ELSEVIER

European Journal of Cancer 41 (2005) 1824–1830

European Journal of Cancer

www.ejconline.com

Experimental therapy of human endometrial cancers with a targeted cytotoxic bombesin analog AN-215: Low induction of multidrug resistance proteins

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Received 4 March 2005; received in revised form 4 April 2005; accepted 8 April 2005
Available online 26 July 2005

Abstract

In this study we have investigated the efficacy and toxicity of targeted cytotoxic bombesin (BN) analog AN-215 and its effects on the expression of three multidrug resistance proteins in experimental human endometrial cancers. Nude mice bearing HEC-1A, RL-95-2 and AN3CA tumours were treated with AN-215 and its cytotoxic radical (AN-201). The BN receptor expression in tumours was followed by RT-PCR analysis and radioligand binding assays. Expression of drug resistance proteins MDR-1, MRP-1 and BCRP were measured by realtime PCR. AN-215 significantly ($P < 0.05$) inhibited the growth of HEC-1A, RL-95-2 and AN3CA tumours while AN-201 was ineffective. The expression of BN receptors was demonstrated in all three tumour models. AN-215 caused a lower induction of MDR-1 in HEC-1A and RL-95-2 cancers than AN-201. MRP-1 and BCRP were not induced by AN-215 or AN-201. Thus, targeted chemotherapy with AN-215 powerfully inhibits the growth of human BN receptor-positive endometrial cancers.

Keywords: Targeted chemotherapy; Endometrial cancer; Bombesin/GRP receptor; Targeted cytotoxic bombesin analog AN-215; Multidrug resistance

1. Introduction

Endometrial carcinoma is the most common neoplasm of the female genital tract and in the USA it accounts for about 40,000 new cases and more than 7000 deaths annually [1–3]. If discovered at an early stage, endometrial cancer has a fairly good prognosis [2]. However, in patients with late stage or recurrent disease, survival rates decrease substantially to 18% and 7.7%, respectively [2,4]. Consequently, new therapeutic strategies are needed for advanced disease.

The discovery of molecular characteristics of tumour cells has led to the development of a new treatment strategy known as targeted therapy, the purpose of which is a direct delivery of antineoplastic drugs to cancer cells and a reduction in systemic toxicity. Modern targeted anti-cancer drugs include monoclonal antibodies against surface structures on malignant cells as well as conjugates consisting of receptor specific ligands linked to toxins, radionuclides or chemotherapeutic agents [5]. Higher intratumoural concentrations of antitumour agents produced by targeting may overcome

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[1] Supported by a fellowship within the post-doctorate program of the German Academic Exchange Service (DAAD).

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doi:10.1016/j.ejca.2005.04.031

chemoresistance of malignant cells and are expected to result in greater therapeutic efficacy.

Cancer cells can develop multidrug resistance (MDR) to a variety of antitumour agents that appear to be structurally and functionally unrelated. One mechanism of action of MDR is the increased efflux of chemotherapeutic agents mediated by transport proteins. The product of the MDR-1 gene, an adenosine triphosphate (ATP)-dependent membrane transporter termed P-glycoprotein (Pgp) and the recently discovered MDR protein 1 (MRP-1) use this mechanism of action [6,7]. Breast cancer resistance protein (BCRP) is another overlapping, but distinct type of MDR, based on drug efflux [8]. Expression of MDR-1 has been detected in most human endometrial cancer specimens as well as in normal endometrial tissue [9–11]. MRP immunoreactivity was detected in normal endometrium and it showed a progressive increase in intensity from endometrial hyperplasia to endometrial carcinoma [12]. Targeting chemotherapeutic drugs directly to tumour cells could overcome MDR based on transmembrane efflux, as it would increase the local concentration of the chemotherapeutic agent.

