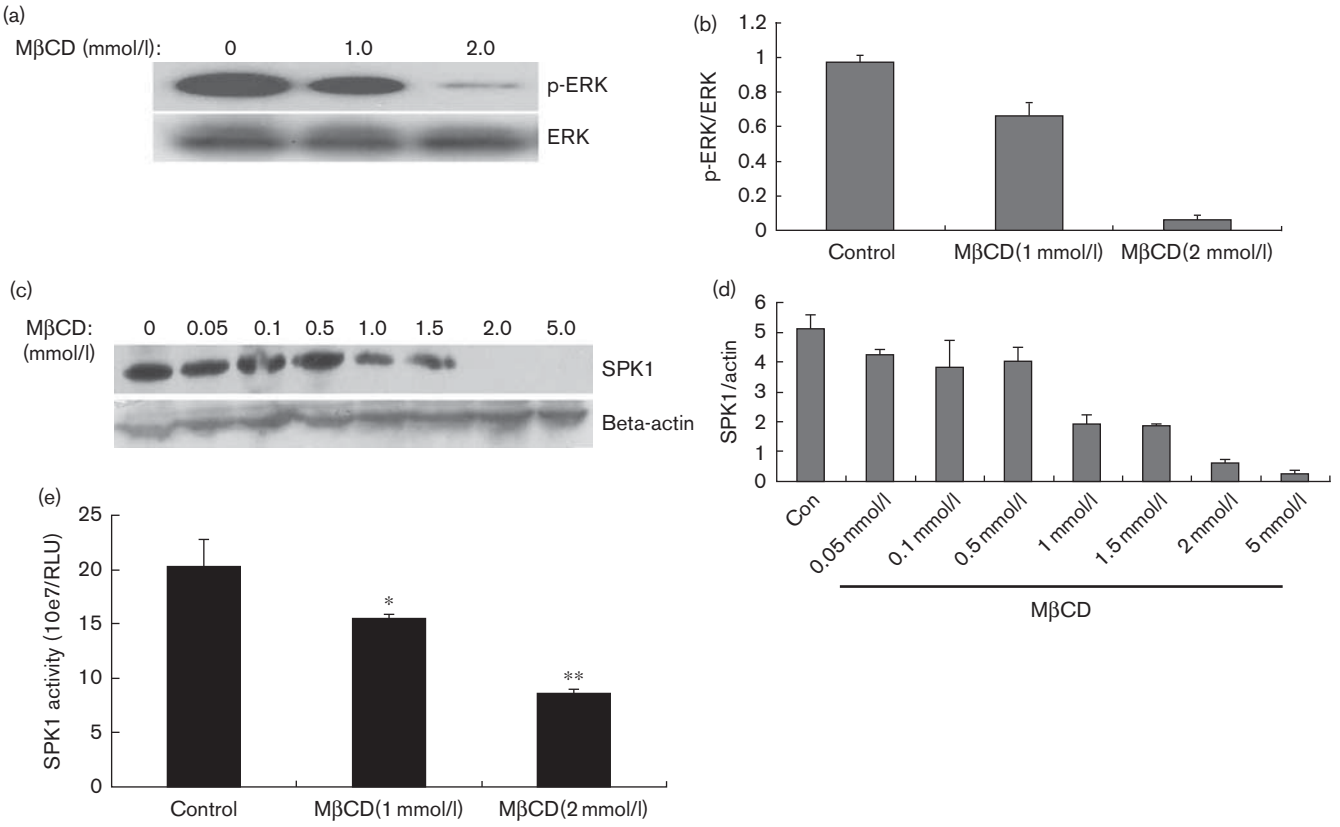
Fig. 2



(a) K562 and K562R cells were incubated with or without methyl-β-cyclodextrin (MβCD) (1 mmol/l) for 24 h. Western blotting of cell extracts was used to detect the expression of membrane-bound LC3 (LC3-II) versus sequestered LC3 (LC3-I), with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) used as a loading control. (c) K562 cells were treated with 0 (control), 0.5, 1, or 2 mmol/l MβCD for 24 h. Cell lysates were prepared and expression of BECN1 and β-actin was detected by immunoblot. (e) Immunofluorescence to detect LC3-positive vesicles (green and indicated with arrows) present in K562 cells treated with 1 mmol/l or 2 mmol/l MβCD for 24 h versus untreated K562 cells (control) was performed. Nuclei (blue) were stained with 4′,6-diamidino-2-phenylindole. Scale bar: 10 μm. Normalized intensities and vesicles measured by densitometry of two independent experiments are depicted as bar graphs (b, d, f, h). (g) K562 cells were cultured for 24 h in the presence or absence of MβCD (1 mmol/l), annexin V, and PI double staining. Values in the bar graphs (b, d, f, h) represent the mean ± standard deviation of three independent experiments. $^{*}P<0.01$ vs. control; $^{\#}P<0.01$ vs. MβCD.

microtubule-associated protein-1 light chain-3 (LC3), which is the mammalian homolog of the yeast autophagy protein Apg8 [23], has been used as a marker of autophagy. The conversion of LC3-I to LC3-II (LC3-II/LC3-I) is a marker of autophagy induction. As shown in Fig. 2a, treatment with MβCD resulted in the conversion of full-length LC3-I to LC3-II, as detected in western blot assays. The ratio of LC3-II to LC3-I increased approximately 1.5-fold in MβCD-treated K562 and K562R cell lysates compared with controls. The autophagy-specific molecule Beclin1 (homologous to the yeast ATG6 protein) belongs to the class III PI3K complex (PI3K III) family and is a central player in the formation of