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Anti-tumour effects of HL-37, a novel anthracene derivative, in-vivo and in-vitro

Song-Qiang Xie, Guo-Qiang Hu, Zhong-Quan Zhang, Mei Xu and Bian-Sheng Ji

Abstract

Many anthracene derivatives possess excellent anti-tumour activity and are extensively used clinically as anti-tumour agents. However, their clinical use is frequently limited by emergence of multidrug resistance (MDR) in tumour cells. Therefore, new agents with the ability to overcome MDR are needed for cancer treatment. HL-37, a novel anthracene derivative, exhibited potent anti-cancer activity in both drug-sensitive (K562) and multidrug-resistant (K562/DOX) leukaemia cells. Mechanistically, we found that HL-37 was neither a substrate nor an inhibitor of P-glycoprotein (P-gp) and could overcome apoptotic resistance via up-regulation of p53 protein and down-regulation of Bcl-xL protein. In addition, HL-37 also induced K562/DOX cell apoptosis and a decrease in G_0/G_1 phase. Moreover, reduction of mitochondrial membrane potential, release of cytochrome *c* and an increased expression of cleaved protein fragment of caspase-3, caspase-9 and caspase-8 were also observed. Importantly, HL-37 was found to be better tolerated and more effective at inhibiting tumour growth than bisantrene in a xenograft mouse model.

Introduction

Multidrug resistance (MDR) is now recognized as one of the most common causes of failture in cancer chemotherapy. The MDR phenotype results from cross-resistance to a variety of structurally and functionally unrelated anti-cancer drugs used for anti-tumour therapy (Wang et al 2007a). MDR in cancer, especially in leukaemia, represents a major obstacle to successful chemotherapy (Zhou et al 2006). Chronic myelogenous leukaemia (CML) is a myeloproliferative disorder. The K562 cell line derived from humans with chronic myeloid leukaemia in blast crisis, representing a widely used in-vitro model system for CML, was also shown to be refractory to apoptosis induced by many anti-tumour drugs (Czyz et al 2005). Recent evidence has shown that membrane pump extrusion of chemotherapeutic agents and resistance to apoptosis are two main mechanisms common to most cancers. The main mechanism of non-pump resistance is the activation of cellular anti-apoptotic defence, such as Bcl-xL up-regulation and p53 down-regulation (Wang et al 2007a). Previous study indicated that the over-expression of the anti-apoptotic members of the Bcl-2 family, particularly the over-expression of Bcl-xL, was related to MDR (Czyz et al 2005). Normal p53 protein is activated in response to DNA damage and its alterations result in cell-cycle arrest or apoptosis. Disturbed or impaired p53 function may prevent the cells from being able to die by apoptosis or to stop cell-cycle checkpoints after damage (Foroutan et al 2007). The inactivation of p53 by mutations can cause resistance to doxorubicin in-vivo and the mutational status of p53 might be associated with drug resistance in human tumours (Yu et al 2007). Also associated, importantly, is over-expression of membrane transporters, such as P-glycoprotein (P-gp), the product of the MDR1 gene (Kostrzewa-Nowak et al 2007). P-gp is a 170 kDa transmembrane phospho-glycoprotein and a transmembrane ATP-dependent efflux pump that has broad substrate specificity. Increased amounts of P-gp confer MDR in cells by reducing intracellular accumulation of

Institute of Pharmacy, Henan University, Kaifeng 475001, China

Song-Qiang Xie, Guo-Qiang Hu, Zhong-Quan Zhang, Mei Xu, Bian-Sheng Ji

Correspondence: B. S. Ji, Institute of Pharmacy, Henan University, Kaifeng 475001, The People's Republic of China.
E-mail: jibiansheng@yahoo.com.cn

a variety of cytotoxic drugs, such as anthracyclines, mitoxantrone, bisantrene and taxanes, as well as vinca alkaloids (Tarasiuk et al 2004). Clinically, P-gp was shown to be expressed in acute myelocytic leukaemia cells in approximately 30% of patients when diagnosed and over 50% at relapse (Gatouillat et al 2007).

