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Antagonists of growth hormone-releasing hormone (GH-RH) enhance tumour growth inhibition induced by androgen deprivation in human MDA-Pca-2b prostate cancers

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Abstract

In the present study, we investigated whether the growth hormone-releasing hormone (GH-RH) antagonist JV-1-38 could enhance the effects of androgen deprivation produced by the anti-androgen Flutamide and luteinising hormone-releasing hormone (LH-RH) agonist Decapeptyl in an experimental model of human androgen-sensitive MDA PCa 2b prostate carcinoma implanted subcutaneously (s.c.) into nude mice. We also evaluated by reverse transcriptase-polymerase chain reaction (RT-PCR) the effects of combined treatment on the mRNA expression for prostate-specific antigen (*PSA*) and measured serum PSA levels. In experiment 1, GH-RH antagonist JV-1-38 greatly inhibited tumour growth in combination with Decapeptyl, but was ineffective when given alone. Thus, combined therapy with JV-1-38 at 20 μg/day and Decapeptyl microcapsules releasing 12.5 μg/day for 29 days inhibited significantly ($P<0.01$) MDA PCa 2b tumour growth by 65%, compared with controls. Combined treatment also significantly ($P<0.05$) decreased serum PSA levels by 52% and reduced tumour weight by 54% vs. controls. In experiment 2, GH-RH antagonist JV-1-38 at 20 μg/day likewise showed powerful growth inhibitory effects when combined with Flutamide (25 mg/kg/day) for 21 days. Combined treatment with JV-1-38 and slow-release pellets of Flutamide significantly ($P<0.001$) inhibited tumour growth by 61% versus controls, and was significantly ($P<0.05$) more effective than Flutamide or JV-1-38 alone. Combination therapy also reduced significantly ($P<0.001$) tumour weight and serum PSA levels by 59 and 47%, respectively. The mRNA expression for *PSA* in MDA PCa 2b tumours was not changed by JV-1-38, Decapeptyl and Flutamide alone or in their respective combinations. Our findings suggest that GH-RH antagonists could enhance the tumour inhibitory effects of androgen deprivation for the primary therapy of patients with advanced prostate carcinoma.

Keywords: GH-RH antagonists; Prostate cancer; Prostate-specific antigen; Androgen deprivation therapy

1. Introduction

Androgens play a crucial role in the tumorigenesis and progression of prostate cancer [1]. Thus, current treatment modalities for the therapy of advanced prostate cancer, are based upon androgen deprivation induced by orchiectomy, depot formulations of agonists of luteinising hormone-releasing hormone (LH-RH) or anti-androgens. These therapies bring about the suppression of tumour growth and the induction of apoptosis [2,3]. In the past decade, LH-RH agonists have become the preferred treatment modality for men with advanced carcinoma of the prostate [2,3]. Chronic administration of LH-RH agonists produces a marked inhibition of the pituitary and the gonadal functions, resulting in medical castration [2,4,5]. Anti-androgens, which exert their effects by interfering with the binding of dihydrotestosterone to the androgen receptor (AR), are currently used prior to and during early therapy with LH-RH agonists to prevent an initial rise in androgen levels (flare-up) following LH-RH treatment [2,3] or together with LH-RH agonists to produce a complete androgen blockade. However, it is well established that all these modalities of androgen deprivation cause only a remission of limited duration and a

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progression to androgen-independent prostate cancer inevitably occurs [1–3]. Therefore, new treatment options are needed to improve the therapy for advanced carcinoma of the prostate.

Antagonistic analogues of growth hormone-releasing hormone (GH-RH) are promising new antitumour agents effective against various experimental malignancies, including human prostate cancers [6]. The antiproliferative effects of GH-RH antagonists are exerted in part indirectly through inhibition of the pituitary growth hormone/hepatic insulin-like growth factor-I (IGF-I) axis as well as through the direct suppression of tumoral IGF-I and-II, and vascular endothelial growth factor (VEGF) [7–9]. In addition, recent evidence points to another mechanism of GH-RH antagonists independent of IGFs and based on the direct blocking of a tumoral autocrine/paracrine GH-RH stimulatory loop [10–12]. This mechanism is likely mediated by truncated splice variants (SVs) of pituitary GH-RH receptors. The presence of these SVs of GH-RH receptors was demonstrated in LNCaP, MDA PCa 2b, C4-2b, PC-3 and DU-145 human experimental prostate cancer lines [8,11,13–15], surgical specimens from patients with locally advanced prostate cancer [15] as well as in a large variety of human cancer lines [2,10,14].