autophagosomes. Beclin1 contains a conserved BH3 domain and plays a direct role in initiating apoptotic signaling [24]. Beclin1 bridges the processes of cell autophagy, cell differentiation, and apoptosis [25]. When Beclin1 expression was monitored by western blot after treatment with MβCD, a dose-dependent increase in Beclin1 expression was detected (Fig. 2c). Furthermore, laser scanning confocal microscopy confirmed the accumulation of LC3-positive structures (green) in cells treated with 1 mmol/l and 2 mmol/l MβCD for 24 h

Fig. 3



(a) K562 cells were treated with 0, 1, or 2 mmol/l methyl-β-cyclodextrin (MβCD) for 24 h. (b) Cell lysates were prepared, and the expression of phosphoextracellular signal-regulated kinase (ERK) and ERK was detected by western blot. Detection of β-actin was used as a loading control. (c) Cell lysates collected from K562 cells treated with various concentrations of MβCD as indicated, were analyzed for expression of sphingosine kinase 1 (SPK1). Detection of β-actin was used as a loading control. Quantification of protein levels is demonstrated in the bar graph (d), with the mean ± standard deviation as shown. (e) SPK1 activity for untreated K562 cells (control) versus K562 cells treated with 1 or 2 mmol/l MβCD, as indicated, was measured using a Sphingosine Kinase Activity Assay Kit. The data presented represents the mean ± standard deviation. $*P<0.05$, $**P<0.01$ vs. control.

(Fig. 2e, middle and right panels, respectively) compared with untreated cells, which contained fewer LC3-positive vacuoles (Fig. 2e, left panel).

In an attempt to investigate the role of autophagy in MβCD-induced cell death, we used the pharmacological agent CQ to inhibit autophagy. As shown in Fig. 2g, MβCD-induced cell death was significantly attenuated when MβCD (1 mmol/l) was combined with CQ (10 μmol/l), whereas treatment with CQ alone had no effect. These results suggest that MβCD induces autophagic cell death.

