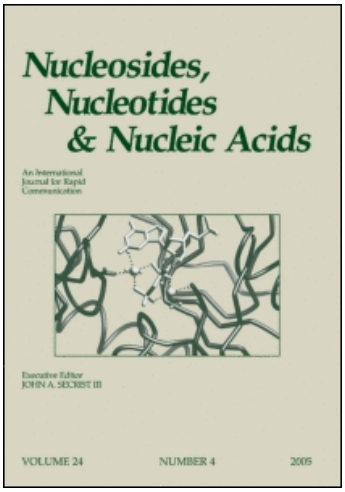

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[a] State Key Laboratory of Bioreactor Engineering, Institute of Biochemistry, East China University of Science and Technology, Shanghai, China [b] Jiangxi-OAI Gemeinsames Institute, Nanchang University, Nanchang, China

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An Antisense Oligodeoxynucleotide-Doxorubicin Conjugate: Preparation and Its Reversal Multidrug Resistance of Human Carcinoma Cell Line In Vitro

Yuhong Ren,[1,2] Dongzhi Wei,[1,*] Jianwen Liu,[1] and Wu Su[1]

[1]State Key Laboratory of Bioreactor Engineering, Institute of Biochemistry, East China University of Science and Technology, Shanghai, China
[2]Jiangxi-OAI Gemeinsames Institute, Nanchang University, Nanchang, China

ABSTRACT

An antisense oligodeoxynucleotide—doxorubicin conjugate was synthesized by an aminocaproic acid linker. The synthetic conjugate was identified by HPLC analysis and UV-vis spectra. Properties of the conjugate in vitro conditions were investigated. The results demonstrated that the conjugate was remarkably stabilized by doxorubicin. When incubated in Dulbecco Phosphate-Buflered Saline (pH 7.4) at 37°C, the conjugate was more stable than doxorubicin or the mixture of doxorubicin and antisense oligodeoxynucleotide. When incubated in 10% fetal serum at 37°C, the conjugate showed a remarkable stabilization as compared to the unmodified oligodeoxynucleotide. Melting experiments demonstrated that the covalent attachment of doxorubicin strongly stabilized the binding of the oligodeoxynucleotide to its complementary sequence. In addition, in vitro reversion of multidrug resistance by the conjugate was assayed in a human carcinoma cell line (KB-A-1) resisting to doxorubicin. The result showed that the conjugate displayed very high reversal multdrug resistance activity in KB-A-1 cells in vitro. The conjugate lowered the IC50 value

*Correspondence: Dongzhi Wei, State Key Laboratory of Bioreactor Engineering, Institute of Biochemistry, East China University of Science and Technology, Shanghai 200237, China; Fax: +86-21-64250068; E-mail: dzhwei@ecust.edu.cn.

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from 21.5 μM to 2.2 μM with a fold-reversal factor of 10. In contrast, a slight decrease of the IC50 value was observed when they combined with the ''free'' antisense oligodeoxynucleotide: the IC50 value was down from 21.5 μM to 16.8 μM. This study suggested that antisense oligodeoxynucleotide-doxorubicin conjugate might be helpful in multidrug resistance reversal.

Key Words: Antisense oligodeoxynucleotide; Doxorubicin; Antisense oligodeoxynucleotide-doxorubicin conjugate; Synthesis, multdrug resistance reversal; KB-A-1 cell.

INTRODUCTION

Antisense strategy is a simple approach to down-regulate the gene expression by inhibiting transcription or translation via sequence-specific blinding of either DNA or RNA, respectively.[1,2] But the efficiency of the synthetic oligodeoxynucleotide (ODN) in regulating gene express in living cell depends on its thermodynamic stability, resistance toward nuclease and cellular uptake. Much research has indicated that a synthetic ODN coupled with DNA intercalators such as acridine,[3] adriamycin,[4] naphthyl imide,[5] psoralen[6] and pyrene[7] might increase its stability.