To overcome MDR and improve the efficacy of chemotherapy, several strategies have been developed. One of the effective ways to overcome P-gp-mediated drug resistance is either to block its drug pump function or to inhibit its expression. A variety of agents are able to inhibit P-gp-mediated drug efflux (Kostakis et al 2005), including calcium-channel blockers such as verapamil (Salmon et al 1991) or the immunosuppressant ciclosporin and its non-immunosuppressive analogue PSC-833 (Egashira et al 1999). Although various MDR-reversal agents have been identified and some are undergoing clinical trials, clinical application of these agents has not been extensively pursued up to date, owing to their unwanted and sometimes life-threatening side effects (Saponara et al 2007), or serious drug–drug interaction between the P-gp inhibitor and leukaemia-treating drugs that are P-gp substrates (Chau et al 2007). Therefore, developing novel anti-cancer drugs that are not a substrate for P-gp (Ozben 2006) or that overcome apoptotic resistance (Wang et al 2005) is another notable strategy for overcoming MDR.

Apoptosis can be triggered by several stimuli and controlled by two major pathways, namely the membrane death receptor pathway and the mitochondrial pathway (Pan et al 2006). In the former, procaspase-8 is activated as an apoptotic initiator and then activated caspase-8 activates downstream procaspase-3. In the latter, the loss of mitochondrial membrane potential (MMP) induces cytochrome *c* release from mitochondrion to cytosol, where it binds to apoptotic protease activation factor (Apaf)-1. Meanwhile, procaspase-9 also binds to the protein Apaf-1 and this interaction activates procaspase-9. Activated caspase-9 further activates downstream procaspase-3 that eventually leads to apoptosis (Wang et al 2007b).

Therefore, new agents with the ability to overcome MDR are needed for leukaemia treatment. A variety of anthracene-containing derivatives possess excellent anti-cancer activity, such as bisantrene and mitoxantrone, as well as anthracyclines, and are extensively used for treatment of cancer (Tarasiuk et al 2005). However, their clinical use is frequently limited by serious side effects or by emergence of MDR in tumour cells (Zhang et al 1994). Many researchers have explored ways to modify the structure of anthracycline to generate various analogues to reduce side effects and reverse MDR. However, these efforts have had only limited success (Wunz et al 1990; Zhu et al 2005). Despite this, in fact, an intense search for novel anthracene derivatives beneficial to the design of anti-cancer drugs has been sparked and has received extensive attention. In an effort to design and synthesize novel types of agents, many researchers have now turned their attention to non-anthracycline anthracene-containing derivatives, due to the chemical reactivity of the anthracene skeleton, which allows its modification with a variety of substituents in the reactive positions 9 and 10, and the anthracene ring system is suitable for intercalation into the DNA helix (Kozurkova et al 2007). Recently, there have been reports of the design and synthesis of many anthracene derivatives that possess remarkable ability to overcome MDR activity (Bu et al 2005; Zhang et al 2006; Pourpak et al 2007). HL-37, a novel anthracene derivative, was found to display an excellent anti-cancer activity in both drug-sensitive (K562) and multidrug-resistant leukaemia cells (K562/DOX). However, the precise mechanism of the action mentioned above was not well elucidated. This study investigates the anti-tumour activity and apoptotic pathway of HL-37 in-vitro and in-vivo.

Materials and Methods

Materials

Human myelogenous leukaemia K562 cells and their multidrug-resistant K562/DOX cells were purchased from Shanghai Institutes for Biological Science, Chinese Academy of Sciences (Shanghai, China). RPMI 1640 and fetal calf serum (FCS) were purchased from Gibco (USA). Primary antibodies against caspase-3, caspase-8, caspase-9, p53, Bcl-xL, cytochrome *c* and peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibody were purchased from Santa Cruz Biotechnology (USA). Primary monoclonal antibody against P-gp (C219) was purchased from Calbiochem-Novabiochem (Seattle, WA). All other chemicals used in the experiments were commercial products of reagent grade.