Specific receptors for bombesin/gastrin releasing peptide (GRP) peptides have been found in various human malignancies and cancer cell lines, including breast, prostatic and ovarian cancers and other tumours [13]. Consequently, we have developed a cytotoxic bombesin analog AN-215 by covalently linking a highly potent derivative of doxorubicin (DOX), 2-pyrrolino-DOX (AN-201) [14] to a bombesin-like carrier, Gln–Trp–Ala–Val–Gly–His–Leu–ψ (CH_2–NH)–Leu–NH_2 [15]. AN-215 shows high affinity to BN/GRP receptors, retains the antiproliferative effect of its cytotoxic moiety [15] and has been successfully used for the experimental therapy of various human cancers, such as prostatic, gastric and other tumours [13].

Receptors for BN/GRP were previously detected in AN3CA, KLE, HEC-1A and Ishikawa human endometrial cancer cells in vitro [16]. Consequently, the current study was designed to investigate the efficacy of targeted chemotherapy with AN-215 in AN3CA, HEC-1A as well as RL-95-2 cancer cell lines in vivo. In addition, we compared the effects of the treatment with AN-215 and the non-targeted cytotoxic radical AN-201 on the expression levels of MDR-1, MRP-1 and BCRP.

2. Materials and methods

2.1. Peptides and cytotoxic radical

Cytotoxic radical 2-pyrrolino-DOX (AN-201) and the cytotoxic bombesin analog AN-215, consisting of 2-pyrrolino-DOX-14-O-hemiglutarate linked to the amino terminal of Gln–Trp–Ala–Val–Gly–His–Leu–ψ (CH_2–NH)–Leu–NH_2 (RC-3094) were synthesised in our laboratory as previously described [15]. Bombesin antagonist RC-3095 (D-Tpi^6, Leu^{13}ψ (CH_2NH)) ^{14}LeuBN was also synthesised in our laboratory [17]. The compounds were dissolved in 5% (w/v) aqueous D-mannitol solution (Sigma, St Louis, MO, USA) before intravenous (i.v.) injection.

2.2. Cell lines

Human endometrial cancer cell lines HEC-1A, RL-95-2 and AN3CA were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were grown at 37 °C in humidified 95% air 5% carbon dioxide atmosphere, passaged weekly and routinely monitored for mycoplasma contamination using a detection kit (Boehringer Mannheim, Mannheim, Germany). All culture media were purchased from Gibco (Grand Island; NY, USA).

2.3. Animals

Five- to six-week-old female athymic nude mice (Ncr *nu/nu*) were obtained from the National Cancer Institute (NCI, Bethesda, MD, USA). The animals were housed in sterile cages under laminar flow hoods in a temperature-controlled room with a 12 h light/12 h dark schedule. They were fed autoclaved chow and provided water ad libitum.

2.4. Experiments

Exponentially growing cells from each cell line were implanted into 5 female donor nude mice by subcutaneous injection of 10^7 cells in both flanks. Tumours resulting after 4 weeks in donor animals were aseptically dissected and mechanically minced. Three mm^3 pieces of tumour tissue were transplanted subcutaneously (s.c.) in the experimental animals by a trocar needle. Tumour volume (length × width × height × 0.5236) and body weight were measured weekly. The total leukocyte count (WBC) was determined with the Unopette microcollection kit (Becton Dickinson, Franklin Lakes, NJ).

At the end of each experiment, animals were sacrificed under anesthesia, tumours were excised and weighed and necropsy was performed. Tumour specimens were snap frozen and stored at −70 °C. The procedures were in accordance with institutional guidelines for the welfare of animals in experimental research. The Institutional Animal Care and Use Committee reviewed the protocols for the animal experiment and gave full approval.

In experiment 1, when HEC-1A tumours had reached a volume of approximately 50 mm^3, mice were assigned to three experimental groups and were given a single i.v. injection of the corresponding agent into the jugular vein on day 1: Group 1 (control) received vehicle solution (5% mannitol) (5 mice); group 2 was injected with cytotoxic

analog AN-215 at 250 nmol/kg (6 mice); and group 3 was given cytotoxic radical AN-201 at 250 nmol/kg (5 mice).

In experiment 2, when RL-95-2 tumours had reached a volume of approximately 70–75 mm^3, mice were divided into 3 experimental groups: group 1 (control) received vehicle solution (10 mice); group 2 was injected with cytotoxic analog AN-215 at 200 nmol/kg (10 mice); and group 3 was given cytotoxic radical AN-201 at 200 nmol/kg (10 mice).