Doxorubicin (DOX), one of anthracycline antibiotics that induces genetic damage leading to cell death, is one of the most useful drugs in cancer chemotherapy. However, its clinical efficiency is limited by its cardiotoxicity and suppression of bone marrow function.[8,9] Moreover, DOX often causes a multidrug resistance (MDR) phenomenon, which reduces the intracellular drug content and results in therapy failure.[10,11] Many DOX analogs are synthesized in order to mitigate the toxicity and the MDR phenomenon, but no clearly improved drug has been found.[12] In the past few years, a prevailing approach to overcome MDR is inhibiting MDR gene expression by AS ODN.[13–15]

Recently, Chi et al.[16] reported that combining AS ODN (G3139) with free DOX and liposomal DOX increased their antitumor activity. Their results indicated that there exists a synergistic action of AS ODN and DOX. Similar results were also reported by other studies.[17,18] In our laboratory, we have found that an AS ODN, 5 TCCTCCATTGCGGTCCCCTT-3 the 30–11 region of mdr1 gene which associated with MDR in a human epidemic carcinomata cell line, could control expression of the mdr1 gene.[5] In this study, we have synthesized the AS ODN-DOX conjugate and investigated its properties in Dulbecco Phosphate-Buffered Saline (PBS) or in cell culture. The activity of the conjugate to reverse MDR of KB-A-1 cells in vitro has also been investigated.

MATERIALS AND METHODS

Materials

DOX was a gift from Zhejiang Hisun Pharmacentical Co. Ltd. (Zhejiang China). Other chemicals were obtained from commercial sources. All the solvents were dried and distilled before used. The AS ODN with phosphate group at the 3 end and its

complementary sequence RNA were purchased from Shanghai Bioasia Biotech Co. Ltd.(Shanghai China).

Cell Line and Culture Conditions

A human oral epidermoid carcinoma, DOX-resistance cell line (KB-A-1) was kindly provided by Dr. Ira Pastan and Micheal M. Gottesman (National Institutes of Health, USA). KB-A-1 was cultured in Dulbecco Modified Eagle Medium (DMEM) (Life Technologies, Inc.MA, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco, USA), 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, and 1 μg/ml DOX. The cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Synthesis of Boc-Caproic Acid (Compound 1)

A mixture of 6-aminocaproic acid (0.66 g, 5 mmol) and di-tert-butyl dicarbonate (Boc_2O) (1.11 g, 5.1 mmol) in tert-butyl alcohol/H_2O (1:1, V:V) solution (10 ml) (pH8.5 with a modified by 2 N NaOH) was stirred at room temperature. The reaction was monitored by TLC analysis on silica gel plates using methanol (MeOH)/ethyl acetate (EtOAc) (90:10, V:V) as eluent (Rf 6-aminocaproic acid = 0.18, Rf 6-Boc-aminocaproic acid = 0.64). When the reaction was completed, the solution was concentrated under reduced pressure, the residue was partitioned between EtOAc and H_2O. After being dried by $MgSO_4$, the organic solution was evaporated and obtained compound 1 (0.9 g, 3.9 mmol) yield 78%. ^{1}H-NMR ($CDCl_3$): δ1.39 (s, 9 H, $-CH_3$), 1.28–1.52 (m, 6 H, J 7.1, 7.2, 7.3, $-CH_2-$), 2.15 (m, 2 H, J 7.24, $-CH_2COO-$), 3.01 (m, 2 H, J 7.0, NCH_2-). MS (TOF-ES+) m/z: 254.1 (M + 23).

Synthesis of Aminoalkyl Derivate of DOX (Compound 3)

The mixture of compound 1 (14 mg, 0.06 mmol), N-ethyl-N- (3-dimethylaminopropyl) (EDC) (12 mg, 0.06 mmol), N-hydroxybenzotriazole (8 mg, 0.06 mmol) in dried N, N-dimethylformamide (DMF)/CH_2Cl_2 (1:1, V:V) (4 ml) was stirred for 2 h at 4°C. A solution of Doxorubiciin (35 mg, 0.06 mmol) and N-methylmorpholine (6 mg, 0.06 mmol) in anhydrous DMF (2 ml) was poured into the mixture and the whole was stirred at 4°C. The reaction was monitored by TLC using CH_2Cl_2/MeOH (90:10, V:V) as eluent (Rf DOX = 0.05, Rf aminoalkyl derivate of DOX = 0.54) when the reaction was completed, one drop of AcOH was added to decompose the redundant EDC, then the precipitate was removed by filtration and the filtrate was concentrated under pressure. The residue was kept in $CHCl_3$ (20 ml) and the organic layer washed with cold water (2 ml). After being dried over $MgSO_4$ the organic solution was concentrated, and the residue was purified by column chromatography using silica gel with CH_2Cl_2/MeOH (90:10, V:V) gave compound 2 (30 mg, 0.04 mmol), yield 65%. ^{1}H-NMR ($CDCl_3$): δ1.20 (3 H, d, $-CH_3$), 1.38 (9 H, s,$-(CH_3)_3$), 1.25–1.8 (8 H, m,$-CH_2-$), 2.15 (2 H, m, $-CH_2COO-$), 3.0–3.4 (4 H, m, NCH_2-, $-NHCO-$, $-NHOCO-$), 3.6 (2 H, d, $-CH_2OH$), 3.8 (1 H, m, $-CHN-$), 4.1 (3 H, s, $-OCH_3$), 7.4–8.0 (3 H, d, t, d, aromatic), 13.2–13.8 (2 H, s, s, Ph-OH). MS (TOF-ES+) m/z: 779.3 (M + 23).

