Enhanced sensitivity of hormone-refractory prostate cancer cells to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated cytotoxicity by taxanes

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KEY WORDS

prostate ◗ prostate cancer ◗ TRAIL ◗ taxane

ABSTRACT

Introduction. Prostate cancer is one of the most commonly diagnosed male malignancies in many European countries and the USA. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is one of the most promising candidates for cancer therapy. TRAIL has been recently shown to induce apoptosis in a variety of transformed cells with no toxicity against normal tissues. Prostate cancer cells that are resistant to TRAIL induced apoptosis can be sensitized by chemotherapeutic drugs.
The aim of the study. In this study, we investigate the effect of TRAIL on prostate cancer cells with or without taxanes: paclitaxel or docetaxel.
Materials and methods. Three human prostate cancer cell lines: hormone-refractory cancer cells DU145, PC3 and hormone-sensitivity cancer cells LNCaP were incubated with TRAIL in the presence or absence of the chemotherapeutic agents: paclitaxel and docetaxel. Cytotoxicity was determined by MTT and LDH assays.
Results. Our study confirmed that all prostate cancer cell lines were resistant to TRAIL. Subsequently we examined the cytotoxic effect of TRAIL in combination with taxanes, such as paclitaxel or docetaxel on prostate cancer cells. We reported that co-treatment with taxanes significantly sensitizes prostate cancer cells to TRAIL induced cytotoxicity.
Conclusion. Paclitaxel or docetaxel markedly augmented TRAIL mediated cytotoxicity against prostate cancer *in vitro*. The obtained results suggest that combined treatment of TRAIL and taxanes may provide the basis for a new therapeutic approach to induce cytotoxicity in hormone-refractory prostate cancer cells.

INTRODUCTION

Prostate cancer is one of the most commonly diagnosed male malignancies in many European countries and the USA. In recent years a gradual increase in the incidence of prostate cancer has been observed. It is estimated that it constitutes 12% of freshly diagnosed cases of malignancies in the European Union and 29% in the USA [1, 2, 3].

The basic methods used in the treatment of patients with prostate cancer include radical prostatectomy, radiation therapy or hormonal therapy. The great oncological problem is to prevent the growth and progression of prostate cancer which develops in spite of androgenic blockade as the cancer cells become hormone resistant with time [1]. One of the methods to treat patients with hormone-independent prostate cancer is chemotherapy with taxanes: paclitaxel or its semi-synthetic derivative, docetaxel [4, 5, 6, 7]. Taxanes are antimitotic medications. Their mechanism of action includes mitosis suppression by binding to specific sites on tubulin of microtubules and blocking the disruption of this key cytoskeleton protein. The first substance from this group, paclitaxel, was isolated in 1971 from the cortex of the yew tree [8].

Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) is a type II membrane protein belonging to the TNF family (Tumour Necrosis Factor) [9]. TRAIL is able to induce apoptosis in neoplastic cells without toxic effects against normal tissues [10, 11]. It suggests the role of TRAIL as the effector molecule in the process of cancer cell elimination and its potential use as anticancer therapy [9, 10, 11].

Sensitivity of cancer cells to the cytotoxic effects of TRAIL is a result of different distribution of death receptors TRAIL-R1 and/or TRAIL-R2 which are the sites the ligand binds to on the surface of these cells, as well as the expression of intracellular proteins inhibiting the transduction of signals leading to apoptosis [12].

The preliminary results of phase I and II clinical trials on TRAIL in patients with advanced neoplastic diseases (including prostate cancer) indicate that the direction of these studies is proper [13].

Inducing TRAIL-mediated apoptosis in neoplastic cells is often conditioned by the cooperation of physical factors e.g. ionising radiation or chemical factors such as cytostatics, flavonoids [14, 15, 16]. *In vitro* and *in vivo* studies demonstrated that cytostatics may augment the cytotoxic effects of TRAIL on cancer cells, making them more sensitive to TRAIL-induced apoptosis [17].

THE AIM OF THE STUDY

The aim of the study was to assess the cytotoxic effects of TRAIL combined with the following taxanes: paclitaxel or docetaxel on the prostate cancer cells. Tests were performed on three human prostate cancer cell lines: hormone-refractory cancer cells DU145, PC3 and hormone-sensitivity cancer cells LNCaP.