In experiment 3, when AN3CA tumours had reached a volume of approximately 130 mm^3, mice were assigned to six experimental groups: group 1 was given control vehicle solution (10 mice); group 2 was injected with cytotoxic analog AN-215 at 200 nmol/kg (10 mice); group 3 was given cytotoxic radical AN-201 at 200 nmol/kg (10 mice); group 4 received a mixture of cytotoxic radical AN-201 and the bombesin analog RC-3094 at 200 nmol/kg (5 mice); group 5 carrier RC-3094 alone at 200 nmol/kg (5 mice); and group 6 was injected with 200 μg of the bombesin antagonist RC-3095 i.v. 15 min prior to the injection of cytotoxic analog AN-215 at 200 nmol/kg (5 mice).

2.5. RNA extraction and reverse transcription (RT)-PCR

The methods for isolation of total RNA and RT-PCR for analysis of the expression of mRNA for the BN/GRP receptor have been reported elsewhere [18]. Ten microliter aliquots of the PCR products were separated on 1.8% agarose gel and visualised by ethidium bromide on an ultraviolet transilluminator.

2.6. Receptor binding studies

Binding sites for BN/GRP on HEC-1A, RL-95-2 and AN3CA tumours from the control groups were characterised by ligand competition assay using radiolabeled ^{125}I-[Tyr^4]-BN. Preparation of tumour membrane fractions and receptor binding studies of BN/GRP were performed as described previously [19,20]. The type of receptor binding, dissociation constant (K_d) and maximal binding capacity of the receptors (B_{max}) were calculated as previously described [21].

2.7. Realtime PCR for MDR-1, MRP-1 and BCRP mRNA expression

Total RNA was isolated from approximately 100 mg of tumour tissue for each sample according to the TRI-Reagent® protocol. One microgram of total RNA was subjected to reverse transcription with the Iscript cDNA synthesis kit (BioRad) following the manufactures protocol. Real time PCR was employed to measure drug resistance gene expression using the SYBR Green system (BioRad). Primers for MDR-1 sense 5′-TCT GGA GGA AGA CAT GAC CAG GTA-3′; antisense 5′-GGC ACC AAA ATG AAA CCT GAA TGT-3′], MRP-1 [sense 5′-AGA GAC AGC TCA GCA GCT CCT-3′; antisense 5′-GCC TTG TCA GCC TCC ATC AG-3′], BCRP [sense 5′-TAT CAA TGG GAT CAT GAA ACC TGG-3′, antisense: 5′-GCG GTG CTC CAT TTA TCA GAA C-3′] and β-actin [sense 5′-CTG GAA CGG TGA AGG TGA CA-3′; antisense 5′-AAG GGA CTT CCT GTA ACA ATG CA-3′] were used to measure gene expression. The thermal cycling conditions comprised an initial denaturation step at 95 °C for 3 min, then 40 cycles of two-step PCR including 95 °C for 15 s and 60 °C for 1 min. Data were collected during the 60 °C annealing step and were further analysed by the i-Cycler iQ Optical system software (Bio-Rad). Realtime PCR efficiencies (E) for MDR-1 (target gene 1), MRP (target gene 2), BCRP (target gene 3) and β-actin (reference gene) were calculated from the given slopes in the i-Cycler software according to the following equation: $E = 10^{[-1/\text{slope}]}$ [22]. Three tumour samples from each experiment (control-, the AN-215 and the AN-201 group) were analysed in triplicate. Quantification of the target genes in treated groups relative to the controls was performed using a mathematical model by Paffl [23] which takes into account the efficiencies and the crossing points (CP) for the transcripts of each sample:

$$\text{Ratio} = (E_{\text{target}})^{\Delta CP\ \text{target}\ (\text{control}-\text{treatment})} : (E_{\text{reference}})^{\Delta CP\ \text{reference}\ (\text{control}-\text{treatment})}$$