Compound 2 (30 mg, 0.04 mmol) was dissolved in 3 M HCl EtOAc (0.5 ml) and stirred 30 min at room temperature, then the solution was removed in vacuum and the residue was purified on preparative silica gel plates using CH_2Cl_2/MeOH (95:5, V:V) as eluents. The compound 3 was obtained (21 mg, 0.015 mmol) yield 80%. ^{1}H-NMR ($CDCl_3$): δ1.20 (3 H, d, $-CH_3$), 1.25–1.86 (8 H, m, $-CH_2-$), 2.15 (2 H, m, $-CH_2COO-$), 3.1–3.2 (5 H, m, NCH_2-, $-NHCO-$, $-NH_2$), 3.6 (2 H, d, $-CH_2OH$), 3.8 (1 H, m, $-CHN-$), 4.1 (3 H, s, $-OCH_3$), 7.4–8.0 (3 H, d, t, d, aromatic), 13.2–13.8 (2 H, s, s, Ph-OH). MS (TOF-ES+) m/z: 679.1 (M + 23).

Synthesis of AS ODN-DOX Conjugate

10 A_{260} O.D.(50 nmol) of AS ODN with a phosphate group at 3 end in water (100)was completely precipitated with 8% N-cetyl-N,N,N-trimethylammonium bromide. The sediment was separated by centrifuged and dried in vacuum over P_2O_5. A mixture of this dried N-cetyl-N,N,N-trimethylammonium salt of oligodeoxynucleotide, triphenylphosphine (20 mg, 0.77 mmol), dipyridyldisulfide (20 mg, 1.0 mmol), 1-methylmidazole (8 μl) and anhydrous DMF (50 μl)was subjected to several (3∼5) brief (1 min) heating at 50°C followed by intensive vortexing and incubation at room temperature for 15 min. Then 2 mg of the aminoalkyl derivative of DOX and 2 Φl of anhydrous N, N-diisopropylethylamine were added. After stirred for 1 hour at room temperature, the solution was precipitated with 1.5 ml of 2% $LiClO_4$ in dry acetone. The sediment was re-precipitated by 2% $LiClO_4$ in dry acetone from 3 M $LiClO_4$ water solution and washed by acetone. The residue was dissolved in 1 ml of water. Purified by reverse-phase HPLC using Lichrospher RP-18, 10 μm, 10 × 250 mm column (Merk), Agilent 1100 chromatograph system and Agilent diode-array absorbance detector (Germany). Linear (0 to 60%) gradient of acetonitrile in 0.1 M aqueous triethylammonium acetate, pH 7.0, (flow rate of 4 ml/min) was used. And then was lyophilized and storage at −20°C. The conjugate was characterized by combination of denaturation polyacrylamide gel electrophoresis, reverse-phase HPLC and UV-vis spectroscopy.

In Vitro Stability in Dulbecco's Phosphate-Buflered Saline (PBS)

The solution of DOX, a mixture of AS ODN and DOX, and the conjugate were incubated in PBS (pH 7.4) at 37°C, respectively. The absorbance at 480 nm was detected at each 8 hours within 72 hours with a Model 752 UV-vis spectrophotometer (China).