In a previous study, we showed that GH-RH antagonist JV-1-38 is effective in combination with castration in human androgen-sensitive prostate cancers xenografted into nude mice [8]. However, it is presently unclear if GH-RH antagonists could also exert tumour inhibitory effects in combination with other methods of androgen deprivation. Thus, we investigated in this study whether a combination of GH-RH antagonist JV-1-38 with LH-RH agonist Decapeptyl or anti-androgen Flutamide could inhibit growth of MDA PCa 2b human androgen-sensitive prostate cancers xenografted into nude mice. We also measured the effects of these treatments on serum prostate-specific antigen (PSA) levels in nude mice. To further elucidate the functional activity of the AR after treatment with these compounds, we investigated the mRNA expression of the androgen-responsive protein PSA.

2. Materials and methods

2.1. Peptides and anti-androgens

GH-RH antagonist JV-1-38 was synthesised in our laboratory by solid a phase method and purified as described in Ref. [16]. For daily subcutaneous (s.c.) injection at a dose of 20 μg/day, the compound was dissolved in 0.1% dimethylsulphoxide in 10% aqueous propylene glycol solution. Microcapsules of the LH-RH agonist [D-Trp^6]LH-RH or-Triptorelin (Decapeptyl), dispersed in a polymeric matrix of poly (DL-lactide-co-glycolide) (Ipsen Pharma Biotech, Toulon, France), were suspended in an injection vehicle and 375 μg aliquots, releasing 12.5 μg Decapeptyl/day for 30 days, were injected s.c. in 0.2 ml into each mouse [17]. Flutamide was given by slow-release pellets (Innovative Research of America, Sarasota, FL), at doses of 10 mg releasing 25 mg/kg/day for 30 days. The doses for JV-1-38, Decapeptyl and Flutamide were chosen based on previous studies [8,9,17–19].

2.2. Animals

Male athymic (Ncr *nu*/*nu*) nude mice approximately 6 weeks old, were obtained from the National Cancer Institute (Frederick Cancer Research and Development Center, Frederick, MD) and housed and fed as described in Ref. [8]. All experiments were performed in accordance with institutional ethical guidelines for the care and use of experimental animals.

2.3. Experimental protocols

Human androgen-sensitive prostate cancer cell line MDA PCa 2b was obtained from Dr. N. Navone, the University of Texas, M.D. Anderson Cancer Center (Houston, TX) and maintained in culture as described in Refs. [8,20]. MDA-PCa-2b tumours were initiated and implanted s.c. as described in Refs. [8,20].

2.3.1. Experiment 1

The treatment was started when s.c. tumours had grown to a mean volume of approximately 160 mm^3. Animals were randomly assigned into four groups, containing 8 animals each: 1. Control (vehicle solution); 2. JV-1-38 at 20 μg/day; 3. Decapeptyl microcapsules and 4. Combination (JV-1-38 and Decapeptyl). On day 1, groups 3 and 4 received a single s.c. injection of Decapeptyl microcapsules which released approximately 12.5 μg/mouse per day for 30 days as described in Refs. [17,19]. The other groups received vehicle injection only. The mice were sacrificed on day 29 or when they became moribund.

2.3.2. Experiment 2

When s.c. tumours had grown to a mean volume of approximately 350 mm^3, animals were randomly assigned to four groups, containing 8 mice each: 1. Control (vehicle solution); 2. JV-1-38 at 20 μg/day; 3. Flutamide at 25 mg/kg/day and 4. Combination (JV-1-38 and Flutamide). Flutamide slow release pellets at 10 mg doses releasing 25 mg/kg body weight (BW) per day were implanted on day 1 through a 5 mm incision on the necks of nude mice, according to the manufacturer's instructions. This procedure was performed under Isoflurane anesthesia (Abbott Laboratories, North Chicago,

IL). The experiment was ended on day 21 by euthanising the animals.