ERK/SPK1 expression and activity are attenuated by MβCD in K562 cells

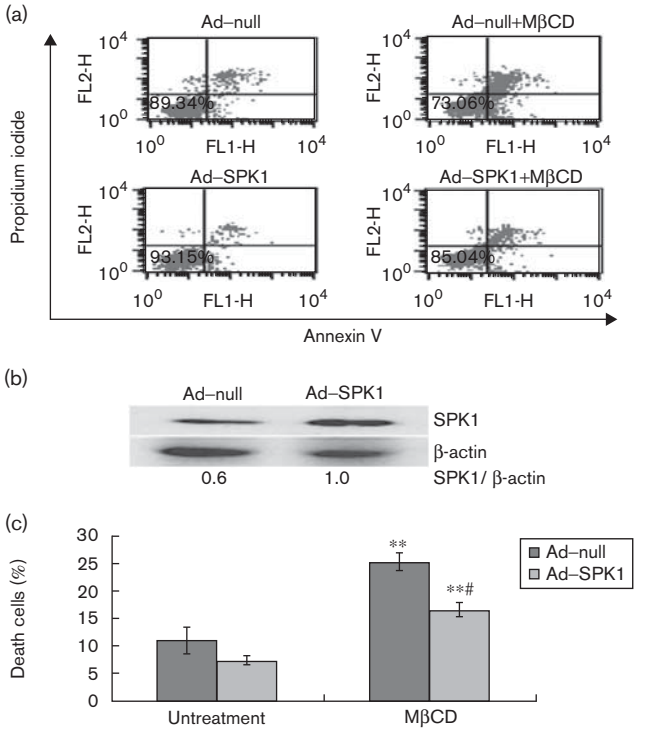
ERK1/2 phosphorylation plays a key role in prosurvival signaling that is mediated by lipid rafts and induced by growth factors and other stress. In this study, ERK1/2 phosphorylation was assessed after treatment of K562 cells with MβCD, and a significant decrease in levels of phosphorylated ERK1/2 was observed (Fig. 3a), consistent with similar findings reported by Sorice *et al.* [9].

Sphingosine is a lipid mediator, which plays a major role in regulating cell growth, survival, invasion, and autophagy. The balance between sphingosine and its metabolic precursor, ceramide, is considered to be a critical determining factor in the fate of the cell [26]. SPK1 is a key regulator of this balance and can therefore function as an oncogene [27]. Chemotherapeutic agents have been shown to downregulate or inhibit SPK1, suggesting that SPK1 could represent an important target in anticancer therapies [12]. In this study, treatment of K562 cells with MβCD was associated with a significant decrease in the expression and activity of SPK1 in a dose-dependent manner (Fig. 3c and e).

Adenovirus-mediated overexpression of SPK1 attenuates MβCD induced cell death

To further investigate the role of SPK1, K562 cells were infected with adenoviral-SPK1 and adenoviral-null viruses before treatment with MβCD (1 mmol/l). After 24 h, cell death was assessed by staining cells with annexin V and PI. As shown in Fig. 4a, there was an absence of

Fig. 4



(a) K562 cells were transfected with adenoviral-null and adenoviral-sphingosine kinase 1 (SPK1) for 48 h before being treated with methyl-β-cyclodextrin (MβCD) (1 mmol/l) for 24 h. Cells were then stained with annexin V and propidium iodide (PI), and the percentage of cells negative for both markers is indicated in each of the dot plot panels. (b) The annexin V+/PI+ percentages are represented as the mean ± standard deviation in the bar graph below. (c) The transfection efficiency of K562 cells with adenoviral-SPK1 was also evaluated by western blotting, with the detection of β-actin used as a loading control. Levels of SPK1 protein were quantified.

serum-induced cell death for 11% of the adenoviral-null-infected cells, which was higher than the rate of cell death observed for adenoviral-SPK1-infected cells under the same conditions (approximately 7%). For cells treated with MβCD, the rate of cell death for the adenoviral-SPK1-infected cells decreased to approximately 12% compared with adenoviral-null-infected cells (Fig. 4b). Western blotting further revealed that expression of SPK1 in adenoviral-SPK1-infected cells increased approximately 1.7-fold compared with adenoviral-null cells (Fig. 4c).