In Vitro Stability in Calf Serum

The conjugate (8 μl, 1 μg/μl) and AS ODN (8 μl 1 μg/μl) were incubated at 37°C in 10% activated fetal calf serum (32 μl), respectively. After incubation for various regular times, the mixture was heated at 70°C for 5 min, and then 20 μl mixture was taken out for denaturation polyacrylamide gel electrophoresis analysis and 2 μl for HPLC analysis.

Thermal Denaturation Experiments

Thermal denaturation of ODN complexes was carried out in cacodylate buffer pH 7.4, containing 140 mM KCl, 2.0 mM sodium cacodylate, 1.5 mM $MgCl_2$, and 0.8 mM

spermine, 0.5 mM EDTA. The concentration of each component was 1.0 μm. The melting curves (absorbance versus temperature) were obtained with the use of a Model UV-2501PC spectrometer (Shimadzu, Japan) at 260 nm. The temperature of the cell holder was regulated by circulating liquid used a Model TB-85 thermo bath (Shimadzu, Japan). A buffer solution of appropriate ionic strength was placed in a reference curette occupying one of the cell hold. Each ODN mixture was contained in a quartz curette with a Teflon cap. The temperature was initiated at 10°C and was increased at 1°C/min increment with a 1 min waiting period for stabilization at each temperature. The duplex melting temperatures (Tm) were evaluated as the maximum of the first derivative of the melting profiles.

Treatment Cells with ODN-DOX Conjugate or Combined with DOX

To assess the reversal of MDR ability of the conjugate to the cells, KB-A-1 cells were seeded in 96-well plates (Costar Corp., Combridge, MA) in 200 μl complete DMEM at the concentration of 1×10^4 cells/well. After incubated at 37°C for 20–22 h to allow the cells to attach, the medium was removed. The cells were washed twice with serum-free medium, and were incubated in a serum-free DMEM medium supplemented with 0.5 μM of the conjugate or a mixture of 0.5 μM of the AS ODN and 0.5 μM of DOX. In control wells, only serum-free medium was added. After incubated for 24 h, the cells were washed once with complete medium, and then exposed to DOX at doses from 0.1 μM to 25 μM for 48 h. At the end of the combination treatment, samples from each group were harvested, counted and assayed for viability (trypan blue dye exclusion).

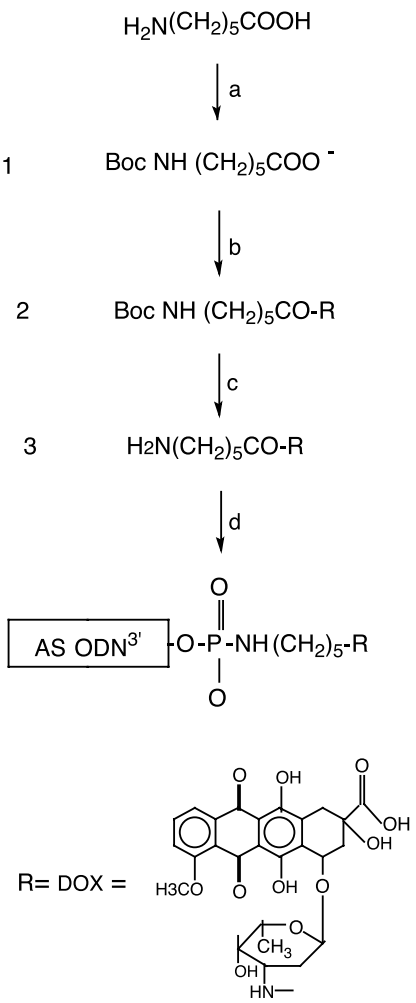
Statistical Analysis

Each experimental value is expressed as the mean ± standard deviation (SD). Students' paired *t* test was used to evaluate the significance of differences between groups, and the criterion of statistical significance was taken as $p < 0.05$ or $p < 0.01$.

RESULTS

Synthesis

The conjugate was synthesized as shown in Scheme 1. Before being reacted with DOX, the amino group of 6-aminocaproic acid should be protected. In general, many protective groups have been used for the amino group. In our studies, Boc group was used because it can be easily cleaved at room temperature. The reaction of 6-aminocaproic acid was completely converted into the Boc group. Isolation yield of the desired compound 1 (N-tert-butyloxycarbonyl- 6-amino-caproic acid) was approximately 82%. The compound 1 was then reacted with DOX in the presence of tertiary base to yield compound 2. The reaction was catalyzed by EDC in 65% yield. Finally, the Boc group was cleaved with 3 M HCl in ethyl acetate to give in nearly quantitative yield of the compound 3.