BW and tumour volumes were monitored weekly as described in Ref. [8]. At the end of the experiment, the animals were euthanised by severing the abdominal aorta. Trunk blood was collected and serum was separated for quantitative measurement of total PSA. Liver, heart, lungs, kidneys, spleen, testicles, prostate and seminal vesicles were carefully removed under a dissecting microscope and weighed. The tumours were excised, weighed, snap-frozen and stored at −70 °C until further analyses.

2.4. RIA for IGF-I and testosterone

Serum samples for IGF-I determination were extracted by using a modified acid/ethanol cryoprecipitation method [12] to eliminate most of the IGF-binding proteins that can interfere in the RIA. Rat IGF-I (Receptor Grade, Gro-Pep, Adelaide, Australia) was used as a standard in the range of 2–2000 pg per tube and also for iodination by the chloramine-T method. Goat anti-rat IGF-I obtained from Diagnostic System Laboratories (DSL Inc., Webster, TX) was used at the final dilution of 1: 20,833 in the RIA. Intraassay and interassay coefficients of variation were less then 10 and 15%, respectively. Serum testosterone was measured by using a commercial kit from DSL Inc. (DSL-4100).

2.5. PSA measurement

Quantitative measurement of total PSA in serum was performed as reported in Ref. [8] using the reagents and protocol Active PSA Coated-Tube IRMA kit DSL-9700 provided by DSL Inc.

2.6. mRNA isolation and RT-PCR

Total RNA was extracted from frozen tissue samples using TRI reagent (Molecular Research Center, Inc., Cincinnati, OH) as described [8]. For amplification of cDNA transcripts, gene-specific primers for *PSA* and *β-actin* were used as described in Ref. [8]. Two microgrammes of total RNA were reverse transcribed by using Moloney murine leukaemia virus reverse transcriptase according to the manufacturer's instructions (Perkin Elmer, Alameda, CA). Polymerase chain reaction (PCR) products were subjected to electrophoresis on a 1.8% agarose gel, then stained with ethidium bromide, and visualised under ultraviolet (UV) light. Bands of PCR products were scanned and analysed semiquantitatively by using the Kodak EDAS 290 imaging system with the Kodak 1D 3.6 Image Analysis Software (Eastman Kodak Comp. Rochester NY). All experiments were repeated at least twice, and mRNA levels for each gene were normalised versus the corresponding levels of mRNA for *β-actin*. Negative controls were run in parallel to exclude genomic DNA contamination.

2.7. Statistical analysis

Data are expressed as means ± standard errors of the means (SEM). Differences between the values were evaluated with the two-tailed Student's *t*-test, a $P < 0.05$ being considered significant.

3. Results

3.1. Inhibition of growth of MDA PCa 2b human androgen-sensitive prostate cancers and serum PSA levels by combined treatment of JV-1-38 and Decapeptyl or Flutamide

In experiment 1, we evaluated whether daily s.c. administration of GH-RH antagonist JV-1-38 in combination with Decapeptyl given in depot microcapsules could exert antiproliferative effects on the growth of s.c. implanted MDA PCa 2b prostate cancers. As shown in Fig. 1a and Table 1, therapy with JV-1-38 or Decapeptyl alone for 29 days inhibited tumour growth only non-significantly, compared with control mice. In contrast, combined treatment with JV-1-38 and Decapeptyl strongly suppressed tumour growth, reducing tumour volume and weight by 65% ($P < 0.01$) and 54% ($P < 0.05$), respectively, compared with the controls (Table 1, Fig. 1a). At the end of the experiment, serum PSA levels in animals treated with combined therapy were significantly ($P < 0.05$) reduced by 52% compared with control values (Table 1), whereas Decapeptyl or JV-1-38 alone had no significant effect. The PSA secretion index (ng/ml serum PSA per mg tumour weight) was not significantly changed after treatment with JV-1-38 (0.85 ± 0.07), Decapeptyl (0.69 ± 0.03) or combined treatment (0.93 ± 0.12) compared with control values (0.89 ± 0.14).