Synthesis route of HL-37

HL-37 (Figure 1) was synthesized by the treatment of pyridinyl-s-triazole thiol (Hu et al 2005) with 2-methoxyethoxy chloromethane followed condensation with 9-formyl anthracene.

Cell culture

K562 cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS at 37°C in a humidified atmosphere of 5% CO_2. K562/DOX cells were cultured in the presence of 0.5 μM doxorubicin and were grown in drug-free medium 2 weeks before the experiments (Ji & He 2007).

Cytotoxicity assay

The anti-proliferative ability of HL-37 was evaluated in K562 cells and K562/DOX cells by the conversion of MTT to a purple formazan precipitate as previously described (Ji et al

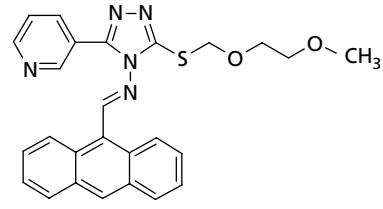

Figure 1 Structure of HL-37 and bisantrene.

2005). Briefly, cells were seeded into 96-well plates (5×10^4 cells/well). Various concentrations of HL-37 were subsequently added and incubated for 48 h. The inhibitory rate was calculated from plotted results using untreated cells as 100%.

Intracellular HL-37 accumulation assay in K562 cells and K562/DOX cells

The K562 cells and K562/DOX cells at a density of 1×10^5 cells/mL in exponential growth were used for the test. Cells were incubated in the presence of 1 μM HL-37 or 1 μM rhodamine (Rh123) at 37°C for 60 min. The intracellular mean fluorescence intensity (MFI) associated with HL-37 or Rh123 was measured with a FACScan flow cytometer (Becton Dickinson). Excitation was performed by an argon ion laser operating at 488 nm; the emitted fluorescence was collected through a 615-nm or 530-nm pass filter for the HL-37 and Rh123 assay, respectively. Data analysis was performed using Cell Quest software (Ji & He 2007).

Flow cytometric apoptosis and cell cycle analysis

The K562/DOX cells at a density of 5×10^5 cells/mL in exponential growth were exposed to indicated concentrations of HL-37 for 36 h, and then the cells were collected and fixed with ice-cold 70% ethanol at −20°C overnight. The cells were treated with 50 $\mu g\,mL^{-1}$ RNase A at room temperature for 30 min, and then 100 $\mu g\,mL^{-1}$ propidium iodide (PI) was added. The remaining DNA content was measured using FACScan flow cytometry (Becton Dickinson) (Ji et al 2005). An additional evaluation of apoptosis was performed by means of the AnnexinV-FITC and PI double stain. Cells were exposed to HL-37 at indicated concentrations for 36 h, then harvested and stained according to manufacturer's instruction (Jin et al 2006).

Assessment of the change of MMP

MMP was measured using flow cytometry with Rh123 and PI double stain (Ren et al 2006). About 1×10^6 cells treated for 24 h were harvested and incubated with Rh123 (10 $\mu g\,mL^{-1}$) at 37°C for 30 min, then PI (10 $\mu g\,mL^{-1}$) was added and incubated for 5 min. The percentages of $Rh123^-/PI^+$ and $Rh123^-/PI^-$ presented the effective collapsed MMP.