Combined effects of MβCD and imatinib on the death of CML cells

Treatment of K562 and K562R cells with MβCD and imatinib was also evaluated in relation to rates of apoptosis. As shown in Fig. 5a, the percentage of apoptotic K562 cells stained with annexin V–FITC in the treatment groups that received imatinib (2 μmol/l) or MβCD (1 or 2 mmol/l) alone, was less than that induced by the combined treatment of MβCD and imatinib. Similarly, the percentage of apoptotic K562R cells detected after treatment with both MβCD and imatinib was also greater than that observed with either treatment alone (Fig. 5c). Depletion of the anti-apoptosis protein Mcl-1(Fig. 5e) and the conversion membrane-bound LC3 (LC3-II) to sequestered LC3 (LC3-I) ratio (Fig. 5g) was also determined. For both assays, a greater effect was observed when cells were treated with the combination of MβCD and imatinib, than with either drug alone. These findings indicate that co-treatment of K562 cells with MβCD and imatinib increases the rates of apoptotic and autophagic cell death.

To further determine the antileukemic effect mediated by MβCD, with and without imatinib, CD34+ stem cells were isolated from bone marrow samples obtained from three newly diagnosed CML patients. As shown in Fig. 5i, the percentage of dead cells present after treatment with MβCD and imatinib alone increased by 16% and 24%, respectively. Furthermore, treatment of the CD34+ stem cells with a combination of MβCD and imatinib induced death in approximately 30% of the cells.

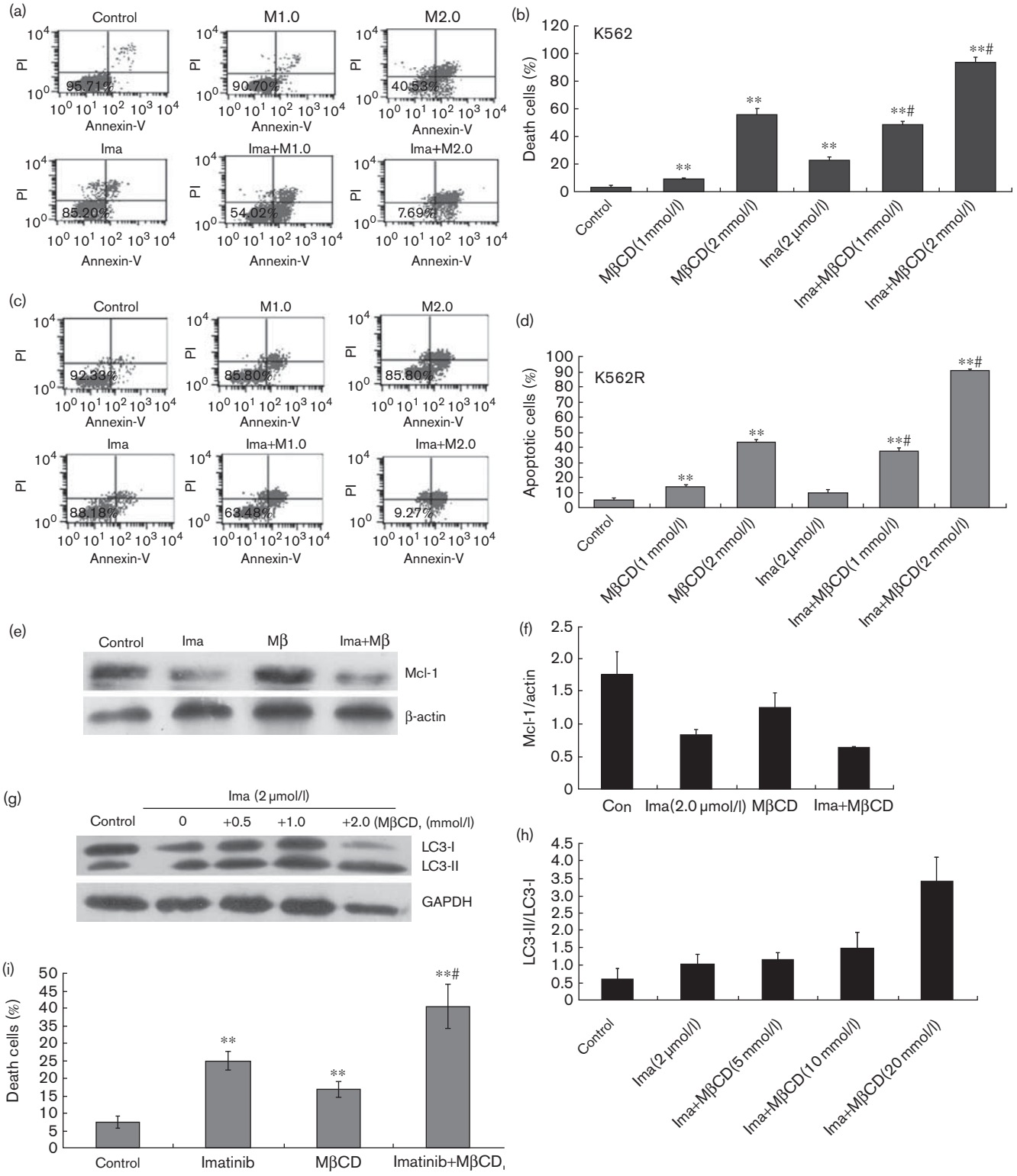
Downregulation of Bcr–Abl/ERK/SPK1 induced by imatinib is augmented by MβCD

