Antimycin A induces death of the human pulmonary fibroblast cells via ROS increase and GSH depletion

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Abstract. Antimycin A (AMA) inhibits the growth of various cells via stimulating oxidative stress-mediated death. However, little is known about the anti-growth effect of AMA on normal primary lung cells. Here, we investigated the effects of AMA on cell growth inhibition and death in human pulmonary fibroblast (HPF) cells in relation to reactive oxygen species (ROS) and glutathione (GSH) levels. AMA inhibited the growth of HPF cells with an IC50 of ~150 µM at 24 h. AMA induced a G1 phase arrest of the cell cycle and it also triggered apoptosis accompanied by the loss of mitochondrial membrane potential (MMP; ΔΨm). AMA increased ROS levels including O2·− in HPF cells from the early time point of 25 min. It induced GSH depletion in HPF cells in a dose-dependent manner. Z-VAD (a pan-caspase inhibitor) did not significantly prevent cell death and MMP (ΔΨm) loss induced by AMA. N-acetylcysteine (NAC; an antioxidant) attenuated cell growth inhibition, death and MMP (ΔΨm) loss in AMA-treated HPF cells and NAC generally decreased the ROS level in these cells as well. Vitamin C enhanced cell growth inhibition, death, GSH depletion and O2·− levels in 100 µM AMA-treated HPF cells whereas this agent strongly attenuated these effects in 200 µM AMA-treated cells. In conclusion, AMA inhibited the growth of HPF cells via apoptosis as well as a G1 phase arrest of the cell cycle. AMA-induced HPF cell death was related to increased ROS levels and GSH depletion.

Introduction

Reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), superoxide anion (O2·−) and hydroxyl radical (‘OH) are involved in diverse cellular proceedings of differentiation, cell proliferation and cell death. Redox status changes in tissues and cells influence the production and metabolism of ROS. ROS are primarily generated during the mitochondrial respiration and are specifically made by various oxidases (1). Superoxide dismutases (SODs) [cytoplasmic (SOD1), mitochondrial (SOD2) or extracellular (SOD3) isoforms] metabolize O2·− to H2O2 (2). Further metabolism of H2O2 by catalase (CAT) or glutathione (GSH) peroxidase (GPX) yields O2 and H2O (3). Particularly, thioredoxin (TXN) system consisting of TXN, TXN reductase (TXNR) and NADPH critically regulates cellular redox homeostasis (4). TXN as a thiol reductase acts as a potent anti-oxidant as well as a scavenger against ROS (4). Oxidative stress due to either overproduction of ROS or accumulation of them can initiate events that lead to cell death.

Antimycin A (AMA) derived from Streptomyces kitazawensis inhibits succinate and NADH oxidases and this agent also impedes mitochondrial electron transport via its binding to complex III (5). The inhibition of electron transport triggers a failure of the proton gradient across the mitochondrial inner membrane, thereby collapsing mitochondrial membrane potential (MMP; ΔΨm) (6,7). This inhibition can lead to the overproduction of ROS (7,8). Accordingly, oxidative stress and the collapse of MMP (ΔΨm) by AMA unlock the mitochondrial permeability transition pore (PTP), which is accompanied by the release of cytochrome c into the cytoplasm to induce apoptosis (9,10). In fact, AMA-induced apoptosis has been reported in a variety of cells including HeLa cervical cancer cells (11), As4.1 juxtaglomerular cells (12), HL60 leukemia cells (13) and Hep3B hepatoma cells (14) and normal endothelial cells (15).

Previously we reported that AMA reduces the growth of Calu-6 and A549 lung cancer cells via apoptosis and cell cycle arrest (16,17). AMA also increases ROS levels in A549 cells (17) and induces GSH depletion in Calu-6 cells (18). However, little is known about the cellular effects of PG on normal primary lung cells. Thus, we examined the effects of AMA on cell growth and death in human pulmonary fibroblast (HPF) cells in relation to ROS and GSH levels. In addition, we investigated the effects of N-acetylcysteine (NAC) and vitamin C...
Materials and methods

Cell culture. HPF cells purchased from PromoCell GmbH (Heidelberg, Germany) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Gibco BRL, Grand Island, NY, USA). HPF cells were used for experiments between passages four and eight.