Western blots

Cells, treated with different concentrations of HL-37 for 24 h or 48 h, were harvested and washed with PBS. Cytosolic and mitochondrial fractions were prepared as described previously (Min et al 2006). The detection of cytochrome *c* in the cytosolic and mitochondrial fractions was analysed by western blot. Total cellular protein was isolated using the protein extraction buffer (containing 150 mM NaCl, 10 mM Tris (pH 7.2), 5 mM EDTA, 0.1% Triton X-100, 5% glycerol and 2% SDS). Protein concentrations were determined using the protein assay kit. Equal amounts of proteins (50 μg/lane) were fractionated using 8–12% SDS-PAGE and transferred to PVDF membranes. The membranes were incubated with primary antibodies against caspase-3, caspase-9, caspase-8, p53, Bcl-xL and P-gp, as well as cytochrome *c* (1 : 5000). After washing with PBS, the membranes were incubated with peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibody (1 : 3000), followed by enhanced chemiluminescence staining through the enhanced chemiluminescence system. Actin was used to normalize for protein loading (Huang et al 2004).

Animal models for therapeutic study

SPF male ICR mice were purchased from the laboratorial animal center of Beijing (Beijing, China). All animal studies were carried out in a pathogen-free barrier zone at Henan university in accordance with the procedure outlined in the Guide for Care and Use of Laboratory Animals. Mice were allowed free access to sterilized animal chow and water and were housed at 23 ± 0.5°C, 10% humidity under a 12-h light–dark cycle. The effects of HL-37 on tumour growth and host survival were measured by evaluating tumour volume, tumour weight and percentage increase in the lifespan of tumour hosts. For solid tumour development, the ICR mice (6–8 weeks old, 18–22 g) were injected subcutaneously with 2×10^6 of sarcoma 180 cells. The day after inoculation, mice were randomized into five groups and were injected intraperitoneally with HL-37 (3.125, 6.25 and 12.5 $mg\,kg^{-1}$), physiologic saline or bisantrene (6.25 $mg\,kg^{-1}$) for 10 consecutive days. On day 11, the mice were killed by cervical dislocation, and solid tumours were removed and weighed. The inhibitory rate was calculated as follows: inhibitory rate (%) = $[(A–B)/A] \times 100$, where A is the mean tumour weight of the negative control group, and B is that of the HL-37-treated or positive group (Liu et al 2004). The size of tumours in all groups was measured using a digital caliper and the volume of tumours was determined using the formula $width^2 \times length \times 0.52$. For calculating the survival time, ICR mice were inoculated intraperitoneally with 1×10^6 sarcoma 180 cells/mouse on day 0 and the treatment with three doses of HL-37 (3.125, 6.25 and 12.5 $mg\,kg^{-1}$, i.p.) was started 24 h after inoculation for 10 consecutive days. The control group was treated with physiologic saline or bisantrene (6.25 $mg\,kg^{-1}$). The median survival time (MST) for each group was observed and the anti-tumour activity of HL-37 was evaluated by measuring the increase in lifespan.

Data analysis

All data are presented as mean ± s.d. and analysed using Student's *t*-test or analysis of variance followed by *q*-test.

Results

Effect of HL-37 on cell proliferation

We firstly examined the effect of HL-37 on K562 cells and K562/DOX cell proliferation by MTT assay. The IC50 values (concentration resulting in 50% inhibition of cell growth) for K562 cells and K562/DOX were 0.09 ± 0.01 μM and 0.11 ± 0.02 μM, respectively, for a 48-h treatment. The data suggested that HL-37 had similar cytotoxicity to drug-sensitive parental K562 cells and K562/DOX MDR cells.

Intracellular HL-37 accumulation

As shown in Figure 2, no difference in HL-37-associated MFI was found between K562 cells and K562/DOX cells, whereas the Rh123-associated MFI in K562 cells was significantly higher than that in K562/DOX cells, indicating that P-gp was over-expressed in K562/DOX cells and HL-37 cannot be transported by P-glycoprotein.