2.7.1. Statistical analysis

Data are expressed as means ± SE. Differences between mean values were evaluated by two-tailed Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. Effects of treatment with cytotoxic compounds on tumour growth in vivo

In experiment 1, a single dose of 250 nmol/kg cytotoxic bombesin analog AN-215 significantly inhibited the growth of HEC-1A human endometrial cancers. In animals treated with AN-215, the final tumour volume and weight were both significantly reduced by 48.0% ($P < 0.05$), respectively and tumour doubling time was significantly prolonged ($P < 0.01$). An equimolar dose of the cytotoxic radical AN-201, did not significantly affect any of these parameters (Fig. 1 and Table 1).

In experiment 2, a single dose of 200 nmol/kg of cytotoxic bombesin analog AN-215 significantly inhibited the growth of RL-95-2 human endometrial cancers as reflected by a decrease in tumour volume and weight of 65.0% ($P < 0.05$) and 60.0% ($P < 0.05$), respectively after 29 days and an extended tumour doubling time

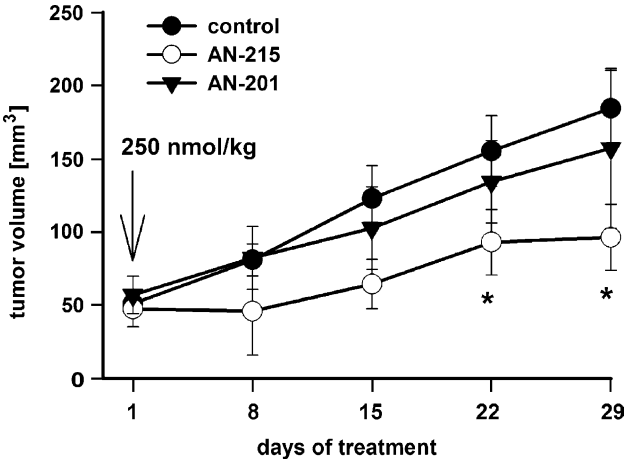

Fig. 1. Effects of targeted cytotoxic bombesin analog AN-215 and the radical AN-201 at doses of 250 nmol/kg on the growth of HEC-1A human endometrial carcinoma xenografted into nude mice (mean ± SE). Arrow indicates treatment. (*$P < 0.05$ two sided Student's *t*-test.)

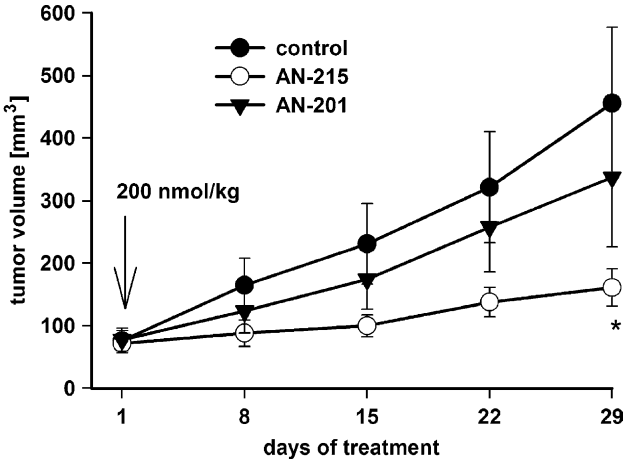

Fig. 2. Effects of targeted cytotoxic bombesin analog AN-215 and the radical AN-201 at doses of 200 nmol/kg on the growth of RL-95-2 human endometrial carcinoma xenografted into nude mice (mean ± SE). Arrow indicates treatment. (*$P < 0.05$ two sided Student's *t*-test.)

($P < 0.01$). An equimolar dose of the cytotoxic radical AN-201 alone had no significant effects on any of these parameters (Fig. 2 and Table 1).