Reagents. AMA purchased from Sigma-Aldrich Chemical Co. was dissolved in ethanol at 20 mM. Pan-caspase inhibitor (Z-VAD-FMK) was obtained from R&D Systems, Inc. (Minneapolis, MN, USA) and was dissolved in dimethyl sulfoxide (Sigma-Aldrich Chemical Co.). NAC and BSO were obtained from Sigma-Aldrich Chemical Co. NAC was dissolved in the buffer [20 mM HEPES (pH 7.0)]. BSO was dissolved in water. Vitamin C purchased from Riedel-de Haen (Hannover, Germany) was also dissolved in water. Based on the previous studies (19,20), cells were pretreated with or without 15 µM Z-VAD, 2 mM NAC, 10 µM BSO or 1 mM vitamin C for one hour prior to AMA treatment.

Cell growth inhibition assays. Cell growth changes were determined by measuring the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich Chemical Co.) dye absorbance as previously described (21). Cells were exposed to the indicated amounts of AMA (2-200 µM) with or without Z-VAD, NAC, vitamin C or BSO for 24 h.

Cell cycle and sub-G1 analysis. Cell cycle and sub-G1 cells were determined by propidium iodide (PI, Ex/Em=488 nm/617 nm; Sigma-Aldrich) staining as previously described (22). Cells were incubated with the indicated amounts of AMA (2-200 µM) in the presence or absence of Z-VAD, NAC, vitamin C or BSO for 24 h. The absence of rhodamine 123 indicated the loss of MMP (∆Ψm) in HPF cells. The MMP (∆Ψm) levels in the cells excluding MMP (∆Ψm) loss cells were expressed as mean fluorescence intensity (MFI), which was calculated by CellQuest software (Becton-Dickinson).

Detection of intracellular ROS levels. Intracellular ROS were detected by a fluorescent probe dye, 2’,7’-dichlorodihydrofluorescein diacetate (H2DCFDA, Ex/Em=495 nm/529 nm; Invitrogen Molecular Probes) as previously described (22). Dihydroethidium (DHE, Ex/Em=518 nm/605 nm; Invitrogen Molecular Probes) is a fluorogenic probe that is highly selective for O2·- - among ROS. Cells were incubated with the indicated doses of AMA (2-200 µM) in the presence or absence of Z-VAD, NAC, BSO or vitamin C for the indicated times. The fluorescence of DCF and DHE was detected using a FACStar flow cytometer (Becton-Dickinson). ROS levels were expressed as MFI.

Detection of intracellular GSH. Cellular GSH levels were analyzed using a 5-chloromethylfluorescein diacetate dye (CMFDA, Ex/Em=522 nm/595 nm; Invitrogen Molecular Probes) as previously described (21,22). Cells were incubated with the indicated doses of AMA (2-200 µM) in the presence or absence of Z-VAD, NAC, BSO or vitamin C for the indicated times. CMF fluorescence intensity was determined using a FACStar flow cytometer (Becton-Dickinson). Negative CMF staining (GSH depleted) cells were expressed as the percent of (-) CMF cells.

Statistical analysis. The results correspond to the mean of three independent experiments (mean ± SD). The data were analyzed using Instat software (GraphPad Prism4, San Diego, CA, USA). The Student’s t-test or one-way analysis of variance (ANOVA) with post hoc analysis using Tukey's multiple comparison test was used for parametric data. Statistical significance was defined as p<0.05.