Effect of HL-37 on cell apoptosis and cell cycle perturbation

Cell cycle analysis was performed using a flow cytometer. The percentage of G_0/G_1 phase cells at 0, 0.02, 0.04 and 0.08 μM HL-37 and 50 μM bisantrene was 75.98 ± 1.39, 66.31 ± 1.09, 57.92 ± 1.13, 51.29 ± 1.04 and 73.36 ± 1.24%, respectively. By contrast, the respective percentage of sub-G_1 cells was 1.38 ± 0.26, 14.75 ± 0.64, 23.91 ± 1.09, 33.42 ± 1.21 and 3.68 ± 0.27%. This suggested that HL-37 could induce G_0/G_1 phase cell decrease and apoptotic cell increase in a dose-dependent manner, but bisantrene could not, in K562/DOX cells.

For a further assessment of apoptosis induced by HL-37, we examined the exposure of phosphatidylserine on the cell surface by using PI and Annexin V-FITC double staining. By using this assay, it is possible to differentiate between viable cells (Annexin V^-/PI^-), early apoptotic cells (Annexin V^+/PI^-) and late apoptotic/necrotic cells (Annexin V^+/PI^+). Flow cytometric analysis revealed that the percentage of Annexin-V-stained cells increased with concentration in HL-37-treated cells. The percentage of early and late apoptotic K562/DOX cells was 2.52 ± 0.19% and 0.95 ± 0.06% in the control, 6.51 ± 0.44% and 3.07 ± 0.28% at 0.02 μM HL-37, 13.61 ± 1.03% and 4.48 ± 0.42% at 0.04 μM HL-37, 29.83 ± 1.52% and 9.13 ± 0.73% at 0.08 μM HL-37 and 3.46 ± 0.23% and 1.36 ± 0.08% at 50 μM bisantrene, respectively.

Effect of HL-37 on MMP change

The disruption of mitochondrial integrity is one of the early events leading to apoptosis. To assess whether HL-37 affects the function of mitochondria, the MMP was analysed by FACScan flow cytometry. After the cells were treated with the desired concentrations of HL-37 for 24 h, the concentration-dependent decreases of MMP were observed in K562/DOX cells. The percentage of $Rh123^-/PI^-$ and $Rh123^-/PI^+$ K562/DOX cells was 0.46 ± 0.03% and 0.38 ± 0.02% in the control, 8.72 ± 0.64% and 0.49 ± 0.04% at 0.02 μM HL-37, 23.61 ± 1.75% and 4.57 ± 0.41% at 0.04 μM HL-37, 35.89 ± 2.78% and 6.36 ± 0.47% at 0.08 μM HL-37 and 0.82 ± 0.03% and 0.47 ± 0.01% at 50 μM bisantrene, respectively. These results indicated that HL-37-induced apoptosis was involved in mitochondrial dysfunction in K562/DOX cells.

