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ORIGINAL ARTICLE

Transformation of the naturally occurring frog skin peptide, alyteserin-2a into a potent, non-toxic anti-cancer agent

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Abstract Alyteserin-2a (ILGKLLSTAAGLLSNL.NH_2) is a cationic, amphipathic α-helical cell-penetrating peptide, first isolated from skin secretions of the midwife toad *Alytes obstetricans*. Structure–activity relationships were investigated by synthesizing analogs of alyteserin-2a in which amino acids on the hydrophobic face of the helix were replaced by L-tryptophan and amino acids on the hydrophilic face were replaced by one or more L-lysine or D-lysine residues. The Trp-containing peptides display increased cytotoxic activity against non-small cell lung adenocarcinoma A549 cells (up to 11-fold), but hemolytic activity against human erythrocytes increases in parallel. The potency of the N15K analog against A549 cells (LC_{50} = 13 μM) increases sixfold relative to alyteserin-2a and the therapeutic index (ratio of LC_{50} for erythrocytes and tumor cells) increases twofold. Incorporation of a D-Lys^{11} residue into the N15K analog generates a peptide that retains potency against A549 cells (LC_{50} = 15 μM) but whose therapeutic index is 13-fold elevated relative to the native peptide. [G11k, N15K] alyteserin-2a is also active against human hepatocarcinoma HepG2 cells (LC_{50} = 26 μM), breast adenocarcinoma MDA-MB-231 cells (LC_{50} = 20 μM), and colorectal adenocarcinoma HT-29 cells (LC_{50} = 28 μM). [G11k, N15K] alyteserin-2a, in concentrations as low as 1 μg/mL, significantly ($P < 0.05$) inhibits the release of the immune-suppressive cytokines IL-10 and TGF-β from unstimulated and concanavalin A-stimulated peripheral blood mononuclear cells. The data suggest a strategy of increasing the cationicity while reducing the helicity of naturally occurring amphipathic α-helical peptides to generate analogs with improved cytotoxicity against tumor cells but decreased activity against non-neoplastic cells.

Keywords Cell-penetrating peptide · Alyteserin-2a · Structure–activity · Anticancer

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Introduction

Skin secretions from Anura (frogs and toads) contain a wide range of compounds with biological activity that has excited interest because of their potential for drug development. Cell-penetrating peptides are present in skin secretions from many, but by no means all, frog species and play an important role in the system of innate immunity that constitutes the animal's first-line defense against invading pathogens and are a component of the chemical defense system against predators (Conlon 2011a). With few exceptions, these frog skin peptides are cationic (molecular charge between +2 and +6), contain at least 50 % hydrophobic amino acids, and have the propensity to adopt an amphipathic α-helical conformation in a membrane-mimetic environment (reviewed in Conlon 2011b).

Although there is no single mechanism by which the peptides produce cell death, their action generally does not involve binding to receptors on the cell membrane or on intracellular targets. A non-specific perturbation of the cell membrane and insertion into lipid bilayer leads to membrane disruption and cell lysis (Almeida and Pokorny 2009; Bocchinfuso et al. 2009).

Cell-penetrating peptides are being considered as candidates for development into anti-infective (Zaiou 2007; Conlon 2012), anti-inflammatory (Yeung et al. 2011), and anti-cancer (Hoskin and Ramamoorthy 2008; Lu et al. 2008; Schweizer 2009) agents. The problems posed by the emergence of multidrug resistance in the treatment of bacterial infections are also encountered in cancer chemotherapy (Chen and Tiwari 2011). Because of their non-specific and destructive mechanism of action, cell-penetrating peptides show therapeutic potential for development into anti-cancer agents in cases where the tumor is not responsive to conventional pharmaceutical therapy. Examples of peptides from frog skin that show potential as anticancer agents include magainin-2 and its derivatives that are tumouricidal against human lung (Ohsaki et al. 1992) and bladder (Lehmann et al. 2006) cancer cells and against suspension cultures of a wide range of hematopoietic cell lines (Cruciani et al. 1991), dermaseptins that are active against hepatoma-derived HepG2 cells (Conlon et al. 2007b) and prostatic adenocarcinoma PC-3 cells (van Zoggel et al. 2012), pentadactylin active against melanoma cells (Libério et al. 2011), analogs of ascaphin-8 and peptide XT-7 that are tumouricidal against HepG2 cells (Conlon et al. 2008) together with aureins (Rozek et al. 2000), brevinin-1 fragments (Won et al. 2006), and temporin-1CEa (Wang et al. 2012) that are active against a range of tumor cell lines. These peptides are not tumor-specific in their cytotoxic action but show fivefold greater potency against tumor cells than against erythrocytes or fibroblasts.