Results

Effects of AMA on cell growth and cell cycle distribution in HPF cells. Firstly, we investigated the effect of AMA on the growth of HPF cells at 24 h. Based on MTT assays, AMA decreased HPF cell growth with an IC50 of ~150 µM in a dose-dependent manner (Fig. 1A). When cell cycle distributions was examined in AMA-treated HPF cells, AMA induced a G1 phase arrest of the cell cycle as compared with control cells (Fig. 1B). Furthermore, 200 µM AMA clearly increased the number of sub-G1 DNA content cells by ~10% as compared with that of control HPF cells (Fig. 1B). However, other doses of AMA did not increase the number of sub-G1 DNA content cells (Fig. 1B).
Effects of AMA on cell death, apoptosis-related proteins and MMP (ΔΨ)_m in HPF cells. It was determined whether AMA induces HPF cell death via apoptosis. As shown in Fig. 2A and C, AMA increased the numbers of Annexin V-FITC-positive cells in a dose-dependent manner. The elevated change was observed between 100 and 200 µM AMA treatment (Fig. 2A and C). Western blotting showed that a 32-kDa precursor (procaspease-3) slightly disappeared in AMA-treated positive cells in a dose-dependent manner. The elevated change was observed between 100 and 200 µM AMA treatment (Fig. 2A and C). Western blotting showed that a 32-kDa precursor (procaspease-3) slightly disappeared in AMA-treated
HPF cells, implying that caspase-3 was activated in these cells (Fig. 2D). The intact 116 kDa moiety of poly(ADP-ribose) polymerase (PARP) was decreased by AMA (Fig. 2D). Apoptosis is closely related to the collapse of MMP (ΔΨm). Correspondingly, 50-200 µM AMA significantly induced the loss of MMP (ΔΨm) in HPF cells (Fig. 2B and E). The percentage of MMP (ΔΨm) loss in 50 or 100 µM AMA-treated HPF cells was higher than those of Annexin V-FITC-positive cells in these cells (Fig. 2A-C and E). The levels of MMP (ΔΨm) in HPF cells excluding MMP (ΔΨm) loss cells were decreased by AMA in a dose-dependent manner (Fig. 2F).

Effects of AMA on ROS, GSH and antioxidant-protein levels in HPF cells. To assess intracellular ROS and GSH levels in AMA-treated HPF cells, we used H2DCFDA, DHE and CMF dyes. As shown in Fig. 3A, all the tested doses of AMA significantly increased ROS (DCF) level in HPF cells at 24 h whereas the level was not clearly changed at the treatment of 2, 10 or 50 µM AMA (Fig. 3C). Treatment with 50 and 200 µM AMA transiently increased O2−• level in HPF cells at 25 min whereas 10 µM AMA decreased the level at this time (Fig. 3D). At 180 min, all the tested doses of AMA increased O2−• levels in HPF cells and 200 µM AMA showed a strong effect (Fig. 3D). When antioxidant-protein levels were assessed in 150 µM AMA-treated HPF cells, the expression of SOD1 was not changed by AMA and SOD2 was slightly increased (Fig. 3E). In addition, AMA downregulated the expression of TXN and TXNR2 in HPF cells and it did not change that of TXNR1 (Fig. 3E).

Treatment with 50, 100 or 200 µM AMA significantly increased GSH depleted cell number in HPF cells (Fig. 3F). However, the lower doses of 2 or 10 µM AMA did not induce

Figure 3. Effects of AMA on ROS and GSH levels in HPF cells. Intracellular GSH and ROS levels were measured using a FACStar flow cytometer. (A and B) Graphs indicate DCF (ROS) levels (%) in HPF cells at 24 h (A) and at the early times (B). (C and D) Graphs indicate DHE (O2−•) levels (%) in HPF cells at 24 h (C) and at the early times (D). (E) Samples of protein extracts (10 µg) were resolved by SDS-PAGE gels, transferred onto PVDF membranes and immunoblotted with the indicated antibodies against SOD1, SOD2, TXN, TXNR1, TXNR2 and β-actin. (F and G) Graphs show (-) CMF (GSH-depleted) cells (%) at 24 h (F) and GSH levels at the early times (G). *p<0.05 compared with the control group.
GSH depletion in HPF cells as compared with control HPF cells (Fig. 3F). At the early time-points of 25-90 min, all the doses of AMA reduced GSH content level in HPF cells (Fig. 3G). There were increases in GSH levels in AMA-treated HPF cells at 120 min and the increases were attenuated at 180 min (Fig. 3G).

**Effects of Z-VAD, NAC, vitamin C or BSO on cell growth, cell death and MMP (ΔΨm) in AMA-treated HPF cells.** We examined the effect of Z-VAD, NAC, vitamin C and BSO on the growth and death of AMA-treated HPF cells. For this experiment, 100 or 200 µM AMA was used as a suitable dose to differentiate the levels of cell growth inhibition and death. Treatment with 15 µM Z-VAD slightly attenuated growth inhibition in AMA-treated HPF cells (Fig. 4). NAC strongly attenuated HPF cell growth inhibition by 100 or 200 µM AMA (Fig. 4). Vitamin C significantly enhanced cell growth inhibition in 100 µM AMA-treated HPF cells but this agent strongly prevented that in 200 µM AMA-treated HPF cells (Fig. 4). BSO slightly increased HPF cell growth inhibition by 100 µM AMA but it did not affect that in 200 µM AMA-treated HPF cells (Fig. 4). BSO alone slightly inhibited HPF control cell growth (Fig. 4).