Scheme 1. Synthesis route of the AS ODN-DOX conjugate.

Synthesis of the AS ODN-DOX conjugate was carried out according to Aitssando Balbi et al.[19] and Kostenko et al.[20] with slight modifications. In the presence of 1-methylimdazole and N, N-diisopropylethylamine, the conjugate was synthesized with 85% yield. Reverse-phase HPLC analysis of the crude mixture obtained by conjugating the AS ODN with compound 3 showed a main peak corresponding to the 3 conjugated oligomer which had a higher retention time (Rt = 14 min 21 sec) than that of 3 phosphorate (Rt = 8 min 10 sec) (Fig. 1). The UV-vis absorption spectrum of the conjugate and DOX were shown as Fig. 2. The denaturation polyacrylamide gel electrophoresis analysis was shown as Fig. 3. It is shown that the conjugate band was slightly above the ODN band. This could be not only due to the increasing molecular weight of the ODN, but also due to changing the ODN charge.

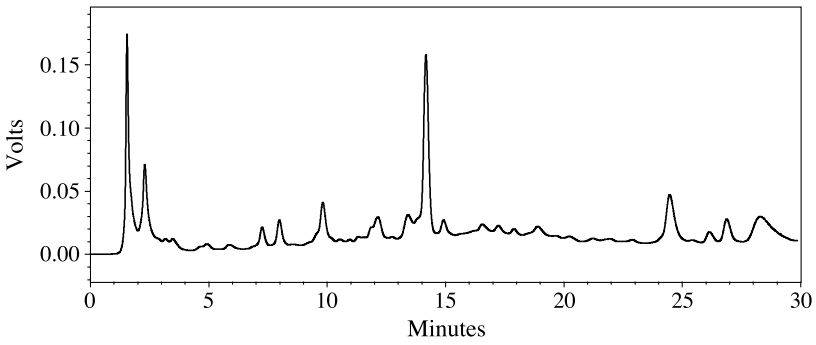


Figure 1. Reverse phase analysis on a Lichrospher100RP18 (5 μm) colum (125 mm × 4 mm) (using a linear gradient of CH_3CN: from eluent A to eluent B within 30 min, with a flow rate of 1 ml/min) of the crude mixture obtained after coupling of p-ODN with DOX aminoalkyl derivative. The retention time is: the AS ODN-DOX conjugate: Rt = 14 min 21 sec, the AS ODN: Rt = 8 min 10 sec, DOX aminoalkyl derivative: Rt = 24 min 38 sec.

Stability of the Conjugate In Vitro

In order to examine their stability, DOX, the mixture of the AS ODN and DOX, and the conjugate in PBS (pH 7.4) at 37°C were monitored at 480 nm (Fig. 4). It was demonstrated that the absorbance of DOX fell to 76.7% of the initial level after incubation for 72 hours. The mixture of the AS ODN and DOX showed slightly better stability. However, the conjugate retained more than 84.7% of the initial absorbance after 72 hours incubation.

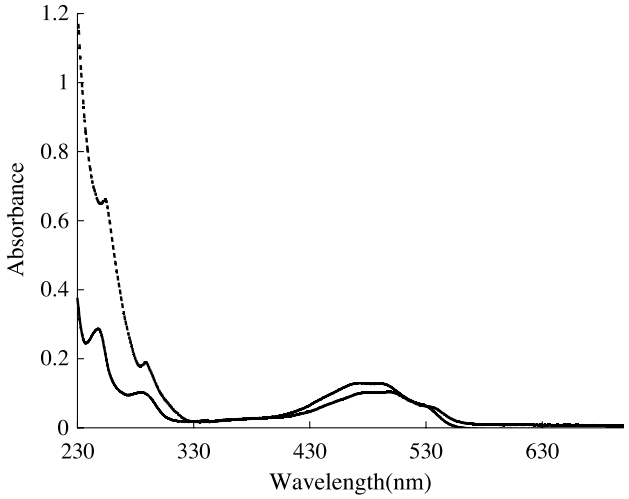


Figure 2. UV-vis spectrum of AS ODN and AS ODN-DOX conjugate in water (-): AS ODN (5.1 μM). (....): AS ODN-DOX (5.6 μM).