Alyteserin-2a (ILGKLLSTAAGLLSNL.NH_2) was first isolated from norepinephrine-stimulated skin secretions from the midwife toad *Alytes obstetricans* (Conlon et al. 2009). The peptide is cationic (molecular charge of +2 at pH 7), contains a high proportion of hydrophobic amino acids, and adopts an amphipathic α-helical conformation in 50 % trifluoroethanol-water. A helical wheel representation (Schiffer and Edmundson 1967) of alyteserin-2a illustrates the amphipathic nature of the α-helical conformation with the hydrophilic Lys^4, Ser^7, and Ser^{14} segregating on one face of the helix and the hydrophobic Ile^1 Leu^2, Leu^5, Leu^6, Leu^{12}, and Leu^{13} residues on the opposite face (Fig. 1). Preliminary data have shown that alyteserin-2a displays relatively low cytotoxic potency against A549 human non-small cell lung adenocarcinoma cells (LC_{50} = 80 μM) and its hemolytic activity against human erythrocytes is also low (LC_{50} = 140 μM) (Conlon et al. 2012). There have been several structure–activity studies of frog skin cell-penetrating peptides aimed at developing analogs with increased antimicrobial activity and decreased cytotoxicity to mammalian cells (reviewed in Conlon 2012). In contrast, there have been relatively few studies that have investigated the effect of structural modifications on anti-cancer activity. The present structure–activity study investigates the effect on tumor cell viability of selective substitutions of amino acids in alyteserin-2a by either L-lysine or D-lysine and by L-tryptophan that produce analogs with differing cationicity, helicity, and hydrophobicity. The aim of the investigation was to develop a non-toxic peptide with high potency against tumor cells that has therapeutic potential as an anti-cancer agent.

Experimental procedures

Peptide synthesis

Alyteserin-2a and all analogs were supplied in crude form by GL Biochem Ltd (Shanghai, China). The peptides were purified to near homogeneity by reversed-phase HPLC on a (2.2 cm × 25 cm) Vydac 218TP1022 (C-18) (Grace, Deerfield, IL, USA) column equilibrated with acetonitrile/water/TFA (28.0/71.9/0.1, v/v/v) at a flow rate of 6 mL/min. The concentration of acetonitrile was raised to 56 % (v/v) over 60 min using a linear gradient. Absorbance was

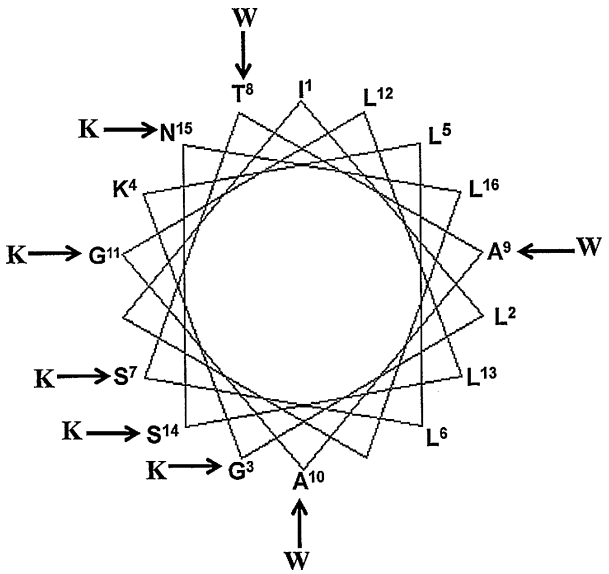

Fig. 1 Schiffer–Edmundson wheel representation of alyteserin-2a demonstrating the amphipathic nature of the α-helical conformation. The *arrows* denote the sites of replacement of amino acids by lysine and tryptophan in order to produce analogs with improved therapeutic indices