MATERIALS AND METHODS

Prostate cancer cells

The tests were performed on three human prostate cancer cell lines purchased at DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany):

Table 1. Cytotoxic activity of taxanes at the concentration of 5-20 µg/ml against prostate cancer cells.

Taxanes [µg/ml]	Cytotoxicity [%]		
	LNCaP cells	DU145 cells	PC3 cells
Paclitaxel 5	21.12 ± 0.95	22.66 ± 1.11	19.68 ± 0.92
Paclitaxel 10	29.91 ± 1.43	32.27 ± 0.75	30.31 ± 0.81
Paclitaxel 20	54.60 ± 1.55	55.37 ± 0.79	51.26 ± 0.73
Docetaxel 5	19.39 ± 1.28	26.29 ± 0.99	18.58 ± 1.24
Docetaxel 10	28.78 ± 1.66	31.99 ± 0.83	28.38 ± 0.91
Docetaxel 20	48.59 ± 1.76	55.35 ± 0.93	40.52 ± 0.93

DU145 line cells, cat. no. ACC-261 – hormone-refractory cells derived from prostate cancer metastasis to the central nervous system in a 69-year-old male,

PC3 line cells, cat. no. ACC-465 – hormone-refractory cells isolated from prostate cancer metastasis into the bone marrow in a 62-year-old patient,

LNCaP line cells, cat. no. ACC-256 – hormone-sensitive cells derived from prostate cancer metastasis to a supraclavicular lymph node in a 55-year-old patient [18].

Cells were cultivated in plastic bottles of 70 ml and 500 ml (Nunc A/S Roskilde, Denmark). The culture medium for LNCaP and DU145 cells was RPMI 1640 (90%) with the addition of heat-inactivated bovine foetal serum (10%). The culture medium for PC3 cells was RPMI 1640 (45%) with the addition of Ham's medium (45%) and heat-inactivated bovine foetal serum (10%). Reagents for a prostate cancer cell culture were purchased at PAA The Cell Culture Company (Pasching, Austria). The cells were cultivated constantly at the temperature 37°C, atmosphere with 5% CO_2, in an incubator with 100% relative humidity, passages were performed three times a week.

Cells adhering to the container bottom were tripsinized and suspensions were prepared which were used during subsequent experiments. The number of prostate cancer cells tested in each experiment was 0.5 x 10^5 (DU145 and PC3 cells) or 1 x 10^5 (LNCaP cells) per 1 ml of the medium.

Cytostatics

Two taxane cytostatics were tested: paclitaxel purchased at Tarchomińskie Zakłady Farmaceutyczne Polfa S.A. (Warsaw, Poland) and docetaxel received free of charge from Aventis Pharma S.A. (Antony, France).

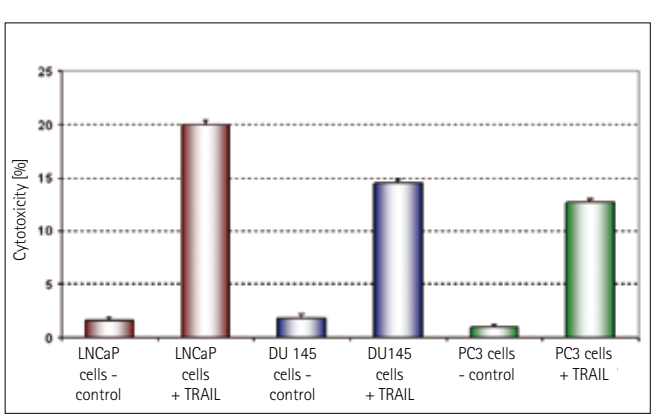

Fig. 1. Cytotoxic activity of TRAIL at the concentration of 100 ng/ml against prostate cancer cells.

TRAIL

Soluble, human recombinant TRAIL by PeproTech Inc. (Rocky Hill, USA) was used in tests.

Cytotoxicity assessment

Mitochondrial dehydrogenase activity (MTT test)

The cytotoxic effects of tested substances on prostate cancer cells were assessed with a MTT test (bromo-3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium) involving the measurement of mitochondrial dehydrogenase activity [19]. The reagents were purchased at Sigma Chemical Company (St. Louis, MO, USA).