In experiment 2, we investigated if a combination therapy consisting of daily s.c. injections of JV-1-38 and slow release pellets of Flutamide could also exert antiproliferative effects on the s.c. growth of MDA PCa 2b tumours in nude mice. As shown in Fig. 1b and Table 1, three weeks of therapy with JV-1-38 alone had no effects on tumour growth in accord with results of experiment 1. Flutamide inhibited slightly tumour growth by 25%, but this difference did not reach statistical significance (Table 1, Fig. 1b). However, the combination of JV-1-38 with Flutamide significantly inhibited tumour volume by 61% ($P < 0.001$), reduced tumour weight by 59% ($P < 0.001$) and extended the tumour doubling time from 6.6 to 12.6 days ($P < 0.001$), compared with controls (Table 1). There were also statistically significant differences ($P < 0.05$) for the inhibition of tumour

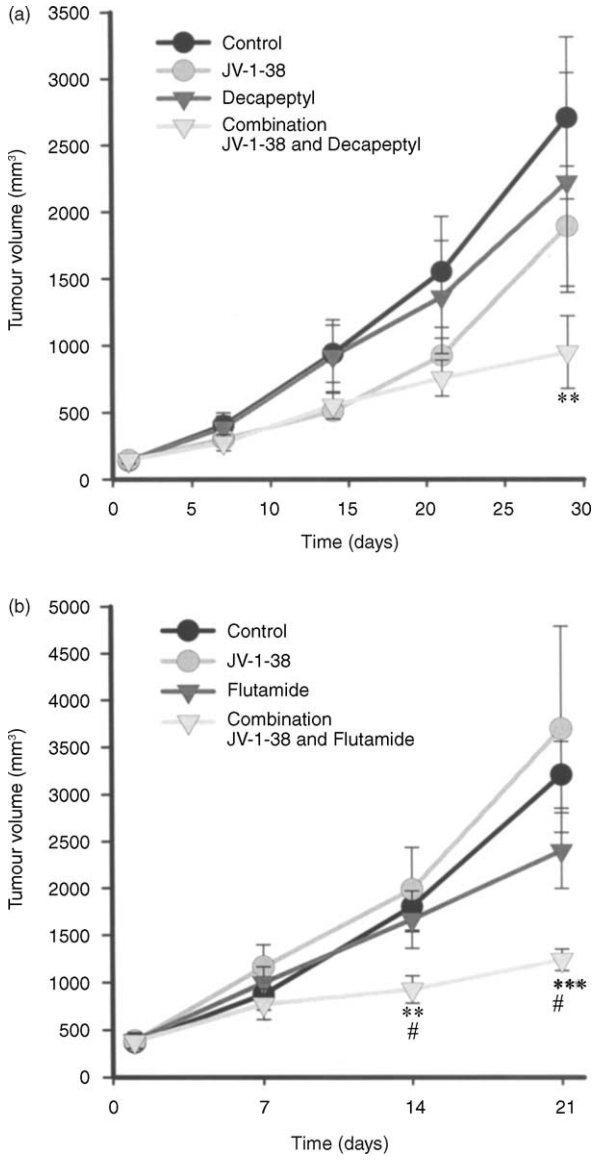

Fig. 1. The effects of daily subcutaneous (s.c.) injections of growth hormone-releasing hormone (GH-RH) antagonist JV-1-38 at doses of 20 μg/day, microcapsules of luteinising hormone-releasing hormone (LH-RH) agonist Decapeptyl releasing 12.5 μg/day, or their combination (experiment 1) (a) and the effects of treatment JV-1-38 at 20 μg/day, slow release pellets of Flutamide at 25mg/kg/day or their combination (b) on the tumoral volume of s.c. xenografted MDA PCa 2b androgen-sensitive prostate cancers. Vertical bars represent standard error of the mean; $^{**}P<0.01$ versus control, $^{\#}P<0.05$ versus Flutamide and JV-1-38 alone.

volume, reduction in tumour weight and extension of tumour doubling time between the combined treatment group and those given Flutamide or JV-1-38 alone (Table 1, Fig. 1b). At the end of the experiment, serum PSA levels in animals treated with the combination therapy were significantly ($P<0.001$) reduced by 47% compared with control values. Combined treatment also lowered serum PSA levels by 30 and 46% compared with levels in animals treated with Flutamide or JV-1-38 alone, respectively (Table 1). As in experiment 1, the