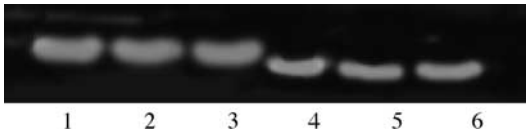


Figure 3. Polyacrylamide gel electrophoresis analysis the stability of AS ODN and AS ODN-DOX conjugate. Lane 1∼3: AS ODN-DOX (40 μM). Lane 4∼6: AS ODN (46 μM).

The stability of the conjugate in fetal calf serum was analyzed by reverse-phase HPLC. As shown in Fig. 5, 15.8% of the conjugate was degraded after 24 hours incubation. And 97.2% of the control AS ODN was degraded under the same condition. These results indicated that attachment of the DOX at the 3 end of the AS ODN could protect it against degradation by nuclease.

Denaturation of Complexes

The cacodylate buffer was chosen due to the limited dependence of pH on temperature. The UV melting curves at 260 nm for the 1:1 (molar ratio) mixtures of the ODN or the ODN-DOX conjugate with the complementary RNA (5 AAGGGGACCG-CAATGGAGGATGTACA) are shown in Fig. 6. Both profiles exhibit a clear melting transition, giving the Tm of 62°C (hypochromicity: 9.6%) for duplex of AS ODN and of 74°C (hypochromicity: 11.4%) for AS ODN-DOX conjugate. This result indicated that the presence of DOX covalently attached to the ODN remarkably increase the stability of the complexes.

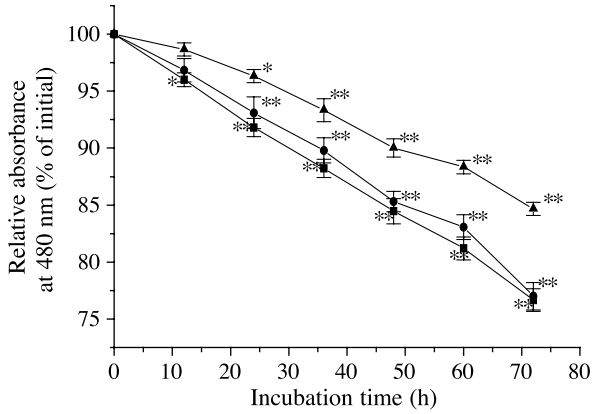


Figure 4. Stability of DOX, DOX plus AS ODN and AS ODN-DOX conjugate in PBS at 37°C in the dark. Estimated from the absorbance at 480 nm. (■) DOX (21 μM). (●) AS ODN (31 μM) plus DOX (21 μM). (▲) AS ODN-DOX conjugate (26 μM). Each value represents the mean ± standard deviation (SD) of three independent experiments. Significant differences from untreated control are indicated by $p < 0.05$ (*) and $p < 0.01$ (**).

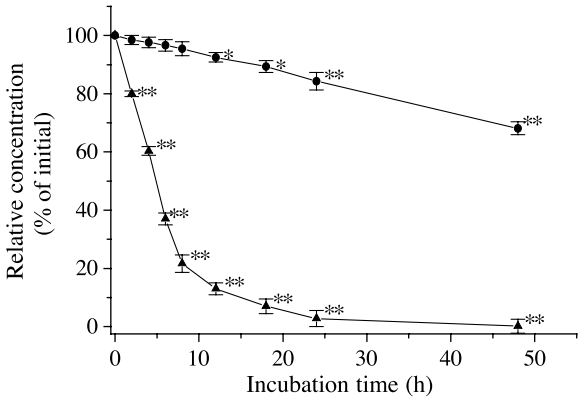


Figure 5. Stability of AS ODN and AS ODN-DOX conjugate in 10% fetal calf serum at 37°C in the dark. Determination by HPLC at 254 nm using a linear gradient of CH_3CN in 0.1 M aqueous triethylammonium acetate, ph 7.0, with a flow rate of 1 ml/min. (▲): AS ODN. (●): AS ODN-DOX conjugate. Each value represents the mean ± standard deviation (SD) of four independent experiments. Significant differences from untreated control are indicated by $p < 0.05$ (*) and $p < 0.01$ (**).