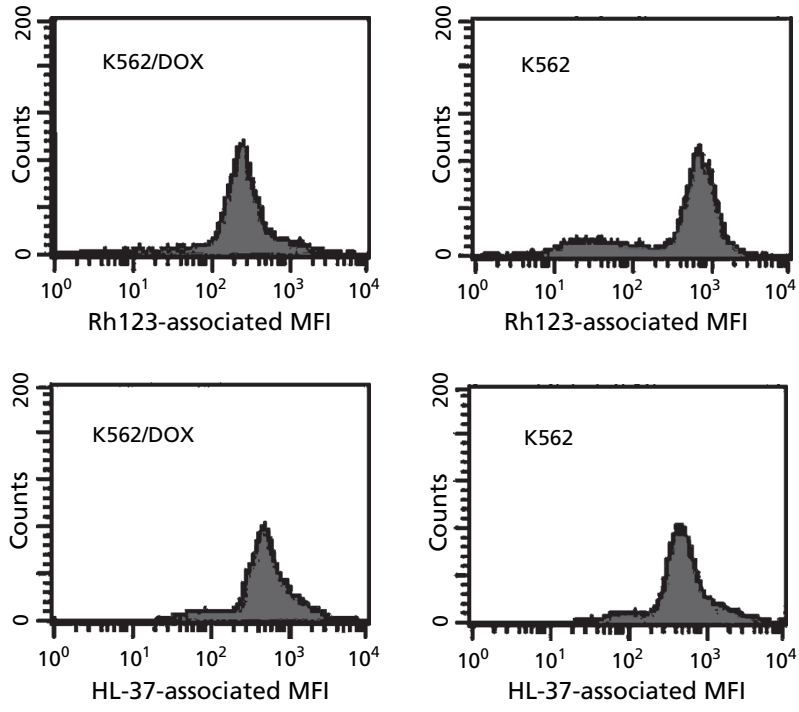

Figure 2 Intracellular mean fluorescence intensity of HL-37 and Rh123. Cells were incubated in the presence of 1 μM HL-37 or 5 μM Rh123 at 37 °C for 60 min. The intracellular MFI associated with HL-37 and Rh123 was measured with a FACScan flow cytometer. Excitation was performed by an argon ion laser operating at 488 nm, and the emitted fluorescence was collected through a 615-nm or 530-nm pass filter for the HL-37 and Rh123 assay, respectively.

Effect of HL-37 on P-gp, p53 and Bcl-xL protein expression

It is well known that K562/DOX cells are apoptosis resistant because of their Bcr-Abl positive, p53 negative and P-gp over-expression. We detected the protein expression of P-gp, p53 and Bcl-xL to discover the possible target of HL-37-mediated apoptosis in K562/DOX cells. These data demonstrated that no effect of HL-37 on P-gp expression was observed on K562/DOX cells, whereas the expression of p53 protein was up-regulated and Bcl-xL protein was down-regulated in a concentration-dependent manner (Figure 3).

Effect of HL-37 on cytochrome *c* and caspases protein expression

A drop in the MMP is usually accompanied by release of cytochrome *c* from mitochondria to cytosol. As shown in Figure 4, HL-37 induced cytochrome *c* release from mitochondria to cytosol concomitant with the related decrease of cytochrome *c* in mitochondria. To determine whether caspases are involved in the HL-37-induced apoptosis in K562/DOX cells, we examined the activation of caspase-8, caspase-9 and caspase-3 in response to HL-37. Our results showed a significant increase in the level of the cleaved fragment of caspase-9, caspase-8 and caspase-3 in the cells treated with HL-37 (Figure 4).

Inhibition of tumour growth in mice bearing sarcoma 180 cells

To evaluate the effect of HL-37 on tumour growth, ICR mice inoculated with sarcoma 180 cells were treated with HL-37. HL-37 reduced tumour weight by 66.72% (0.45 ± 0.04 g), 52.94% (0.64 ± 0.04 g) and 32.13% (0.92 ± 0.07 g), at the dose of 12.5, 6.25 and 3.125 mg kg^{-1} after treatment, respectively, while bisantrene decreased tumour weight by 38.47% (0.83 ± 0.07 g) at the dose of 6.25 mg kg^{-1}, compared with the control (1.35 ± 0.12 g). Similarly, the tumour volume in groups treated with bisantrene (6.25 mg kg^{-1}) and HL-37 (12.5, 6.25 and 3.125 mg kg^{-1}) decreased by 18.62% (504.37 ± 29.67 mm^3), 35.73% (397.84 ± 20.36 mm^3), 25.71% (459.27 ± 30.59 mm^3) and 15.47% (523.68 ± 33.76 mm^3), compared with the control group (618.49 ± 39.48 mm^3), respectively. There was no statistically significant difference in the body weight of mice between the negative control group and the HL-37-treated group. However, bisantrene markedly decreased the body weight of mice at the dose of 6.25 mg kg^{-1}, compared with the negative control group ($P < 0.01$).