Table 1
Effects of treatment with growth hormone-releasing hormone (GH-RH) antagonist JV-1-38, Decapeptyl, Flutamide and their combinations on tumour volume and weight, tumour doubling time, serum prostate-specific antigen (PSA) levels and mRNA expression for *PSA* of subcutaneous (s.c.) implanted MDA PCa 2b prostate cancers in nude mice

Treatment groups	Tumour volume (mm^3)		Tumour doubling time (days)	Tumour weight (mg) (% inhibition)	Final serum PSA (ng/ml)[a]	mRNA expression for *PSA* (% control)
	Initial	Final (% inhibition)				
Experiment 1						
Control	140.8±34.1	2700±608.3	6.1±0.8	2447.2±445.4	1804.8±213	100.0±4.1
JV-1-38	146.6±17.6	1889.1±448.6 (30%)	8.4±0.5	1795.1±389.2 (27%)	1499.3±374.2	105.6±0.6
Decapeptyl	143.1±24.6	2218.3±822.3 (18%)	7.9±0.8	2404.5±942.7	1715.1±736.8	106.7±3.6
Combination (Decapeptyl and JV-1-38)	146.8±30.5	950.5±270.1 (65%)**	15.1±5.7	1116.3±339.7 (54%)*	856.4±259.5*	98.3±7.1
Experiment 2						
Control	370.0±72.0	3208.8±353.5	6.6±0.4	3256.5±276.8	2347.6±253	100.0±5.0
JV-1-38	385.6±74.3	3695.7±1098.4	7.4±0.9	3254.7±846.7	2290.7±634.5	114.9±4.6
Flutamide	389.7±82.4	2401.1±405.2 (25%)	8.1±0.8	2289.0±414.1 (30%)	1767.8±280.4	99.6±6.2
Combination (Flutamide and JV-1-38)	378.7±76.6	1240.9±112.9 (61%)***,#	12.6±1.9***,#	1351.6±112.2 (59%)***,#	1234.8±181.5***	105.2±4.2

[a] Measured at necropsy; $^{*}P<0.05$ versus control, $^{**}P<0.01$ versus control, $^{***}P<0.001$ versus control, $^{\#}P<0.05$ versus Flutamide and JV-1-38 alone.

PSA secretion index was not significantly changed after treatment with JV-1-38 (0.73±0.12), Flutamide (0.79±0.05) or the combined treatment (0.84±0.05) compared with the control values (0.72±0.06).

3.2. Effects of JV-1-38, Decapeptyl or Flutamide and their respective combinations on the organ- and body weights of nude mice bearing MDA PCa 2b prostate cancers

In experiment 1, treatment for 29 days with JV-1-38, Decapeptyl or their combination did not change the body weights in nude mice compared with control animals (Table 2). Similarly, in experiment 2, body weights of animals treated with JV-1-38, Flutamide, or their combination were not changed, compared with those of controls. In contrast, the necropsy revealed significant differences in the weights of the reproductive organs in nude mice after treatment with Flutamide, Decapeptyl or their combination with JV-1-38 compared with controls (Table 2). In experiment 1, the weights of the testicles were significantly ($P<0.01$) reduced by treatment with Decapeptyl alone or in combination with JV-1-38 (Table 2), and the weight of the prostate was also significantly decreased ($P<0.05$) in the group that received Decapeptyl alone (Table 2). Treatment with JV-1-38 did not alter the weight of the reproductive organs in nude mice (Table 2).

In experiment 2, Flutamide also reduced significantly ($P<0.01$) the weight of the testicles and the prostate compared with control animals (Table 2). Combined treatment with JV-1-38 and Flutamide lowered significantly ($P<0.05$) the weight of the prostate and the seminal vesicles versus the control values (Table 2). As in experiment 1, JV-1-38 alone did not change the weight of the reproductive organs compared with controls. The weights of heart, lungs, kidney, spleen and liver were not affected by the treatment (data not shown). Two animals each died in control group, the group treated with Decapeptyl and the combination group in experiment 1, most likely due to tumour cachexia, whereas no animals died in experiment 2.