In experiment 3, a single injection of AN-215 at 200 nmol/kg significantly decreased the growth of AN3CA human endometrial cancer xenografts, the mean tumour volume being 54.0% smaller than that of controls ($P < 0.01$) after 22 days of treatment. Tumour weight was reduced by 57.0% ($P < 0.05$) and tumour doubling time was also significantly prolonged ($P < 0.05$). Equimolar doses of the cytotoxic radical AN-201, a mixture of the bombesin analog carrier RC-3094 and AN-201 and RC-3094 alone had no significant effects. The effect of treatment with AN-215 was nullified by administering 200 μg of the bombesin antagonist RC-3095 15 min prior to the injection of AN-215 (Fig. 3 and Table 1).

3.2. Side effects and toxicity

In experiment 1, AN-215 and AN-201 at doses of 250 nmol/kg caused a significant ($P < 0.05$) decrease in the WBC on day 4 and 10 in both treated groups, however this reduction was more pronounced in animals treated with AN-201. In experiments 2 and 3, AN-201, but not AN-215 at doses of 200 nmol/kg significantly ($P < 0.05$) lowered the WBC (Table 1).

After the injection of AN-201 and AN-215 at 250 or 200 nmol/kg, a small body weight loss of 6.0–9.0% was

Table 1
Effects of therapy with cytotoxic analog of bombesin AN-215 and its cytotoxic radical AN-201 on the growth of HEC-1A, RL-95-2 and AN3CA human endometrial carcinomas xenografted into nude mice

Experiment and cell line	Treatment	Initial tumour volume (mm^3)	Final tumour volume (mm^3) (% inhibition)	Tumour weight (mg) (% inhibition)	Tumour doubling time (days)	WBC (cells/mm^3) on day 8	Deaths in experimental groups
1. HEC-1A	Control	50.9 ± 8.3	184.4 ± 26.0	208.3 ± 26.7	15.0 ± 1.1	8580 ± 416+	–
	AN-215	47.3 ± 12.2	93.0 ± 22.3 (48.0)*	108.2 ± 29.4 (48.0)*	26.4 ± 3.3**	4290 ± 542**+	–
	AN-201	56.8 ± 12.7	157.1 ± 54.5 (15.0)	161.7 ± 60.3 (22.0)	18.6 ± 3.5	2860 ± 110**+	–
2. RL-95-2	Control	75.9 ± 16.6	455.8 ± 121.5	513.7 ± 129.5	12.6 ± 0.9	9955 ± 819	1
	AN-215	71.5 ± 15.1	161.2 ± 29.9 (65.0)*	208.0 ± 2.0 (60.0)*	20.8 ± 2.0**	7150 ± 979	2
	AN-201	77.6 ± 18.6	337.1 ± 111.2 (26.0)	416.1 ± 142.1 (19.0)	15.1 ± 1.1	5445 ± 670**	4
3. AN3CA	Control	137.4 ± 26.8	3812.1 ± 656.5	4768.5 ± 982.2	4.9 ± 0.4	10918 ± 567	–
	AN-215	136.0 ± 21.0	1769.6 ± 285.0 (54.0)*	1929.5 ± 240.3 (57.0)*	6.4 ± 0.5*	7618 ± 1525	–
	AN-201	133.1 ± 28.8	3889.0 ± 979.9 (+2.0)	4981.5 ± 1090.2 (+5.0)	4.9 ± 0.4	5720 ± 273.2**	–
	Mixture	135.5 ± 82.5	3734.0 ± 1398.9 (2.0)	4763.5 ± 1787.9 (0.0)	4.6 ± 0.5	ND	–
	Carrier	140.7 ± 101.5	3928.3 ± 2114.3 (+3.0)	5003.2 ± 2734.6 (+5.0)	4.7 ± 1.0	ND	–
	Blockade	133.4 ± 46.5	3258.5 ± 1029.3 (15.0)	4167.8 ± 1139.4 (13.0)	4.8 ± 0.4	ND	–

In experiment 3, the effects of unconjugated mixture of the radical AN-201 and a BN analog (RC-3094) and the carrier RC-3094 alone are shown. Effects of blockade of BN/GRP receptors with BN antagonist RC-3095 are also demonstrated.
(ND: not determined), (*$P < 0.05$; **$P < 0.01$; two-sided Student's *t*-test), (+: In experiment 1, WBC was determined on day 4).