To examine whether the combined effects of MβCD and imatinib also affect Bcr–Abl/ERK/SPK1 signaling, Bcr–Abl/ERK/SPK1-signaling-associated proteins were examined. When K562 cells were treated with imatinib, a decrease in ERK phosphorylation was observed (Fig. 6a), which was consistent with our previously reported results [14]. However, when K562 cells were treated with MβCD and imatinib, levels of phosphorylated ERK were lower than those achieved with either imatinib or MβCD alone (Fig. 6a). Similarly, expressions of Bcr–Abl, c-Myc, a target oncogene downstream of ERK signal, and tyrosine phosphorylation of Bcr–Abl substrate CrkL(P-CrkL) were all significantly reduced after cotreatment with MβCD and imatinib, compared with treatment with either agent alone (Fig. 6c). In addition, attenuation of SPK1 expression was mainly observed at the highest concentration of MβCD assayed (2 mmol/l) (Fig. 6e). In assays of SPK1 activity, treatment with imatinib was associated with a greater inhibition of SPK1 activity (1/RLU) than MβCD, and the greatest inhibition of SPK1 activity was observed when cells were treated with both MβCD and imatinib (Fig. 6g). Overall, these results suggest that the combination of MβCD and imatinib induces a synergistic downregulation of ERK/SPK1 signaling.