In relation to cell death and the loss of MMP (ΔΨm), Z-VAD did not significantly influence HPF cell death by 100 or 200 µM AMA (Fig. 5A and B). NAC significantly attenuated apoptosis in 100 or 200 µM AMA-treated HPF cells.

![Figure 4. Effects of Z-VAD, NAC, vitamin C or BSO on cell growth in AMA-treated HPF cells. The graph shows cell growth changes in HPF cells as assessed by MTT assays. *p<0.05 compared with the control group. &p<0.05 compared with cells treated with 100 µM AMA.](image)

![Figure 5. Effects of Z-VAD, NAC, vitamin C or BSO on cell death and MMP (ΔΨm) in AMA-treated HPF cells. (A) Each figure shows Annexin V-FITC/PI cells. (B and C) Graphs show the percents of Annexin V-positive staining cells from A, (B) and rhodamine 123-negative [MMP (ΔΨm) loss] cells (C). *p<0.05 compared with the control group. &p<0.05 compared with cells treated with 100 µM AMA.](image)
PARk and YOU: AMA INHIBITS HPF CELL GROWTH

However, NAC did not increase the number of viable (Annexin V-negative and PI-negative) cells in 200 µM AMA-treated HPF cells but it increased that of necrotic (Annexin V-negative and PI-positive) cells in these cells (Fig. 5A). While vitamin C enhanced apoptosis in 100 µM AMA-treated HPF cells, this agent significantly decreased ROS (DCF) levels in 100 or 200 µM AMA-treated HPF cells whereas NAC strongly decreased O$_2^\bullet$- level in these cells (Fig. 6B). Vitamin C increased O$_2^\bullet$- level in 100 µM AMA-treated HPF cells, but BSO did not change the level in these cells (Fig. 6B). In 200 µM AMA-treated HPF cells, Z-VAD, NAC, vitamin C and BSO decreased O$_2^\bullet$- levels and vitamin C-treated group had a significant effect (Fig. 6B). NAC and vitamin C reduced basal O$_2^\bullet$- level in HPF control cells whereas BSO increased the level (Fig. 6B). In relation to GSH levels, NAC significantly decreased GSH depleted cell number in 100 µM AMA-treated HPF cells whereas vitamin C increased the number in these cells (Fig. 6C). Z-VAD and BSO did not affect the cell number (Fig. 6C). In 200 µM AMA-treated HPF cells, Z-VAD, NAC, vitamin C and BSO decrease GSH depleted cell number and NAC or vitamin C-treated groups showed significant effects (Fig. 6C).

Discussion

We demonstrated the molecular mechanism of AMA on HPF cell growth inhibition and death in relation to ROS and GSH levels. AMA dose-dependently decreased HPF cell growth with an IC$_{50}$ of ~150 µM. According to our previous reports (16,17), the IC$_{50}$s of AMA in A549 and Calu-6 lung cancer cells were ~2 and 100 µM at 24 h, respectively. Therefore, HPF cells seemed to be more resistant to AMA than the lung cancer cells of A549 and Calu-6. In addition, calf normal pulmonary artery endothelial cells were more resistant to AMA than Calu-6 cells (16). Since the activity of mitochondria is associated with a susceptibility to apoptosis (23), the difference of susceptibility to AMA among various different cancer and normal cell lines probably is due to the different basal activities of antioxidant enzymes and mitochondria each cell possesses. Furthermore, we observed that AMA altered the levels of SOD2 and TXNR2, which are specifically expressed in mitochondria to eliminate ROS.