measured at 214 and 280 nm and the major peak in the chromatogram was collected manually. The final purity of all peptides tested was >98 % as determined by symmetrical peak shape and electrospray mass spectrometry. The Trp-containing peptides had restricted solubility in physiological buffers and required the use of dimethylsulfoxide (10 μL) to facilitate solubilization prior to dilution to the required concentration. The primary structures, molecular charges at pH 7, calculated hydrophobicities (Wimley and White 1996), and predicted α-helical domains (Muñoz and Serrano 1994) of the peptides used in this study are shown in Table 1.

Cytotoxicity assays

Human non-small cell lung adenocarcinoma A549 cells were maintained at 37 °C in RPMI 1640 medium containing 2 mM L-glutamine and supplemented with 10 % fetal calf serum (FBS, Biowest, Nouaille, France), and antibiotics (penicillin 50 U/mL; streptomycin 50 μg/mL). Human breast adenocarcinoma MDA-MB-231 cells and human colorectal adenocarcinoma HT-29 cells were maintained in DMEM medium supplemented with antibiotics (penicillin 50 U/mL; streptomycin 50 μg/mL) and 10 % fetal calf serum. Human hepatoma-derived HepG2 cells (kindly provided by Prof. Haider Raza, United Arab Emirates University) were cultured as previously described (Conlon et al. 2008). In all experiments, cell viability was higher than 99 % using trypan blue dye exclusion.

Cells were seeded in 96-well plates at a density of 5×10^3 cells/well. After 24 h, all tumor cells were treated for 24 h with increasing concentrations of alyteserin-2a and its analogs (1–100 μM) in triplicate. Control cultures were treated with 0.1 % DMSO. The effect of the peptides on cell viability was determined by measurement of ATP concentrations using a CellTiter-Glo Luminescent Cell Viability assay (Promega Corporation, Madison, WI, USA). Luminescent signals were measured using a GLOMAX Luminometer system. The LC_{50} value was taken as the mean concentration of peptide producing 50 % cell death in three independent experiments.

Hemolysis assay

Peptides in the concentration range 3–400 μM were incubated with washed human erythrocytes (2×10^7 cells) from a healthy donor in Dulbecco's phosphate-buffered saline, pH 7.4 (100 μL) for 1 h at 37 °C. After centrifugation (12,000×*g* for 15 s), the absorbance at 450 nm of the supernatant was measured. A parallel incubation in the presence of 1 % v/v Tween-20 was carried out to determine the absorbance associated with 100 % hemolysis. The

Table 1 Primary structures and physicochemical properties of the peptides used in this study

Peptide	Amino acid sequence	Charge	GRAVY	Helical domain
Alyteserin-2a	ILGKLLSTAAGLLSNL.NH_2	+2	0.136	9–14
G3K	ILKKLLSTAAGLLSNL.NH_2	+3	0.074	9–14
S7K	ILGKLLKTAAGLLSNL.NH_2	+3	0.082	9–14
S7k	ILGKLLkTAAGLLSNL.NH_2	+3	0.082	ND
G11K	ILGKLLSTAAKLLSNL.NH_2	+3	0.074	7–16
G11k	ILGKLLSTAAkLLSNL.NH_2	+3	0.074	ND
S14K	ILGKLLSTAAGLLKNL.NH_2	+3	0.082	8–16
N15K	ILGKLLSTAAGLLSKL.NH_2	+3	0.100	8–16
N15k	ILGKLLSTAAGLLSkL.NH_2	+3	0.100	ND
S7K, G11K	ILGKLLKTAAKLLSNL.NH_2	+4	0.021	4–16
S7k, G11k	ILGKLLkTAAkLLSNL.NH_2	+4	0.021	ND
G11K, N15K	ILGKLLSTAAKLLSKL.NH_2	+4	0.039	4–16
G11k, N15K	ILGKLLSTAAkLLSKL.NH_2	+4	0.039	ND
T8W	ILGKLLSWAAGLLSNL.NH_2	+2	0.260	8–14
A9W	ILGKLLSTWAGLLSNL.NH_2	+2	0.262	10–14
A10W	ILGKLLSTAWGLLSNL.NH_2	+2	0.262	Non-helical
A10W, G11k	ILGKLLSTAWkLLSNL.NH_2	+3	0.201	ND
A10W, N15K	ILGKLLSTAWGLLSKL.NH_2	+3	0.220	11–16
A10W, N15k	ILGKLLSTAWALLSkL.NH_2	+3	0.220	ND