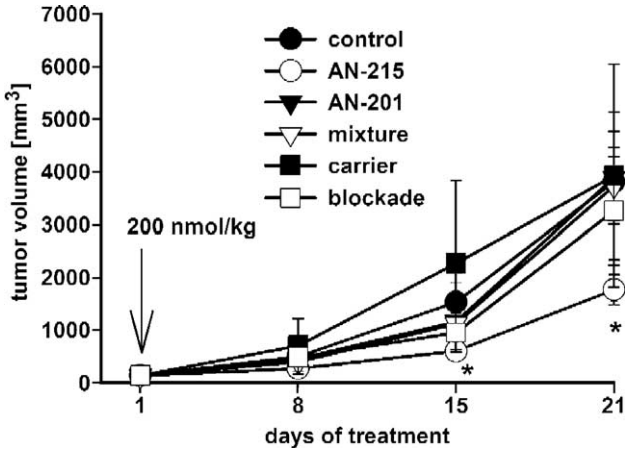

Fig. 3. Effects of targeted cytotoxic bombesin analog AN-215, the radical AN-201, a mixture of AN-201 and bombesin antagonist RC-3095 and RC-3095 alone, administered at doses of 200 nmol/kg, on the growth of AN3CA human endometrial carcinoma xenografted into nude mice. Effects of AN-215 after blockade of the bombesin/GRP receptors with the bombesin antagonist analog RC-3095 are also shown (mean ± SE). Arrow indicates treatment. (*$P < 0.05$ two sided Student's *t*-test.)

observed on treatment day 8. This loss was significant for AN-215 and AN-201 ($P < 0.05$) in experiment 2. However, on day 15 the body weights of the animals treated with these cytotoxic compounds no longer differed from the control groups. No death due to drug-related toxicity occurred in experiments 1 and 3. In experiment 2, four animals died after treatment with AN-201, and two after injection of AN-215.

3.3. Expression of mRNA for the BN/GRP receptor

RT-PCR analyses demonstrated the expression of mRNA for the GRP receptor in HEC-1A, RL-95-2 and AN3CA tumours of control animals. No PCR products were amplified from the negative controls, ruling out the possibility of genomic contamination (Fig. 4).

3.4. BN/GRP receptor binding studies

In membranes of HEC-1A, RL-95-2 and AN3CA tumours from the respective control group, receptor analyses revealed a single class of high affinity binding sites for bombesin. Mean binding affinities (K_d) and mean maximal binding capacities (B_{max}) are shown in Table 2.

3.5. MDR-1 and MRP-1 and BCRP mRNA expression by RT PCR

mRNA for MDR-1, MRP-1 and BCRP was detected in all 3 cell lines (Fig. 5). The PCR products were of the

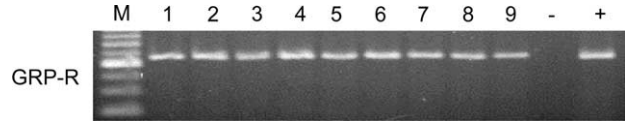

Fig. 4. Expression of the BN/GRP receptors subtype 1 as shown by RT-PCR. Lane M is molecular marker, lanes 1–3 show RL-95-2 control samples, lanes 4–6 HEC-1A control samples and lanes 7–9 AN3CA control samples. The negative control is total RNA and the positive control is the PC3 human prostate cancer cell line.

expected sizes of 95 bp for MDR-1, 127 bp for MRP-1, 140 bp for BCRP and 140 bp for β-actin (Fig. 5). The efficiencies (*E*) were 1.989 for MDR-1, 1.999 for MRP-1, 1.987 for BCRP and 1.997 for β-actin.