Suppression of cell growth due to AMA can be explained in part by its capacity to affect the arrest during the cell cycle. For instance, 100 µM AMA inhibited HPF cell growth by >40% whereas this concentration increased the percentage of Annexin V staining cells by ≤10% as compared with the control cells. Therefore, G1 phase arrest of the cell cycle in AMA-treated HPF cells was considered as a pathway to suppress their growth. Similarly, AMA induces a G1 phase arrest in A549 and Calu-6 cells (16,17). On the other hand, AMA induces the S phase arrest in HeLa cells (24) and non-specifically induces the arrest in all the phases in As4.1 juxta-glomerular cells (25). These results suggest that the inhibition of mitochondrial electron transport by AMA can alter the cell cycle progression and the specificity of cell cycle arrest depends on differences in cell types. In addition, 2-100 µM AMA showing HPF cell growth inhibition did not increase the portion of sub-G1 DNA content cells but these doses increased

Effects of Z-VAD, NAC, vitamin C or BSO on ROS and GSH levels in AMA-treated HPF cells. Next, ROS and GSH levels

Figure 6. Effects of Z-VAD, NAC, vitamin C or BSO on ROS and GSH levels in AMA-treated HPF cells. (A-C) Graphs indicate ROS (as determined by DCF) levels (%) (A), DHE (O$_2^\bullet$-) levels (%) (B) and (-) CMF (GSH-depleted) cells (%) (C). *p<0.05 compared with the control group. #p<0.05 compared with cells treated with 100 µM AMA. &p<0.05 compared with cells treated with 200 µM AMA.
The Annexin V staining cell number. These results support the notion that Annexin V staining cells are a marker to detect early apoptotic cells ahead of observing the sub-G1 cells.

AMA induces the collapse of MMP ($\Delta \Psi_m$) during apoptosis in various cells including cancer cells (11,12,15-17). Likewise, AMA induced the loss of MMP ($\Delta \Psi_m$) in HPF cells and decreased its level in the viable HPF cells. Particularly, the percentage of MMP ($\Delta \Psi_m$) loss by 50 or 100 $\mu$M AMA was higher than those of Annexin V-FITC-positive cells, implying that AMA primarily damages the mitochondria function in HPF cells, resulting in the loss of MMP ($\Delta \Psi_m$) and consequently induction of apoptosis. The activation of caspase-3 and the cleavage of PARP protein are known to be essential in AMA-induced cell death (16). According to our results, AMA increased caspase-3 activity and decreased PARP protein in HPF cells. Z-VAD slightly attenuated growth inhibition and MMP ($\Delta \Psi_m$) loss in 100 $\mu$M AMA-treated HPF cells. However, Z-VAD did not prevent HPF cell death from AMA insult. Therefore, caspase activation was not tightly related to AMA-induced HPF apoptosis but its activation partially affected cell growth and mitochondria. Our previous report demonstrated that 15 $\mu$M Z-VAD including other caspase inhibitors significantly prevented apoptosis in AMA-treated Calu-6 cells (16). It is possible that the dose of 15 $\mu$M Z-VAD was not enough to prevent AMA-induced HPF cell death because the mechanism of cell death can be different between cancer and normal cells.

AMA can disturb the natural oxidation/reduction equilibrium in various cells via causing a breakdown in MMP ($\Delta \Psi_m$). AMA increases ROS levels in HeLa cells (11), endothelial cells (15), As4.1 juxtaglomerular cells (25) and A549 cells (8,17). Likewise, ROS levels (as determined by DCF) were dose-dependently increased in AMA-treated HPF cells at 24 h and ROS levels were also strongly increased at the earlier time-point of 25 min. In addition, $O_2^\cdot$ - level as determined by DHE was increased in AMA-treated HPF cells. Especially, 200 $\mu$M AMA strongly augmented $O_2^\cdot$ - level in HPF cells as compared with 100 $\mu$M AMA-treated cells. In addition, 200 $\mu$M AMA transiently increased $O_2^\cdot$ - level at 25 min. All the tested doses of AMA increased $O_2^\cdot$ - levels at 180 min. The increased $O_2^\cdot$ - levels in AMA-treated HPF cells probably resulted from the enhanced production of $O_2^\cdot$ - itself rather than the reduction of SOD activity because MMP ($\Delta \Psi_m$) loss was dose-dependently observed in these cells and the expression of SOD1 and SOD2 were not downregulated by AMA. Moreover, it is likely that 200 $\mu$M AMA directly damaged mitochondria and increased the generation of $O_2^\cdot$ - at 25 min, consequently resulting in an increase in ROS (DCF) levels at the early time phases of 25-180 min. TXN and TXNRs can stimulate cell proliferation and confer resistance to anticancer drugs (26,27). Our results showed that AMA downregulated TXN and TXNR2 levels. Therefore, the downregulation of TXN and TXNR2 due to treatment with AMA might make HPF cells more sensitive to this agent. In addition, it seems that AMA altered the levels of SOD2 and TXNR2 in the mitochondria of HPF cells since these antioxidant enzymes specifically maintain the redox state in mitochondria.