Grand average of hydropathicities (GRAVY) of the peptides is calculated using the hydrophobicity scales for amino acid residues of Wimley and White (1996). Secondary structure prediction is made using the AGADIR program (Muñoz and Serrano 1994)

ND not determined

LC_{50} value was taken as the mean concentration of peptide producing 50 % hemolysis in three independent experiments. The therapeutic index of the peptides is defined as the ratio of LC_{50} for erythrocytes to the LC_{50} for tumor cells.

Antimicrobial assays

Minimum inhibitory concentration (MIC) of the peptides against reference strains of *Escherichia coli* (ATCC 25726) and *Staphylococcus aureus* (ATCC 25923) was determined in duplicate in three independent experiments by standard microdilution methods using 96-well microtiter cell-culture plates as previously described (Conlon et al. 2012).

Measurement of cytokine release

Peripheral blood mononuclear (PBM) cells were isolated from heparinized venous blood of healthy donors by density gradient centrifugation (Histopaque 1077, Sigma, Germany). The separated cells were washed three times in RPMI 1640 culture medium containing 20 mM Hepes and suspended in the supplemented culture medium (10 % autologous serum, 2 mM L-glutamine, 100 IU/mL penicillin G and 100 μg/mL streptomycin). Cell number and viability were determined using acridine orange/ethidium bromide double staining. Freshly isolated cells were grown in 96-well plates at a starting density of 0.5×10^6 cells/well. Cells were incubated in the presence of the test peptides (1, 10 and 20 μg/mL) or in medium alone (control). This group is designated Con A^-. The group designated Con A^+ consisted of cells treated in the same way but in the presence of concanavalin A (5 μg/mL). Cells were cultured for 24 h at 37 °C in a humidified atmosphere containing 5 % CO_2. After incubation, cells were centrifuged, supernatants collected and kept at −20 °C. Cytokine concentrations in the supernatants (diluted 1:6) were measured by ELISA according to manufacturer's recommended protocols using an OptEIA assay kit (Cat No 555157) from BD Biosciences (San Diego, CA, USA) for IL-10 and a DuoSet ELISA development kit (Cat No DY240) from R & D Systems (Minneapolis, MN, USA) for TGF-β.

Statistical analysis

Statistical analyses were performed using commercially available software (SPSS version 13.0; SPSS Inc., Chicago, IL, USA). The distributions of data were evaluated for normality using Kolmogorov–Smirnov test and then retested with Chi-square test. Comparison of quantitative parametric data between two study groups was done by application of unpaired *t* test. Differences between the paired data were evaluated using the paired *t* test. A *P* value <0.05, from two-sided tests, was considered statistically significant.

Results

Effect of L-lysine substitutions on anti-tumor and hemolytic activities of alyteserin-2a

Alyteserin-2a shows relatively weak cytotoxic activity against A549 human non-small cell lung adenocarcinoma cells ($LC_{50} = 80$ μM) and human erythrocytes ($LC_{50} = 140$ μM). The effect of substitution of the amino acid residues on the hydrophilic face of alyteserin-2a (Gly^3, Ser^7, Gly^{11}, Ser^{14}, and Asn^{15}) by L-Lys is shown in Table 2. The two- to fourfold increase in antitumor potency of the [S7K], [G11K], [S14K], and [S7K, G11K] analogs is mirrored by a comparable increase in hemolytic activity against human erythrocytes. The substitution $Asn^{15} \rightarrow$ L-Lys produces the greatest (6-fold) increase in cytotoxicity against the A549 cells. This analog shows differential antitumor activity being fourfold less potent against erythrocytes than against A549 cells representing a therapeutic index of 3.9 compared with 1.8 for alyteserin-2a. Increasing cationicity further by substitution of both Gly^{11} and Asn^{15} on the hydrophilic face of the helix by L-lysine results in an analog that is the most active against A549 cells ($LC_{50} = 6$ μM), representing a 13-fold increase in potency over alyteserin-2a, but is also the most hemolytic ($LC_{50} = 16$ μM).