In HEC 1-A tumours, administration of AN-215 and AN-201 caused a 2.8- and a 4.0-fold induction of the MDR-1 gene, respectively. In RL-95-2 tumours, therapy with AN-215 and AN-201 was associated with an 11.2- and 16.1-fold induction of MDR-1 mRNA. In AN3CA tumours, MDR-1 expression was found to be 43.9- and 39.5-fold increased after injection of AN-215 and AN-201, respectively. AN-215 and AN-201 did not

Table 2
Binding characteristics of bombesin to BN/GRP receptors in HEC-1A, RL-95-2 and AN3CA human endometrial cancers xenografted into nude mice

Tumour line	K_d (nM)	B_{max} (fmol/mg protein)
HEC-1A	5.5 ± 10.1	331.9 ± 21.7
RL-95-2	4.56 ± 0.5	384.7 ± 31.1
AN3CA	7.18 ± 0.6	614.6 ± 10.4

Binding characteristics were obtained from 12-point displacement experiments using ^{125}I-[Tyr^4]BN. All values represent mean ± SE of triplicate determinations.

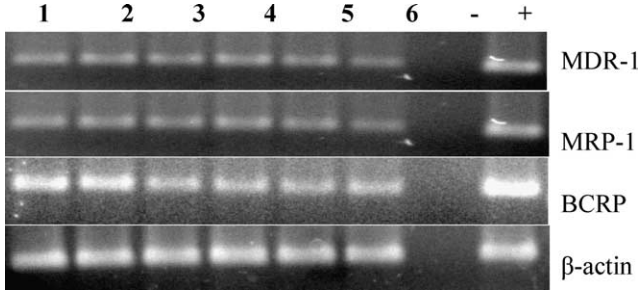

Fig. 5. Expression of mRNA for MDR-1, MRP-1 and BCRP in human endometrial cancers analysed by RT-PCR. PCR products were separated on a 1.8% agarose gel and stained with ethidium bromide. Specific bands at the expected of 95 bp for MDR-1, 127 bp for MRP-1 and 140 bp for BCRP were found in all three human endometrial cancer cell lines. Lanes 1–2 show HEC-1A control samples, lanes 3–4 RL-95-2 control samples, lanes 5–6 AN3CA control samples. The negative control is total RNA The positive controls for MDR-1, MRP-1 and BCRP were the A498 human renal cell cancer cell line, the H-460 human lung cancer line and the MCF-7 human breast cancer line, respectively.

significantly induce MRP-1 or BCRP in any experiment (data not shown).

4. Discussion

Most endometrial cancer specimens as well as the normal endometrial tissue express the MDR-1 gene [9–11]. Thus, endometrial carcinoma belongs to a class of tumours, which originate in organs normally over-expressing the MDR-1 gene and intrinsic resistance to chemotherapy tends to be a common feature of these cancers. Targeted chemotherapy was developed to selectively deliver cytotoxic radicals to malignant cells and thus to achieve a higher intratumoural concentration of cytotoxic drugs. This novel therapeutic approach results in increased antitumour effect and may overcome multidrug resistance of neoplastic cells. Accordingly, in previous studies with LHRH receptor positive human endometrial carcinomas, a strong inhibition of tumour growth was observed after treatment with the cytotoxic LHRH analogs AN-152 and AN-207, which consist of DOX or 2-pyrrolinoDOX linked to [D-Lys^6]-LHRH, respectively [24,25]. The non-targeted radicals DOX and 2-pyrrolinoDOX however, had only minor effects [24,25]. As the expression of receptors for BN/GRP in HEC-1A, Ishikawa, KLE and AN3CA human endometrial cancer cell lines has recently being reported in a study [16], we decided to evaluate the efficacy of our targeted cytotoxic bombesin analog AN-215 in human endometrial cancers xenografted into nude mice. In addition to the HEC-1A and AN3CA lines we investigated a third cell line, RL-95-2. All three cell lines expressed mRNAs for the BN/GRP receptor and displayed specific high affinity binding sites for ^{125}I-labeled [Tyr^4] bombesin. In accordance with the presence of BN/GRP receptors, targeted cytotoxic bombesin analog AN-215 significantly inhibited the growth tumours of all three cell lines xenografted into nude mice. In contrast, the non-targeted cytotoxic radical AN-201 was ineffective at equimolar doses. To demonstrate that the significant antitumour effects of AN-215 are due to the specific delivery of the cytotoxic radical AN-201 to tumour cells through its conjugation to the bombesin analog carrier RC-3094, in a comprehensive study with AN3CA cancers, we included a group of animals that received the carrier RC-3094 and cytotoxic radical as an unconjugated mixture. As no antitumour effects were observed in this group, we can conclude that bombesin analog RC-3094 did not act as a hormone to potentiate the effects of radical AN-201. In addition, we showed that RC-3094, alone at a single dose of 200 nmol/kg, similarly had no effects.