In Vitro Reversal of MDR in KB-A-1 Cells Treated with the Conjugate

In order to assess the ability of the conjugate to increase DOX cytotoxicity in KB-A-1 cells and thus to reverse MDR, KB-A-1 cells were first exposed to the conjugate and then to DOX. The results were shown in Fig. 7. When KB-A-1 cells were treated with DOX alone, only at the higher dose (15 μM) could the inhibition of cells growth observed (about ∼ 28% inhibition compared to the control). However, when treated

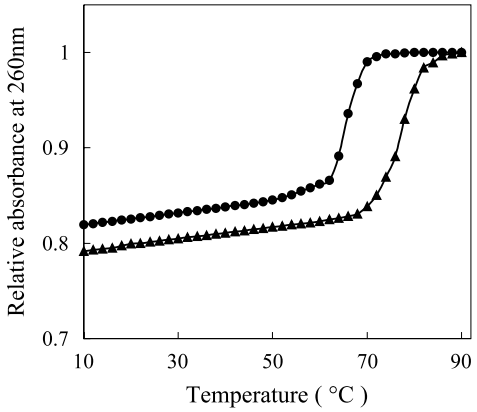


Figure 6. Melting curves for the mixture of AS ODN and AS ODN-DOX conjugate with the complementary ODN. The pH was 7.4 with the cacodylate buffer. The ODN concentration was 1.0 μM in all cases. (●): AS ODN. (▲):AS ODN-DOX conjugate.

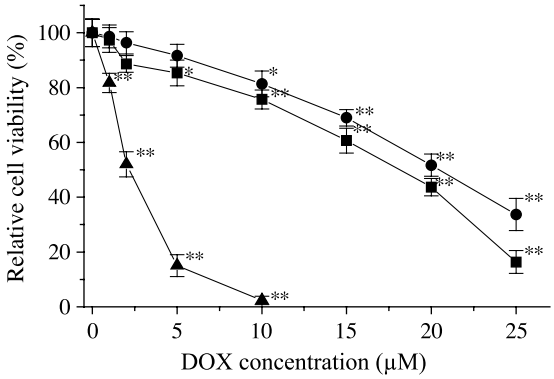


Figure 7. Reveral effect of the conjugate or the AS ODNs + DOX on the MDR in KB-A-1 cell growth. Cells were treated with 0.5 μM the conjugate (▲), the mixture of the 0.5 μM AS ODN AND 0.5 μM DOX (■). DOX alone (●). Cell viability was measured by trypan blue dye exclusion assay. Relative cell viability was calculated by dividing the absolute survival of the treated samples by that of the control samples. Each value represents the mean ± standard deviation (SD) of four independent experiments. Significant differences from untreated control are indicated by $p < 0.05$ (*) and $p < 0.01$ (**).

with the conjugate, a significant increase of DOX cytotoxicity was observed: the inhibition of the cells growth was about 47% at the lower DOX dose (2.0 μM). In contrast, a slight effect was observed when the cells were treated with the mixture of the ''free'' AS ODN (0.5 μM) and DOX (0.5 μM). The DOX IC50 values (dose of DOX that caused 50% of cells growth inhibition) were calculated from the percentage of growth inhibition caused by the different treatments on KB-A-1 cells. The DOX IC50 value for the conjugate was 2.2 μM. In contrast, the DOX IC50 value for the AS ODN mixed with DOX was 16.8 μM, and for DOX alone the value was 21.5 μM. The reversal index of the conjugate (= the DOX IC50 value for DOX alone/the DOX IC50 value for the conjugate) was nearly 10. However, the reversal index of the AS ODN was 1.3. These data demonstrated that the conjugate displayed an evident reversal effect on MDR cells.

DISCUSSION

It has been reported that the contribution of a linker group is very important to the properties of the conjugate.[21,22] Previous studies showed that the $-(CH_2)_5-$ linker of ODN-acidine conjugates led to more stable complexes than other linkers.[21] In our case, we chose 6-aminocaproic acid as the linker. The UV-vis absorption spectrum of the conjugate, which exhibited the expected absorbance radio at $\lambda = 260$ nm and $\lambda = 480$ nm according with the published λ_{max} of DOX (Fig. 2). A slight red-shift of the absorption maximum of DOX was observed similar to the oligonucleotide-daunomycin conjugates.[6] The denaturation polyacrylamide gel electrophoresis analysis showed that the conjugate migrated slightly slower compared to the ODN (Fig. 3). The result suggested that the conjugate had similar character of the ODN and its molecular weight was slightly bigger than the ODN. These results confirmed that DOX was linked to the AS ODN.