Effect of L-tryptophan substitutions on antitumor and hemolytic activities of alyteserin-2a

Increasing the hydrophobicity of alyteserin-2a while maintaining amphipathicity by substitution of Thr^8, Ala^9, and Ala^{10} by L-tryptophan results in analogs showing a 4- to 11-fold increase in potency against A549 cells (Table 2). Hemolytic activity increases in parallel with anti-tumor activity so that the therapeutic index of the peptides is not increased relative to the native peptide. Replacing the Asn^{15} residue in [A10W] alyteserin-2a by L-lysine has only a minor effect on anti-tumor activity but produces a twofold increase in hemolytic activity.

Effect of D-lysine substitutions on anti-tumor and hemolytic activities of alyteserin-2a

In order to produce analogs of alyteserin-2a with increased cationicity but decreased helicity, a series of peptides were synthesized containing one or more helix-destabilizing D-lysine residues at positions 7, 11, and 15 (Table 3).

Table 2 Cytotoxicities of L-lysine-substituted and L-tryptophan-substituted analogs of alyteserin-2a against human non-small cell lung adenocarcinoma A549 cells and erythrocytes

Peptide	A549 cells	Erythrocytes	Therapeutic index
Alyteserin-2a	80	140	1.8
G3K	100	105	1.1
S7K	35	28	0.8
G11K	20	24	1.2
S14K	30	55	1.8
N15K	13	50	3.9
S7K, G11K	20	38	1.9
G11K, N15K	6	16	2.7
T8W	12	13	1.1
A9W	21	39	1.8
A10W	7	11	1.6
A10W, N15K	6	6	1.0

Data are expressed as LC_{50} values (μM)

Substitution of Ser^{7} by D-lysine has a deleterious effect on anti-tumor potency, but the analog is still more hemolytic than the naturally occurring peptide (LC_{50} = 105 μM). In contrast, [G11k] alyteserin-2a shows a threefold increased potency against A549 cells but the analog also shows high hemolytic activity (LC_{50} = 42 μM). The di-substituted analog [S7k, G11k] alyteserin-2a is less hemolytic than alyteserin-2a (LC_{50} = 185 μM), but is only slightly more potent than the native peptide. Similarly, substitution of Asn^{15} by D-lysine has a marked effect on hemolytic activity resulting in threefold decrease in potency but the analog also shows relatively weak anti-tumor activity. Combining the substitutions Gly^{11} → D-Lys and Asn^{15} → L-Lys produces an analog with an appreciably improved therapeutic index (13.0). The peptide shows a 5.3-fold increase in potency against A549 cells coupled with a 1.4-fold decrease in hemolytic potency (Fig. 2). Incorporating the substitution Ala^{10} → L-Trp into the [G11k] and [N15k] analogs increases anti-tumor potency but does not improve the therapeutic index. In the case of the [A10W, G11k, N15k] analog, the increase in hemolytic activity was less pronounced so that the therapeutic index of the peptide increases to 4.0.

The cytotoxicities of the Lys^{15}-substituted analogs against human hepatocarcinoma HepG2 cells, breast adenocarcinoma MDA-MB-231 cells, and human colorectal adenocarcinoma HT-29 cells are shown in Table 4. The [G11k, N15K] analog displays relatively high cytotoxic activities against these cells with potencies in the range 20–28 μM and therapeutic indices in the range of 7.0–9.8.

Antimicrobial activities of the N15-substitited analogs

The growth-inhibitory potencies of the N15-substituted analogs against reference strains of *S. aureus* and *E. coli* are shown in Table 4. The [G11K, N15K] peptide shows the greatest antimicrobial potency, but the [G11k, N15K] peptide has the highest therapeutic index.