Lactate dehydrogenase activity (LDH test)

The cytotoxic effects of tested substances on prostate cancer cells were assessed by measuring lactate dehydrogenase (LDH) activity in the test by Roche Molecular Biochemicals (Mannheim, Germany). Lactate dehydrogenase is released from the cytoplasm into the culture medium as a result of cell membrane damage and cell lysis. The LDH activity increase in cell culture supernatants correlates with the rate of damaged cells.

Statistical analysis of results

The tests were performed in the same experimental conditions. The measurements were performed in four independent experiments. A database using Excel 2000 calculation sheet was prepared basing on the obtained results.

The significance level was $p(\alpha) \leq 0.05$. The following standard tests were applied:

- the cytotoxicity assessment with regard to the different TRAIL levels – the Kruskal-Wallis one-factor analysis of variance (ANOVA),
- specific statistical analysis of the differences in cytotoxicity between two groups – the *U* Mann-Whitney test,
- analysis of simultaneous effects of two factors (cytotoxicity dependence on two factors: cytostatics and TRAIL) – a test based on a two-factor analysis of variance.

RESULTS

Cytotoxic effects of TRAIL on prostate cancer cells

The cytotoxic effects of TRAIL at the concentration of 100 ng/ml following a 48-hour incubation with relation to prostate cancer cells were: 14.5 ±1.1% of killed DU145 cells, 12.7 ±1.1% of killed PC3 cells and 20.0 ±1.3% of killed LNCaP cells. Fig. 1 presents the results of cytotoxic effects of TRAIL determined in the MTT test.

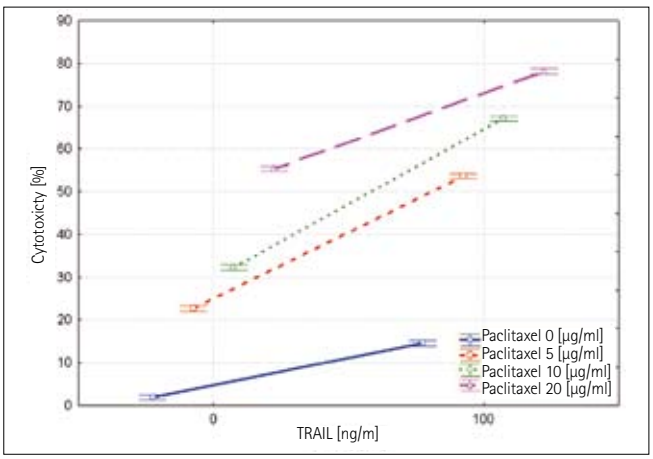

Fig. 2. Cytotoxic activity of TRAIL in combination with paclitaxel against prostate cancer cells DU145.

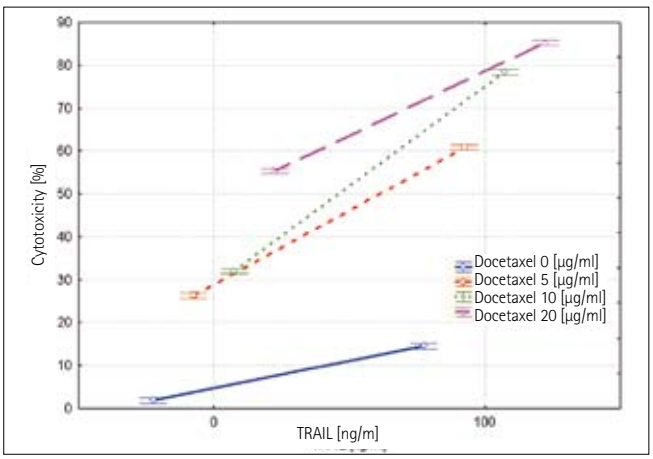

Fig. 3. Cytotoxic activity of TRAIL in combination with docetaxel against prostate cancer cells DU145.

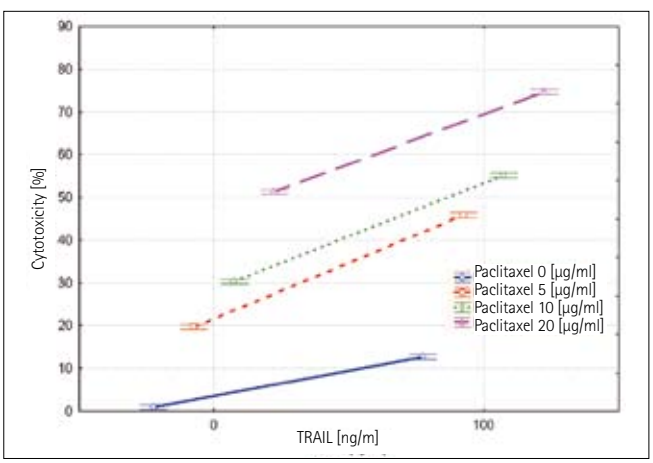

Fig. 4. Cytotoxic activity of TRAIL in combination with paclitaxel against prostate cancer cells PC3.