NAC generally attenuated ROS levels in AMA-treated or -untreated HPF cells. It also prevented cell growth inhibition, apoptosis and MMP ($\Delta \Psi_m$) loss in 100 or 200 $\mu$M AMA-treated HPF cells. Interestingly, NAC induced necrotic cell death in 200 $\mu$M AMA-treated HPF cells. In addition, 5 mM NAC strongly induced necrotic cell death in 200 $\mu$M AMA-treated HPF cells, and it decreased ROS (DCF) levels, but strongly increased $O_2^\cdot$ - level in these cells (data not shown). Treatment with 2 or 5 mM NAC was likely to change cell death pathway (switching apoptotic cell death to necrotic cell death) in 200 $\mu$M AMA-treated HPF cells via affecting different ROS levels (DCF or DHE levels). In addition, the other antioxidant vitamin C slightly attenuated ROS (DCF) level in 100 or 200 $\mu$M AMA-treated HPF cells. Interestingly, vitamin C enhanced cell growth inhibition, apoptosis and MMP ($\Delta \Psi_m$) loss in 100 $\mu$M AMA-treated HPF cells and increased $O_2^\cdot$ - level in these cells. However, vitamin C strongly attenuated $O_2^\cdot$ - level in 200 $\mu$M AMA-treated HPF cells and this agent reduced growth inhibition, apoptosis and MMP ($\Delta \Psi_m$) loss in these cells. The change of $O_2^\cdot$ - level rather than ROS (DCF) level due to vitamin C seemed to be more closely related to AMA-induced HPF cell death. These results implied that vitamin C can be an oxidant or an antioxidant in HPF cells depending on the doses of AMA. Although vitamin C possesses strong antioxidant properties, the mechanism underlying these properties is still unclear in vivo and in vitro (28,29). Z-VAD did not significantly change ROS and cell death levels in AMA-treated HPF cells. BSO showing a slight increase in HPF cell death by 100 $\mu$M AMA increased strongly ROS (DCF) level, but this agent did not affect ROS level and cell death in 200 $\mu$M AMA-treated HPF cells. Collectively, AMA-induced HPF cell death is tightly correlated with the generation of ROS. BSO alone induced cell death in HPF control cells which was accompanied by the production ROS including $O_2^\cdot$ -. Therefore, ROS increased by BSO might be related to HPF cell death (30,31).

AMA increased the number of GSH-depleted cells in HPF cells. As expected, NAC showing an anti-apoptotic effect on AMA-treated HPF cells significantly prevented GSH depletion in these cells. In addition, vitamin C augmented GSH depletion in 100 $\mu$M AMA-treated HPF cells and this agent attenuated the depletion induced by treatment with 200 $\mu$M AMA. BSO showing non-effects on AMA-induced HPF cell death did not change GSH depleted cell number in these cells. These results suggest that the intracellular GSH content has a decisive role on AMA-induced HPF cell death. It is of note that BSO did not significantly affect GSH contents in AMA-treated or -untreated HPF cells. However, our previous reports demonstrate that BSO enhanced GSH depletion as well as cell death in AMA-treated Calu-6 cells (32) and gallic acid-treated HPF cells (33). These data imply that BSO differently influences GSH content levels depending on cell types. The decreased GSH levels in AMA-treated HPF cells at the early time phases (25-90 min) probably resulted from its consumption against increased ROS levels by AMA. On the whole, GSH and ROS levels were inversely related in AMA-treated HPF cells, implying that GSH and ROS in these cells influence each other in the early time-points.

In conclusion, AMA inhibited the growth of HPF cells via apoptosis as well as a G1 phase arrest of the cell cycle. AMA-induced HPF cell death was related to increases in ROS level and GSH depletion. Our current results provide practical information to appreciate the cellular effect of AMA on normal lung cells in relation to ROS and GSH.
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References