3.3. Effects of treatment with JV-1-38 and Decapeptyl or Flutamide on the expression of mRNA for PSA in MDA PCa 2b human androgen-sensitive prostate cancers

In both experiments, using gene-specific primers, we investigated the expression of mRNA for *PSA* in MDA PCa 2b tumours. PCR products of 722 bp, corresponding to the mRNA for *PSA* were detected in MDA PCa 2b tumours in all treatment groups in both experiments 1 and 2 (Fig. 2a and b). Densitometric analyses of the RT-PCR products of experiment 1 (Fig. 2a, Table 1) and experiment 2 (Fig. 2b, Table 1) showed no significant differences in the mRNA expression for *PSA*

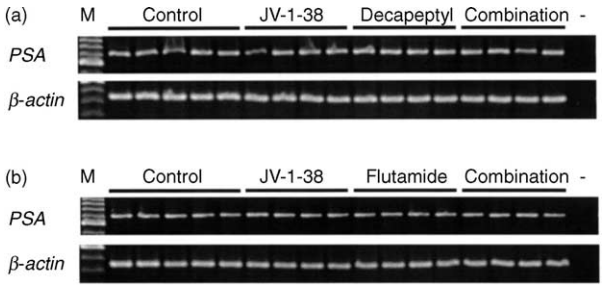

Fig. 2. The effect of JV-1-38, Decapeptyl or their combination (experiment 1) (a), and the effects of JV-1-38, Flutamide or their combined treatment (experiment 2) (b) on the expression of mRNA for human *PSA* (722 bp) in human MDA PCa 2b prostate cancers as revealed by reverse-transcriptase-polymerase chain reaction (RT-PCR). Lane M is the 100 bp molecular marker. The PCR products were resolved on a 1.8% agarose gel and stained with ethidium-bromide. mRNA for *β-actin* (459 bp) was used as an internal control:-negative control.

Table 2
Effects of treatment with JV-1-38, Decapeptyl, Flutamide and their respective combinations on the body weight and the weight of the reproductive organs of nude mice

Treatment groups	BW (g)	Weights of the reproductive organs		
		Testicles (mg)	Seminal vesicles (mg)	Prostate (mg)
Experiment 1				
Control	25.8±0.8	160.3±5.4	110.6±13.7	64.0±4.5
JV-1-38	26.7±1.5	163.8±8.3	147.9±29.8	72.9±10.9
Decapeptyl	24.1±0.7	107.0±5.4** (33%)	83.4±10.2 (25%)	50.3±3.9 (21%)*
Combination (Decapeptyl and JV-1-38)	25.2±0.8	124.0±3.8** (23%)	105.2±13.5	62.2±10.2
Experiment 2				
Control	24. 4±0.9	181.6±4.9	67.3±5.8	64.0±6.3
JV-1-38	24.2±0.8	182.6±9.9	74.7±11.9	63.1±7.2
Flutamide	23.5±1.0	155.4±7.1 (14%)**	57.0±8.1 (15%)	43.8±3.8 (32%)**
Combination (Flutamide and JV-1-38)	23.0±0.5	173.4±10.4 (4%)	48.0±5.5 (29%)*	46.0±5.5 (28%)*

*$P<0.05$ versus control, **$P<0.01$ versus control. BW, body weight.

after treatment compared with controls (Table 1, Fig. 2). Negative controls were used to rule out the possibility of genomic DNA contamination. The amplification with human *β-actin* yielded a single product of 459 bp.

3.4. Effects of treatment with JV-1-38 and Decapeptyl or Flutamide on the serum levels of IGF-I and testosterone in nude mice

In experiment 1, the serum levels of IGF-I in nude mice treated with JV-1-38, Decapeptyl, or their combination were 123.8±11.2, 102.0±9.5 and 150.2±17.2 ng/ml, respectively. These values were not significantly different from the control group, in which serum IGF-I was 118.6±12.3 ng/ml. In experiment 2, serum levels of IGF-I in the groups that received JV-1-38, Flutamide and their combination were 207.6±9.7, 256.6±13.6 and 259.1±9.9 ng/ml, respectively, and did not differ significantly from controls (232.3±6.9 ng/ml). Testosterone values from experiment 1 were not available. However, in a related experiment, treatment with Decapeptyl microcapsules releasing 12.5 μg/day for 30 days lowered serum testosterone significantly ($P<0.05$) to 142±26 pg/ml compared with 228±24 pg/ml in the control group. In experiment 2, testosterone levels in the control group were 256±63 pg/ml. Treatment with Flutamide significantly increased serum testosterone ($P<0.01$) to 579±67 pg/ml and there was also a non-significant increase to 431±55 pg/ml ($P=0.064$) in the group that received a combination of JV-1-38 and Flutamide, while JV-1-38 alone had no effect, the level being 271±42 pg/ml.