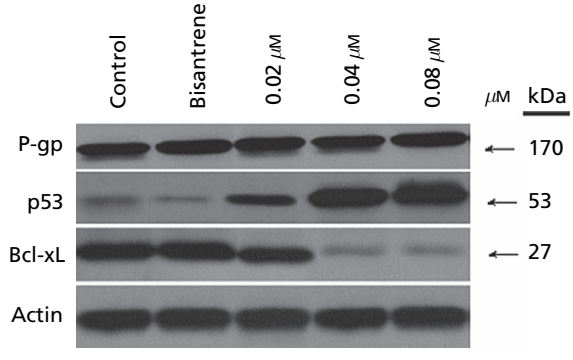

Figure 3 Protein expression of P-gp, p53 and Bcl-xL in K562/DOX cells treated with HL-37 at indicated doses for 48 h. Equal amounts (50 μg/lane) of cellular protein were fractionated on 8–12% SDS-PAGE gels and transferred to PVDF membranes as described in Materials and Methods. Actin protein was blotted as a control.

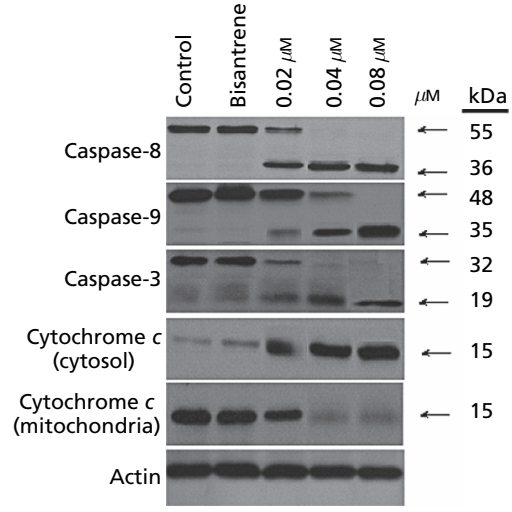

Figure 4 Protein expression of caspase-3, -8 and -9 in K562/DOX cells treated with HL-37 at indicated doses for 48 h (cytochrome *c* for 24 h). Equal amounts (50 μg/lane) of cellular protein were fractionated on 8–12% SDS-PAGE gels and transferred to PVDF membranes as described in Materials and Methods. Actin protein was blotted as a control.

Enhancement of survival time in sarcoma 180 ascitic tumour

Mean survival time (MST) in the control mice was 14.2 ± 1.1 days, while it was increased in a dose-dependent manner by treatment with HL-37. HL-37 increased the lifespan by 1.6-(23.4 ± 1.3 days), 2.1-(29.4 ± 1.2 days) and 2.4-(34.4 ± 1.3 days) fold at the doses of 3.125, 6.25 and 12.5 mg kg^{-1}, and bisantrene at the dose of 6.25 mg kg^{-1} increased lifespan by 2.0-fold (28.5 ± 1.2 days), compared with that of the control group, respectively.

Discussion

Anthraquinone derivatives have proved useful as chemotherapeutic drugs in treating many kinds of cancers. This has led to numerous synthetic and pharmacological studies on the tumoricidal mechanism of these analogues. However, the emergence of MDR is an obstacle for effective leukaemia treatment. Many novel anthracene derivatives have therefore been synthesized to overcome MDR (Tarasiuk et al

2004; Pourpak et al 2007). Our results showed that the IC50 values of HL-37 in both drug-sensitive and drug-resistant cells are proximal; this result is similar to that found for amonafide, a unique topoisomerase II catalytic inhibitor, in murine leukaemia cells (L1210 and L1210/MDR) (Chau et al 2007), suggesting that HL-37 may overcome MDR in K562/DOX cells. Furthermore, HL-37 exhibited stronger cytotoxic activity in K562/DOX cells as compared with 3′-azidodaunorubicin, a novel daunorubicin analogue (Zhang et al 2006).