Effects of alyteserin-2a and [G11k,N15K] alyteserin-2a on the release of IL-10 and TGF-β from PMN cells

Both alyteserin-2a (1–20 μg/mL) and [G11k, N15K]alyteserin-2a (1–20 μg/mL) significantly ($P < 0.05$) inhibit the release of TGF-β from unstimulated and ConA-stimulated PBM cells (Fig. 3). [G11k, N15K] alyteserin-2a (1–20 μg/mL), but not alyteserin-2a, also significantly inhibits the release of IL-10 from both unstimulated and ConA-stimulated PBM cells (Fig. 3). Cell viability was not decreased in the presence of 20 μg/mL of either peptide.

Table 3 Cytotoxicities of D-lysine-substituted analogs of alyteserin-2a against human non-small cell lung adenocarcinoma A549 cells and erythrocytes

Peptide	A549 cells	Erythrocytes	Therapeutic index
S7k	>100	105	<1.0
G11k	28	42	1.5
N15k	75	310	4.1
S7k, G11k	65	185	2.9
G11k, N15K	15	195	13.0
A10W, G11k	17	21	1.2
A10W, N15k	20	27	1.4
A10W, G11k, N15k	17	68	4.0

Data are expressed as LC_{50} values (μM)

Discussion

The cytotoxic potencies of cationic α-helical peptides against microorganisms and mammalian cells are determined by complex interactions between cationicity, hydrophobicity, conformation (α-helicity), and amphipathicity (Kindrachuk and Napper 2010; Huang et al. 2010). These parameters are not independent variables so that alteration of peptide structure by a selected amino acid substitution may change several physicochemical properties simultaneously. Differences in membrane properties between cancer cells and non-neoplastic cells may be exploited to design peptides that show tumoricidal selectivity. The plasma membrane of cancer cells typically carries an elevated negative charge compared with non-transformed cells because of a higher proportion of anionic phospholipids such as phosphatidylserine (Dobrzynska et al. 2005), O-glycosylated mucins (McDermott et al. 2001), sialic acid-containing glycolipids (Riedl et al. 2011), and heparan sulfate proteoglycans (Iozzo and Sanderson 2011). Consequently, increasing the cationicity of a cell-penetrating peptide should promote interaction with tumor cells compared with erythrocytes whose plasma membrane contains primarily zwitterionic phospholipids and so enhance anti-tumor potency relative to hemolytic activity. As shown in Table 2, except in the case of the [G3K] analog, increasing cationicity while maintaining amphipathicity by appropriate substitutions by L-lysine leads to the predicted increased tumoricidal potency, but the concomitant increase in hemolytic activity means that only the [N15K] and the [G11K, N15K] analogs show a significant increase in therapeutic index.

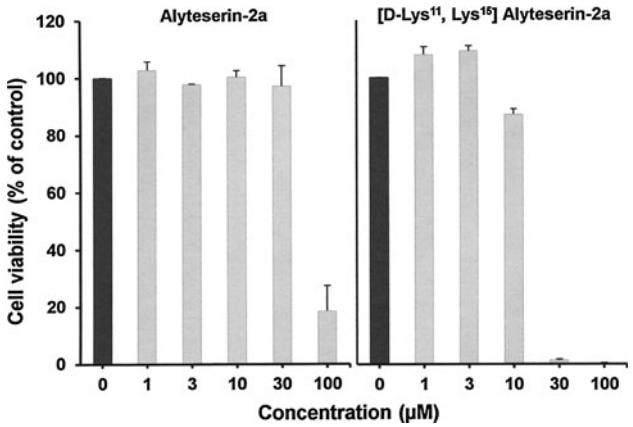

Fig. 2 Effects of alyteserin-2a and [G11k, N15K] alyteserin-2a on the viability of A549 lung adenocarcinoma cells after 24 h exposure. All experiments were repeated at least three times. *Columns* mean; *bars* SEM

Studies with magainin-2 analogs (Dathe et al. 1997; Wieprecht et al. 1997; Tachi et al. 2002) and model amphipathic α-helical peptides (Dathe and Wieprecht 1999; Stark et al. 2002; Chen et al. 2007) have shown that increasing mean hydrophobicity generally results in an increase in cytotoxicity against mammalian cells. Consistent with these observations, increasing hydrophobicity while maintaining amphipathicity by appropriate substitutions of amino acids by L-tryptophan produces peptides with appreciably increased tumoricidal activity (up 13-fold in the case of the [A10W, N15K] analog), but hemolytic activity increases in parallel so that the peptides exhibit little or no tumor selectivity (Table 2).