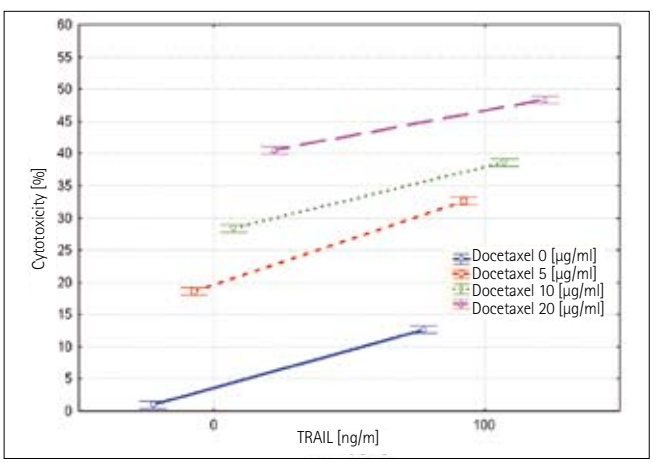

Fig. 5. Cytotoxic activity of TRAIL in combination with docetaxel against prostate cancer cells PC3.

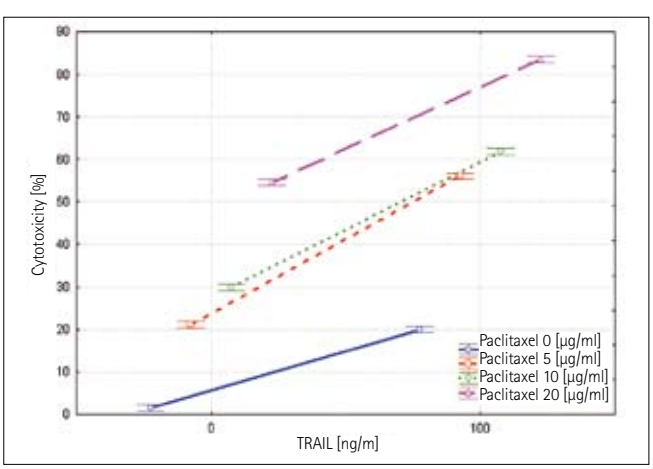

Fig. 6. Cytotoxic activity of TRAIL in combination with paclitaxel against prostate cancer cells LNCaP.

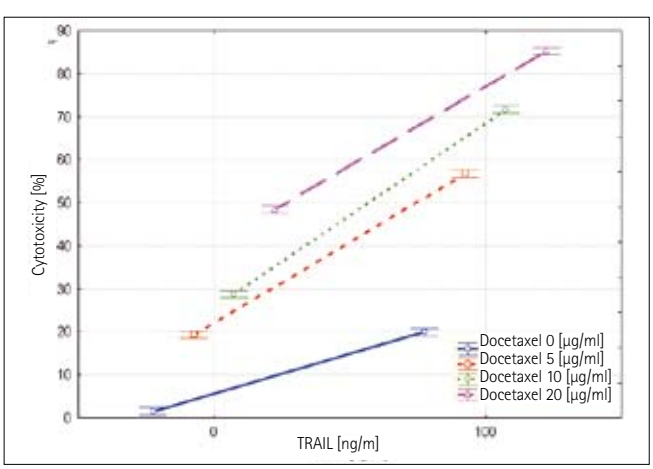

Fig. 7. Cytotoxic activity of TRAIL in combination with docetaxel against prostate cancer cells LNCaP

The ligand concentration higher than 100 ng/ml had no significant influence on its cytotoxicity increase with relation to prostate cancer cells which were tested. TRAIL at the concentrations of 100-200 ng/ml did not induce the lysis of prostate cancer cells determined in the LDH test.

During further stages TRAIL at the concentration of 100 ng/ml was used.