The results presented in this study showed that the presence of DOX covalently linked to the AS ODN on the 3 phosphate group significantly increased the stability of DOX and the AS ODN either in PBS or in cell culture. In PBS, after incubated for 72 h at 480 nm, the absorbance of the conjugate decreased less than that of free DOX (Fig. 4). The observed stabilization of the conjugate in PBS was due to the amino group of DOX forming more stable amide derivate. The results also showed that the AS ODN linked to DOX had remarkably improved its resistance to endonuclease in cell culture. The inhibition to endonuclease degradation of the conjugate is nearly 40-fold as compared to that of the ree AS ODN in cell culture condition (Fig. 5). It is known that 3 exonuclease activity is mainly responsible for the degradation of ODNs in calf serum.[23] This 3 exonuclease activity is similar to that of snake venom phosphodiesterase, which degrades ODNs with a free 3 hydroxyl group from the 3 end. Therefore, the conjugate with DOX attached to the 3 phosphate may prevent the attack from other 3 exonucleases.

In our study, the results also suggested that the conjugate strongly stabilized the complex formed from the AS ODN and its complementary sequence. A considerable increase of Tm of the intramolecular structures of the conjugate is observed: Δ Tm for the conjugate is +12°C compared to the AS ODN (Fig. 6). The result is in agreement with the ODN-acridine conjugates 3. The possible reason is that the presence of an additional positively charged substituent on the 3′-phosphate group affects the stability of the complexes. It is considered that substitution of the 3 phosphate group by the positively charged group not only adds a positive charge to the ODN but also removes one of the negative charges originally borne by the terminal phosphate group.[4]

In the present study, we also investigated the reversal of MDR in KB-A-1 cells treated with the conjugate combination with DOX in vitro. The KB-A-1 cell line is a strong DOX resistant cell line (the IC50 value for DOX is up to 21.5 μM). The ability of the conjugate to elevate the DOX cytotoxicity in KB-A-1 cells and thus to reverse MDR resistance was assessed in combination experiments in which KB-A-1 cells were first exposed to the conjugate and then to DOX. The results demonstrated that a strong decrease of the IC50 value was observed in KB-A-1 cells treated with the combination of the conjugate and DOX. The conjugate lowered the IC50 value from 21.5 μM to 2.2 μM with a fold-reversal factor of 10. In contrast, a slight decrease of the IC50 value was observed when DOX was combined with the ''free'' AS ODN: the IC50 value was down from 21.5 μM to 16.8 μM (Fig. 7).

Antisense strategy is an efficacious approach to overcome MDR.[12,13] Our previous studies showed that the AS ODN could inhibit the expression of the mdr1 gene but not strongly as expected.[24] This might be due to the poor absorbability of the AS ODN, the rapid degradation of the AS ODN, or the poor binding affinity to the target nucleic acid. In this case, however, our data suggested that coupling DOX with the AS ODN efficiently elevated the antisense activity against DOX resistant KB-A-1 cells. The conjugate led to an IC50 that was about 8-fold less than that of the ''free'' AS ODN (16.8 μM verse 2.2 μM). Although the mechanism by which the conjugate inhibits the KB-A-1 cell growth is still unknown, this might be explained in that the conjugate increases cellular uptake, inhibits degradation by exonucleases, and improves binding affinity to the complementary sequence. These results suggested the possibility of utilizing this conjugate to overcome MDR. Further research is needed to explore the antitumor potential of the conjugate in vitro and in vivo.

In conclusion, we synthesized the AS ODN-DOX conjugate and found that the conjugate was very stable either in PBS or in cell culture. This study also showed that the

conjugate displayed very high reversal MDR activity in KB-A-1 cells in vitro. Further studies such as the antitumor activity of the conjugate in vivo using severely combined inmunodeficient mice bearing human resistant tumour xenografts are being explored.

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