To further demonstrate that the action of AN-215 was mediated by bombesin receptors, mice bearing AN3CA xenografts were pretreated with an excess of bombesin antagonist RC-3095 to block the bombesin receptors prior to the administration of AN-215. We found that the antitumour activity of AN-215 was strongly decreased by RC-3095, demonstrating that the unoccupied bombesin receptors are obligatory for targeted therapy with AN-215.

The toxicities of targeted cytotoxic bombesin analogs AN-215 and its radical AN-201 were compared with respect to the incidence of death, weight loss and WBC suppression. Two mice died after the injection of AN-215 and 4 deaths occurred after treatment with AN-201. In all experiments, minor body weight loss of 6.0–9.3% occurred 8 days after treatment in groups treated with AN-215 and AN-201, but this effect was transient and by day 15 animals recovered. The myelotoxicity is generally the most serious side effect and the dose-limiting factor of chemotherapy. At doses of 250 nmol/kg, both AN-215 and AN-201 significantly lowered the WBC, the decrease being more pronounced in animals treated with AN-201. Therefore in experiment 2 and 3 the dose was lowered to 200 nmol/kg, which was equally effective, but at that dose only AN-201 significantly lowered the WBC, while AN-215 caused only a minor decrease. The significant fall in WBC after treatment with AN-215 at 250 nmol/kg was probably due to high esterase activity in the serum of nude mice, which can cause the cleavage of the ester bond in AN-215 and release AN-201. As a result, AN-201 could reach a level in the circulation, which would cause a decrease in WBC. We have also previously demonstrated that the inhibition of the esterase activity in mice significantly reduced the toxicity of AN-215 [26,27]. The overall toxicity of AN-215 in animals is smaller than that of its cytotoxic radical. Due to the esterase activity being much lower in humans than in mice [26], the hematotoxicity of AN-215 (due to AN-201) may be reduced in patients.

In recent in vitro studies, MDR-1 protein levels in the HEC-1A endometrial cancer line were investigated after treatment with targeted cytotoxic LHRH analog AN-152 and its cytotoxic radical DOX [28]. Both compounds induced surface expression of MDR-1 gene product Pgp, but the effect of AN-152 was smaller than that of DOX [28,29]. In the present study, targeted cytotoxic bombesin analog AN-215 also caused a weaker induction of MDR-1 in HEC-1A and RL-95-2 human endometrial cancers than its cytotoxic radical AN-201. Thus, development of chemoresistance mediated by MDR-1 may be delayed by targeted chemotherapy in some types of tumours. No major induction of MRP-1 and BCRP mRNA occurred after treatment with AN-215 and AN-201, respectively.

In conclusion, cytotoxic bombesin analog AN-215 is more effective and less toxic than its non-targeted cytotoxic radical AN-201 and could be considered for therapy of bombesin/GRP receptor-positive endometrial cancers. Our findings support the merit of clinical trials

with AN-215 in patients with advanced or recurrent endometrial cancers. AN-215 should be available clinically in the near future.

Conflict of interest statement

None declared.

Acknowledgements

This work is dedicated to the late Ana-Maria Comaru-Schally, M.D., F.A.C.P. who died recently from thyroid cancer, for her intellectual, spiritual and personal contribution and for the inspiration she provided to this project.

The study was supported by the Medical Research Service of the Veterans Affairs Department and by a grant from Zentaris AG, Frankfurt on Main, Germany, (all to AVS) to Tulane School of Medicine. We wish to thank Patricia Armatis, Miriam Ducruet and Harold Valerio for excellent experimental assistance and Dudley R. Callais for help in preparation of the manuscript.

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