Cytotoxic effects of taxanes on prostate cancer cells

Two taxane cytostatics were tested: paclitaxel and docetaxel. The final concentrations of studied substances were 5 µg/ml, 10 µg/ml and 20 µg/ml. The incubation time for cells with a cytostatic was 48 hours. Tab. 1 presents the results of the paclitaxel and docetaxel cytotoxic effects on prostate cancer cells.

The cytotoxic effects of paclitaxel were as follows: for the concentration of 5 μg/ml from 19.7 ±0.9% to 22.7 ±1.1% of killed prostate cancer cells, for the concentration of 10 μg/ml from 29.9 ±1.4% to 32.3 ±0.8% of killed prostate cancer cells, for the concentration of 20 μg/ml from 51.3 ±0.7% to 55.4 ±0.8% of killed prostate cancer cells.

The cytotoxic effects of docetaxel were as follows: for the concentration of 5 μg/ml from 18.6 ±1.2% to 26.3 ±1.0% of killed prostate cancer cells, for the concentration of 10 μg/ml from 28.4 ±0.9% to 32.0 ±0.8% of killed prostate cancer cells, for the concentration of 20 μg/ml from 40.5 ±0.9% to 55.4 ±0.9% of killed prostate cancer cells.

The cytotoxic effects of studied taxanes on prostate cancer cells mainly depended on the concentration of the studied substance. The type of the studied cytostatic and the type of a studied prostate cancer cell line had an insignificant effect on the number of neoplastic cells which were killed. Only in the case of hormone-resistant PC3 cells a slightly low activity was observed when docetaxel was used. On the other hand, in the case of another hormone-resistant cell line, DU145 and hormone-sensitive prostate cancer cell line, LNCaP the cytotoxic effects of paclitaxel and docetaxel were similar. Taxanes at the studied concentrations did not induce the lysis of prostate cancer cells determined in the LDH test.

Cytotoxic effects of TRAIL combined with taxanes on prostate cancer cells

The studied prostate cancer cells were incubated with TRAIL and cytostatics for 48 hours. Both, paclitaxel and docetaxel, augmented the cytotoxic effects of TRAIL on prostate cancer cells. Figures 2-7 present the results of cytotoxicity for the studied combination of taxanes with TRAIL on cancer cells obtained in the MTT test.

The maximum cytotoxic effects were obtained when TRAIL was combined with taxanes at the highest concentration of 20 μg/ml. TRAIL induced death of 12.7 ±1.1% to 20.0 ±1.3% prostate cancer cells, whereas when combined with paclitaxel at the concentration of 20 μg/ml the number of killed cells increased to 78.1 ±0.9% for DU145 line (Fig. 2), 74.6 ±1.3% for PC3 line (Fig. 5) and 83.5 ±1.1% for LNCaP line (Fig. 6). Similar results were obtained for the combination of TRAIL with docetaxel at the concentration of 20 μg/ml. The cytotoxic effects of the above combination were respectively: 85.2 ±1.1% of killed DU145 line cells (Fig. 3) and 85.3 ±1.2% of killed LNCaP line cells (Fig. 7); only in the case of PC3 cells (Fig. 4) a significantly lower number of killed cells was observed (48.3 ±0.8%).

In the case of lower levels of 5 μg/ml and 10 μg/ml both cytostatics had similar potentializing effects on the TRAIL effects on prostate cancer cells. At these levels taxanes augmented TRAIL cytotoxicity by 40.1% for paclitaxel and 40.8% for docetaxel, on average, when compared to the activity of the ligand alone.

At all studied concentrations paclitaxel and docetaxel augmented the TRAIL effects on hormone-sensitive LNCaP line cells and hormone-refractory DU145 and PC3 line cells.

The studied taxanes did not induce the lysis of prostate cancer cells either alone or combined with TRAIL.

DISCUSSION

In recent years a gradual increase in the incidence of prostate cancer has been observed, as well as in the associated morbidity [1, 2, 3]. The main therapeutic target in patients with advanced diseases is to prevent the growth and progression of prostate cancer which develops in spite of hormonal therapy. Great hopes are being related to the introduction of taxanes into chemotherapy of patients with hormone-refractory prostate cancer [4, 5, 6]. The results of phase II and III clinical trials for docetaxel and phase II clinical trials for paclitaxel justify the use of these cytostatics [4, 21]. Taxanes are classified as antimitotic medications they inhibit mitosis in cancer cells by binding to specific sites on tubulin of microtubules and preventing it from disintegration [8].