An increase in the degree of α-helicity of a cell-penetrating peptide also increases cytolytic activity against mammalian cells (Dathe et al. 1996; Giangaspero et al. 2001; Pacor et al. 2002). Prediction of the secondary structure of the alyteserin-2a analogs using the AGADIR program (Muñoz and Serrano 1994), an algorithm based on the helix/coil transition theory which predicts the helical behavior of monomeric peptides, indicates that the substitution Gly^{11} → L-Lys has the greatest effect on promoting helix stability (Conlon et al. 2012). Thus, increased stability of the α-helical conformation will contribute to the observed increased antitumor and hemolytic activities of the [G11K] analog. Previous studies using analogs of naturally occurring frog skin antimicrobial peptides (Conlon et al. 2007a, 2008) and model amphipathic α-helical peptides (Chen et al. 2005) have shown that incorporation of a helix-destabilizing D-lysine residues results in decreased hemolytic activity. Consistent with these data, incorporation of D-lysine at position 11 of [Lys^{15}] alyteserin-2a produces an analog with relatively high potency (15–28 μM) against cell lines derived from lung, breast,

Table 4 Cytotoxicities of Lys^{15}-substituted analogs of alyteserin-2a against a range of human tumor cell lines and reference strains of bacteria

	MDA-MB-231	HT-29	HepG2	*E. coli*	*S. aureus*
Alyteserin-2a	65 (2.2)	80 (1.8)	85 (1.6)	256 (<1.0)	64 (2.2)
N15K	18 (2.8)	32 (1.6)	17 (2.9)	>256 (<1.0)	32 (1.6)
N15k	80 (3.9)	125 (2.5)	90 (3.4)	128 (2.4)	32 (9.7)
G11K, N15K	10 (1.6)	18 (0.9)	8 (2.0)	16 (1.0)	2 (8.0)
G11k, N15K	20 (9.8)	28 (7.0)	26 (7.5)	32 (6.1)	8 (24.3)

Data are expressed as LC_{50} values (μM). The values in parentheses represent the therapeutic indices of the peptides