It is well known that the MDR of K562/DOX cells is due to the expression of P-gp. There are two general types of assay used to document the presence of P-gp – Western blot assay for quality of P-gp but not its activity and exclusion of Rh123 assay for functional activity of P-gp (Kanofsky & Sima 2000). Our data demonstrated that P-gp is over-expressed in K562/DOX cells via the two assays mentioned above. To elucidate the reason for the similar IC50 values, we detected the intracellular accumulation of HL-37 in K562 cells and K562/DOX cells and the effect of HL-37 on P-gp expression. The results showed that the intracellular accumulation of HL-37 was similar in K562 cells and K562/DOX cells and no effects of HL-37 on P-gp expression was found, suggesting that HL-37 was neither a substrate nor an inhibitor of P-gp, which was similar to findings with amonafide (Chau et al 2007) and ethonafide, an anthracene-containing derivative (Pourpak et al 2007). To explore the mechanism responsible for the cytotoxicity of HL-37, the apoptosis and cell cycle distribution of K562/DOX cells were assessed. Flow cytometry analysis revealed that HL-37 markedly induced K562/DOX cell apoptosis and S-phase cell cycle arrest accompanied by a decrease in G_0/G_1 phase, and the proportion in G_2/M slightly increased. These effects were similar to those of mitoxantrone, an anthracene derivative (Potter & Rabinovitch 2005).

K562/DOX cells are characterized by a chromosomal translocation, resulting in constitutively activated Bcr-Abl tyrosine kinase. The kinase activity of Bcr-Abl maintains a high expression level of the anti-apoptotic protein Bcl-xL, which confers resistance to apoptosis induced by anti-cancer drugs (Czyz et al 2005). Our study indicated that HL-37 could induce the protein expression of Bcl-xL down-regulation. The protein p53 plays a pivotal role in cellular responses to DNA damage and defects in K562/DOX cells. We noticed that there was a significant increase in p53 protein level after application of HL-37. These data demonstrated that HL-37 could overcome the apoptotic resistance in K562/DOX cells. These effects were similar to those observed with arsenic trioxide (Wang et al 2005).

A previous report showed that doxorubicin could trigger apoptosis in cancer cells via both the death receptor and the mitochondrial pathway (Li et al 2006). To reveal the precise molecular mechanism of HL-37-induced apoptosis in K562/DOX cells, we observed the effect of HL-37 on MMP, cytochrome *c*, and activity of caspases. Our results showed that HL-37 decreased the MMP, and increased the level of cytochrome *c*, in the cytoplasm with a corresponding decrease of cytochrome *c* in mitochondria. To determine whether caspases are involved in the HL-37-induced apoptosis in K562/DOX cells, the protein expression of caspase-8, -9 and -3 was measured. Our data demonstrated that HL-37 could activate caspase-9, caspase-8 and caspase-3, and the cleaved fragment of caspase-9, caspase-8 and caspase-3 were detected.

Citarelia et al (1982) reported that bisantrene displayed significant activity against experimental tumours in-vivo when given by the intraperitoneal route and that the optimal dose is $6.25\,mg\,kg^{-1}$. We found that HL-37 was highly active against intraperitoneally and subcutaneously inoculated sarcoma 180 tumours in mice when administered intraperitoneally for 10 consecutive days. In addition, HL-37 had little effect on the body weight of ICR mice compared with the control group, although bisantrene markedly extenuated body weight at the same dose. This was similar with thonafide against SCID mice xenograft MCF-7 breast or A549 non-small cell lung cancer model (Pourpak et al 2007).

In conclusion, our data demonstrated that HL-37 displayed strong anti-tumour activity in-vitro and in-vivo. HL-37 was neither a substrate nor an inhibitor of P-gp and could overcome apoptotic resistance in-vitro. HL-37 induced K562/DOX cell apoptosis via the mitochondrial pathway and membrane death receptor pathway. Importantly, HL-37 possessed an excellent sensitivity toward multidrug-resistant cells and a better tolerance in ICR mice bearing sarcoma 180 tumours. This may be important for the action mechanism of anthracene derivatives and further efforts to explore their development appear warranted.

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