4. Discussion

Extensive oncological investigations have clearly demonstrated tumour inhibitory effects of GH-RH antagonists in various experimental models of cancer including human androgen-independent prostate cancers [2,6–9,21]. The PC-3 and DU-145 prostate cancer cell lines used in these studies did not express the AR, which is present even in the most advanced forms of androgen-independent prostate cancer in man [22], but we subsequently demonstrated in a recent work that the GH-RH antagonist JV-1-38 could inhibit the growth of AR-positive human androgen-sensitive LNCaP and MDA PCa 2b prostate cancers in castrated nude mice [8]. Interestingly, treatment of intact mice bearing these androgen-sensitive tumours with JV-1-38 alone did not inhibit tumour growth. In view of these findings, we investigated in the present study if the combination of JV-1-38 with clinically more common methods for androgen deprivation, based on LH-RH agonists or the non-steroidal anti-androgen Flutamide, would also inhibit growth of MDA-PCa-2b prostate cancers xenografted into nude mice. This model of prostate cancer progression is androgen-sensitive, retains functional differentiation and shows common features of advanced clinical prostate cancer, such as PSA secretion and a doubly mutated androgen receptor [20,23]. These two mutations (L701H and T877A), which render these cells responsive to progesterone and corticosteroids, have been frequently detected in clinical specimens of androgen-refractory prostate cancers [23–25]. We used the MDA PCa 2b model of prostate cancer progression because, in contrast to the other widely used androgen-sensitive cell line LNCaP, it produces higher levels of serum PSA in nude mice [37]. Therefore, this model of prostate cancer seemed to be suitable to examine the effects of treatment with the compounds in question on tumour growth and PSA level. However, the results obtained must be extended in other androgen-dependent prostate cancer lines. Related studies should also be carried out in androgen independent models.

In the first experiment, we demonstrated that GH-RH antagonist JV-1-38 in combination with LH-RH agonist Decapeptyl strongly inhibits the growth of androgen-sensitive MDA PCa 2b prostate cancers. In contrast, treatment with JV-1-38 or Decapeptyl alone, had only non-significant inhibitory effects on tumour growth. The lack of significant inhibition with Decapeptyl can be explained by the low androgen-sensitivity of this prostate cancer cell line compared with androgen-dependent prostate cancer cell lines [25]. However, the dosage used was effective to significantly decrease serum testosterone levels and reduce the weight of the testicles, seminal vesicles and prostate. The synergistic action of LH-RH agonists and GH-RH antagonists could possibly be explained by effects described by two recent studies [26,27], which indicated that in a low-dose androgen environment, the AR-mediated transcription might be enhanced by peptide hormones, such as bombesin/gastrin-releasing peptide, LH-RH and their second messenger cyclic adenosine monophosphate (cAMP). The mitogenic action of GH-RH receptors is also mediated by cAMP, as well as by protein kinase C and mitogen-activated protein kinase [10,28–30]. Recent investigations suggest that tumoral SVs of pituitary GH-RH receptors might use signal-transduction pathways similar to those for LH-RH to mediate the effects of autocrine/paracrine GH-RH, but these mechanisms are incompletely understood [11,12,29–31]. Thus, inhibition of growth mediated by treatment with LH-RH agonists, combined with GH-RH antagonists, might block a synergistic activation of the AR by inhibiting tumoral autocrine/paracrine stimulatory signal-transduction pathways in a low-dose androgen environment.