Discussion

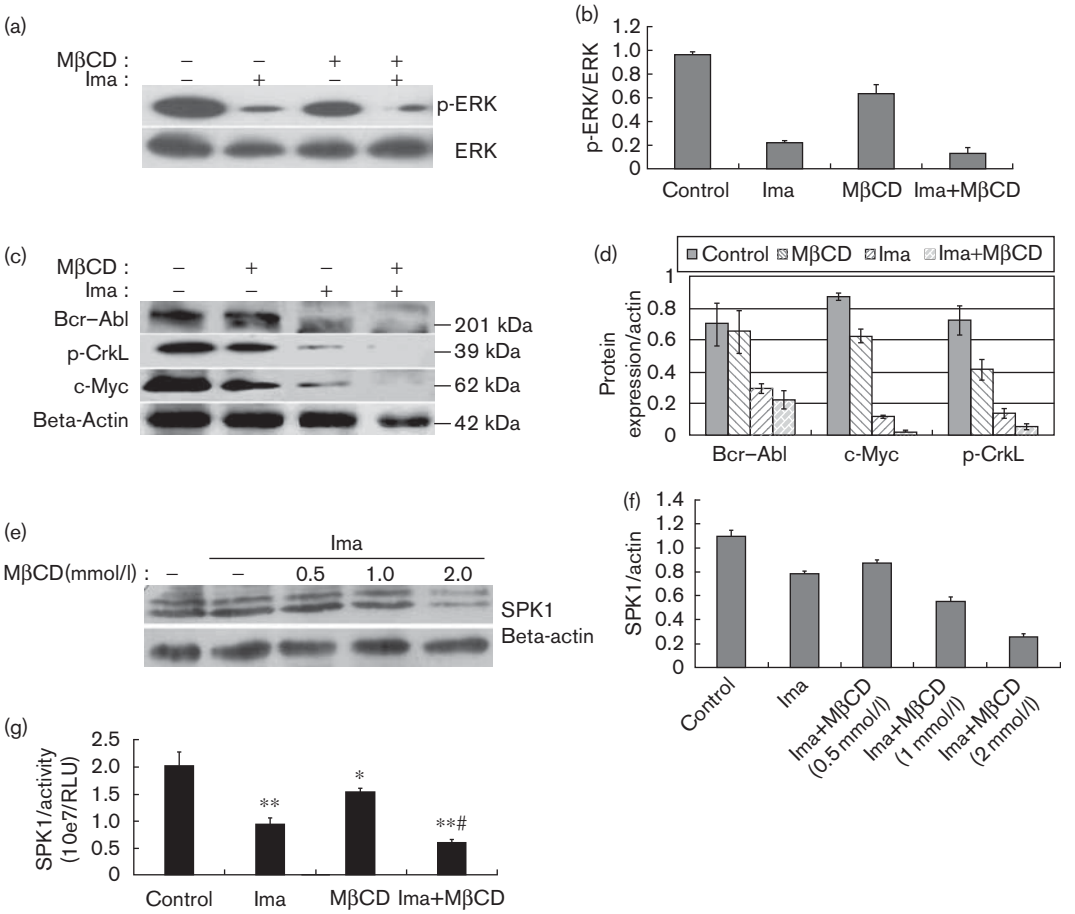
Imatinib mesilate (imatinib), which occupies the ATP-binding site of Bcr–Abl and maintains it in an inactive conformation, is widely used in the treatment of CML. However, resistance to imatinib can occur, particularly during the blast crisis phase, thus presenting a major obstacle in the treatment of CML for some patients. To overcome this resistance, several approaches have been

Fig. 5



Apoptosis induced in K562 (a) and K562R (c) cells was detected by flow cytometry after the staining of cells treated with imatinib (Ima) alone, or in combination with methyl-β-cyclodextrin MβCD at 1 mmol/l (M1.0) or 2 mmol/l (M2.0). The mean ± standard deviation of the annexin V + /PI + population present in each treatment group are presented in the right bar graphs (b, d, f, h). K562 cell lysates were prepared and immunoblotted for expression of Mcl-1 (e) and LC3-II versus LC3-I (g) after treatment with imatinib (Ima, 2 μmol/l) and/or MβCD. Detection of β-actin was used as a loading control. Quantities of proteins are described as right bar graphs (b, d, f, h). (i) CD34 + CML cells were incubated with MβCD and/or imatinib (Ima, 2 μmol/l) and stained with annexin V and propidium iodide (PI). The mean ± standard error of the mean of three samples is presented in the bar graphs (b, d, f, h). $^{**}P < 0.01$ vs. control, $\#P < 0.01$ vs. imatinib.