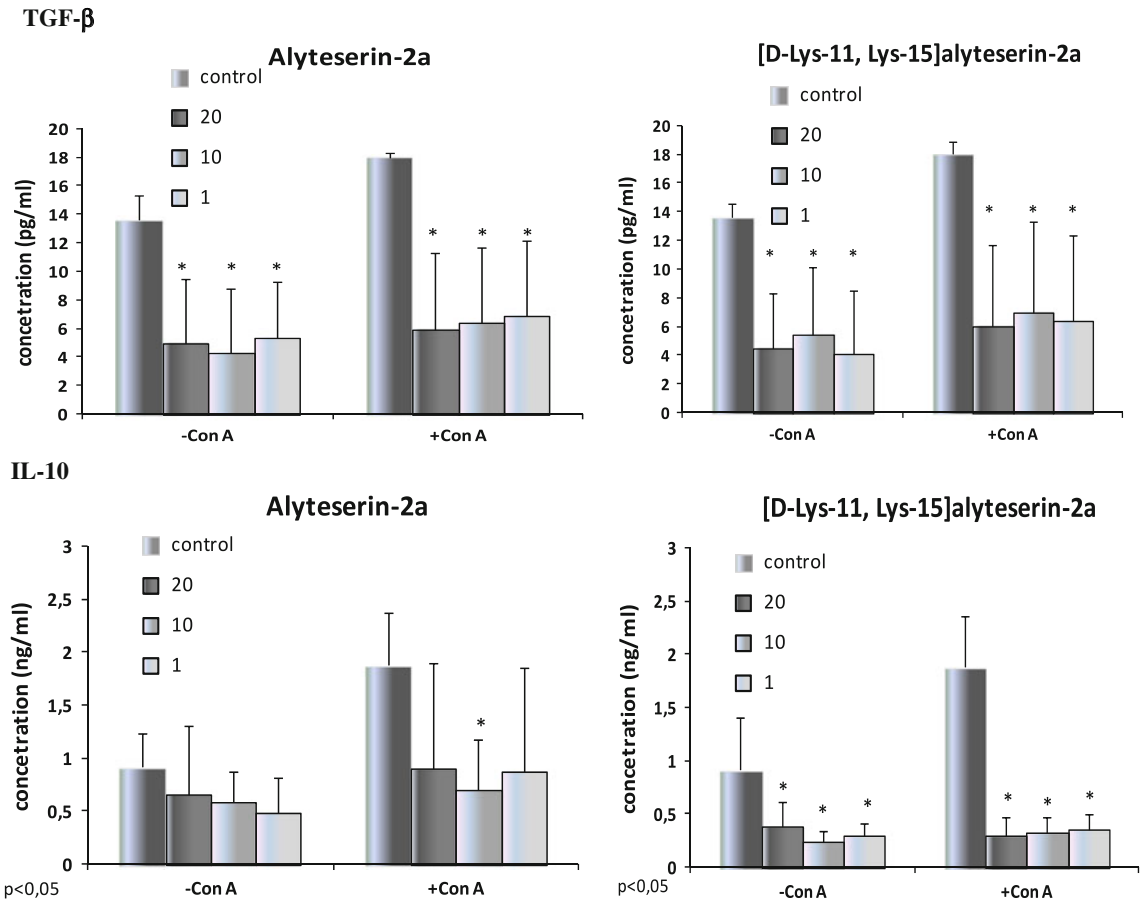

Fig. 3 Effects of alyteserin-2a and [G11k, N15K] alyteserin-2a (1, 10, and 20 μg/mL on the release of the immune-suppressive cytokines TGF-β and IL-10 from unstimulated (−conA) and concanavalin A-stimulated (+conA) peripheral blood mononuclear (PBM) cells. *$P < 0.05$ versus control

liver, and colon tumors, but with decreased hemolytic activity (LC_{50} = 195 μM) compared with the native peptide (Table 3). This activity is combined with good solubility at physiological pH so that the compound shows promise for development into a drug for treatment of tumors that have developed resistance to commonly used anti-cancer agents.

Cancer cells from a variety solid and hematopoietic tumors synthesize and release factors that facilitate growth and promote immune tolerance to escape host immune surveillance. These immune-suppressive mediators activate myeloid-derived suppressor cells, producers of the anti-inflammatory cytokines TGF-β and IL-10 (Srivastava et al. 2012). TGF-β is a potent regulator of tumorigenesis acting as a tumor suppressor at the onset of disease and a tumor promoter at later stages. Interruption of TGF-β signaling pathways is a promising target for cancer therapy (Calone and Souchelnytskyi 2012). Elevated circulating concentrations of IL-10 have been measured in patients with a wide range of tumors and serum IL-10 levels correlate with tumor progression and the presence of metastases (Galizia et al. 2002). Agents that inhibit TGF-β and IL-10 release may enhance the efficacy of other forms of chemotherapy for the treatment of certain malignancies (Sredni 2012). Consequently, the ability of [G11K, N15K] alyteserin-2a to inhibit the release of both TGF-β and IL-10 at concentrations as low as 1 μg/mL from PBM cells (Fig. 3) enhances its therapeutic potential as an anticancer agent.

The present study complements and extends earlier structure–activity work that led to the development of the analog [S7k, G11k] alyteserin-2a that showed high antimicrobial potency (minimum inhibitory concentration <10 μM) against both Gram-negative and Gram-positive bacteria including clinical isolates of multidrug-resistant *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* and a biofilm-producing strain of *Staphylococcus epidermidis* (Conlon et al. 2012). However, this analog shows relatively low cytotoxic potency against A549 tumor cells (LC_{50} = 65 μM) (Table 3). [G11k, N15K] alyteserin-2a also shows high antimicrobial potency, particularly against *S. aureus* (MIC = 8 μM) (Table 4). Solid tumors have been shown to act as a focus for polymicrobial

infections, involving both anaerobic and aerobic microorganisms especially when in proximity to sites such as the gastrointestinal tract and oral cavity where bacteria exist as part of the normal flora (Rolston et al. 2007). Thus, the dual anticancer and antimicrobial activities of [G11k, N15K] alyteserin-2a may complement each other in a therapeutic regime.

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