In the second experiment, we investigated if JV-1-38 in combination with slow-release pellets of the non-

steroidal anti-androgen Flutamide could also inhibit the growth of MDA PCa 2b prostate cancers. Treatment with Flutamide alone inhibited tumour growth only non-significantly, even at a relatively high dose of 25 mg/kg BW per day. It remains unclear whether this lack of significant inhibition is related to the very low binding affinity of the AR for dihydrotestosterone in this cell line or to the mutated AR in the MDA PCa 2b cells, which could extend the ligand binding specificity of the AR, thus allowing these receptors to be activated by anti-androgens [25,32]. Certain growth factors, such as IGF-I, keratinocyte growth factor or epidermal growth factor (EGF) can activate the AR in the absence of its cognate ligand [33]. Our studies have shown that GH-RH antagonists exert their antitumour action in part by inhibiting the tumoral expression of IGF-I, -II and vascular endothelial growth factors (VEGF) [7–9]. Previously [8], we also found that the expression of tumoral IGF-II was decreased more by combined treatment with GH-RH antagonists and castration, than by the GH-RH antagonist alone. Ye and colleagues [34] reported an enhanced inhibition of MDA-PCa-2a androgen-sensitive prostate cancer cells by a combined treatment with hydroxyflutamide and the anti-EGF receptor monoclonal antibody C225. Thus, one possible mechanism for the synergistic antitumour action of JV-1-38 and Flutamide could be the combined inactivation of the AR or its downstream signalling mechanisms and the downregulation of autocrine/paracrine growth factors such as IGF-I, -II or EGF in the tumour tissue. Serum IGF-I levels were also measured in both experiments and with the relatively low dose of GHRH antagonist used, no significant decreases were detected in the groups that received JV-1-38.

Serial serum PSA measurements are the most reliable indicators for monitoring the response to therapy and for signalling the relapsed disease in prostate cancer [35,36]. The MDA PCa 2b prostate cancer cell line, is one of the few PSA-producing human prostate cancer lines available [23]. Previous work indicates that these MDA PCa 2b cells coordinately regulate both tumour growth and mRNA expression of PSA, in contrast to the androgen-sensitive cell line LNCaP [37]. Thus, in our study we tested whether treatment with JV-1-38 and androgen deprivation could affect mRNA expression for the androgen-responsive protein PSA, which might provide an insight into the functional activity of the AR. We also measured serum PSA levels after therapy and calculated the serum PSA index. Combination of JV-1-38 with Decapeptyl or Flutamide significantly decreased serum PSA levels compared with control values. This reduction in PSA levels correlated well with the decrease in tumour volume and weight. In previous studies [8,38], we and others also found a similar correlation in the tumour volume of MDA PCa 2b and LNCaP human androgen-sensitive prostate cancers and the PSA levels. However, RT-PCR analysis showed that the mRNA expression for *PSA* remained unchanged after treatment with Flutamide and Decapeptyl alone or in combination with GH-RH antagonists, in accord with the unchanged PSA secretion index. These findings indicate that the antiproliferative effects of androgen-deprivation therapy combined with GH-RH antagonists do not require an inactivation of the AR.

Collectively, our observations demonstrate that GH-RH antagonists can greatly potentiate the tumour inhibitory effects of the anti-androgen Flutamide and LH-RH agonist Decapeptyl on androgen-sensitive prostate cancers. GH-RH antagonists might interfere with the mechanisms involved in progression of prostate cancer towards androgen independence and could be used clinically as agents preventing or delaying the relapse in prostate cancer patients receiving androgen deprivation therapies [39]. The mechanisms involved in this synergistic inhibitory effect on tumour growth seem to only partly involve the AR as indicated by the unchanged mRNA expression of the androgen-responsive protein PSA. An inhibition of downstream cellular signalling mechanisms such as cAMP or the decreased expression of autocrine growth factors could be implicated in these effects, but further investigations are needed to elucidate the mechanisms of action of combined treatment. Since GH-RH antagonists, used as single drugs, also inhibit the growth of androgen-independent prostate cancers [2,6–9,39], they might be considered for the development of new approaches to the therapy of patients with prostatic carcinoma who had relapsed following conventional androgen deprivation treatment. In conclusion, the present work suggests that GH-RH antagonists could be used to enhance the effects of androgen deprivation in the management of androgen-dependent prostate carcinoma.

5. Conflict of interest

Tulane University has applied for a patent on the GH-RH antagonist JV-1-38 cited in this paper. Dr. Andrew V. Schally and Dr. Jozsef L. Varga are co-inventors on that patent, but the MS deals with experimental work on prostate cancer, which is an academic project.

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