Fig. 6



(a) Cell extracts of K562 cells treated with methyl-β-cyclodextrin (MβCD) (1 mmol/l) and/or Imatinib (2 μmol/l) (Ima) were immunoblotted for the expression of P-extracellular signal-regulated kinase (ERK) and ERK. The expression levels of P-ERK were quantified relative to ERK. (c) After treatment with MβCD (1 mmol/l) and/or Imatinib (2 μmol/l), K562 cell lysates were prepared and immunoblotted for the expression of Bcr – Abl, c-Myc, and P-CrkL. (e) Western blot analysis of sphingosine kinase 1 (SPK1) expression in cell lysates of K562 cells treated with imatinib alone or in combination with various concentrations of MβCD as indicated. (d) SPK1 activity was assayed for K562 cells exposed to MβCD and/or imatinib. $*P<0.05$, $**P<0.01$ vs. control, $\#P<0.01$ vs. imatinib. Normalized intensities measured by densitometry of three independent experiments are depicted as bar graphs (b, d, f, g).

developed, including the combination of imatinib with other anticancer reagents. Studies have described a synergistic cytotoxic effect on human CML cells where imatinib is combined with fludarabine (a nucleoside purine analog) or bortezomib (a proteasome inhibitor) [15,28]. Similarly, in this study, the combination of imatinib with the lipid raft inhibitor, MβCD, resulted in a marked effect on human CML cells compared with the administration of either agent alone.

MβCD is a member of the cyclic oligosaccharides family and disrupts lipid rafts by depleting sources of cholesterol. Our data also verified its effect on disrupting lipid rafts in K562 cells (Supplement 1, *http://www.anti-cancerdrugs.com*). Most of the effects of MβCD, previously reported, have been associated with its ability to abolish cytokine-induced cell proliferation and survival, and to enhance cancer cell apoptosis due to cholesterol depletion [5,9]. In this study, induction of cell death was seen in K562, K562R, and primary CML cells treated with MβCD. Moreover, MβCD-induced apoptosis appears to have been caspase independent. Autophagic cell death, another type of programmed cell death, was also observed after treatment with MβCD. These results are consistent with those of a previous report [29], where a reduction in cholesterol levels led to the activation of autophagy through a PI3K-dependent mechanism. To elucidate the mechanisms underlying the effects observed for the treatment of CML cells with MβCD, levels of ERK and SPK1 were monitored. Expression of these two molecules, as well as the activity of SPK1, was found to be downregulated after treatment with MβCD, indicating that ERK/SPK1 signaling is involved in MβCD-induced cell death. Finally, treatment with MβCD in combination with imatinib was observed to induce more imatinib-sensitive and imatinib-resistant CML cell death than

either treatment individually and was associated with a greater reduction in Bcr–Abl/ERK/SPK1 activity.

Recent studies have suggested that acquired resistance to imatinib is due to mutations in the Bcr–Abl kinase domain [30,31]. Cells expressing the Bcr–Abl mutation, T315I, for example, are not associated with a growth advantage in the absence of imatinib, but can be selected clearly with imatinib treatment [32]. Furthermore, another Bcr–Abl kinase inhibitor, ON012380, has been shown to inhibit the activity of Bcr–Abl T315I [33]. In this study, MβCD was observed to reduce expression of Bcr–Abl at high doses (data not shown). Although there is no experimental evidence to indicate the mechanisms by which MβCD can affect mutant forms of Bcr–Abl and imatinib-refractory primary CML cells, it is conceivable that MβCD increases the susceptibility of these cells to apoptosis due to a disruption of signaling from lipid rafts. In addition, combinations of different inhibitors have been shown to deplete levels of Bcr–Abl, and levels of Bcr–Abl T315I, more than either agent alone [34]. These results indicate that imatinib resistance can be overcome by the synergistic activity of combined drugs. Additional studies are needed to elucidate the interactions between imatinib, MβCD, and Bcr–Abl mutations.

In conclusion, disruption of lipid rafts by MβCD in human CML cells induced cell death, partly due to down-regulation of ERK/SPK1 signaling. These effects were enhanced by coadministration of imatinib and MβCD. The results observed in this study have the potential to advance research into the optimal treatment of CML.

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Conflicts of interest

There are no conflicts of interest.

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