When molecular aspects of the development of hormone-refractory prostate cancer are analysed it can be seen that disturbances of apoptosis regulation are one of the main reasons why cancer cells become androgen-independent, apart from mutations in the genes for androgen receptors [1]. The main mechanism of taxane action is the induction of apoptosis in cancer cells, apart from mitosis suppression [8]. Scientists from the Michigan University and the University of South Florida confirmed that paclitaxel and docetaxel induce apoptosis in the cells of hormone-sensitive prostate cancer LNCaP and hormone-refractory prostate cancer PC3 [22, 23]. Based on the results of our experiments we confirmed that both studied cytostatics were characterised by a similar high anticancer activity on the cells of all three lines of prostate cancer, including hormone-sensitive LNCaP and hormone-refractory DU145 and PC3.

Intensive phase I and II preclinical and clinical trials on Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) indicate a new, alternative direction in the treatment of patients with hormone-refractory prostate cancer. TRAIL is able to induce apoptosis in cancer cells without toxic effects on normal body cells [10, 11, 13]. Treatment with TRAIL combined with cytostatics would make it possible to increase the effectiveness of anticancer treatment and to reduce doses of administered medications and to reduce systemic toxicity, maintaining at the same time the same anticancer activity.

Based on the results of performed tests it can be stated that both studied taxanes augmented the cytotoxic effects of TRAIL on prostate cancer cells. All three prostate cancer cell lines used in the study demonstrated resistance to TRAIL and the number of killed cells did not exceed 20%. Paclitaxel and docetaxel at every concentration supported the cytotoxic effects of TRAIL in hormone-sensitive and hormone-refractory prostate cancer cells. These observations are consistent with the results obtained by other authors. Asakuma et al. confirmed that paclitaxel augments TRAIL-induced apoptosis in renal cancer cells by decreasing the expression of the antiapoptotic protein – Akt [24]. Whereas Lee et al. observed that paclitaxel decreases the levels of antiapoptotic proteins, bcl-2 in cervical cancer cells and intensifies the action of TRAIL ligand [25]. Shankar et al. in their *in vitro* and *in vivo* studies proved that paclitaxel and other antimitotic medications such as vincristine and vinblastine increase the number of cells undergoing TRAIL-induced apoptosis in prostate cancer cell line PC3 [26]. Nimmanapalli et al. demonstrated that paclitaxel increases the expression of death receptors TRAIL-R1 and TRAIL-R2 on the surface of prostate cancer cell lines DU145, PC3 and LNCaP what has a significant effect on the increased cytotoxic effects of TRAIL [23]. Scientists from the Anhui Medical University observed synergistic effects of TRAIL combined with paclitaxel on lung cancer cell line A549 [27]. Yoo et al. proved that docetaxel intensifies TRAIL-induced apoptosis in the cells of hormone-refractory prostate cancer line C4-2B [28].

The fact that microtubules are the strategic target for chemotherapeutics of the highest activity towards hormone-refractory prostate cancer suggests that this mechanism of action is of clinical significance. According to Kim et al. TRAIL suppresses mitosis in cells and

intensifies the action of antimitotic medications with their uptake sites on microtubules [29]. For that reason using TRAIL combined with paclitaxel or docetaxel on hormone-refractory prostate cancer cells makes it possible to increase significantly the cytotoxic effects when compared to the action of each of these substances alone.

The obtained preliminary results prove that paclitaxel and docetaxel significantly supported the cytotoxic effects of TRAIL on studied prostate cancer cells. It indicates that using a combination therapy with TRAIL and taxanes in the future may be a potential method to treat patients with hormone-refractory prostate cancer [30].

Taxanes are associated with a great number of adverse effects such as suppression of bone marrow haematopoietic activity, induction of hypersensitivity reactions, neurotoxicity, cardiotoxicity or hepatotoxicity [31]. We have observed that combining taxanes with TRAIL induced positive cytotoxic effects also when lower levels of these substances were used. Such measures may decrease *in vivo* the toxic effects of paclitaxel or docetaxel. However, the question whether observed cytotoxic effects of a combination therapy with taxanes and TRAIL is associated with additive or synergistic actions of separate factors remains to be answered. Taking into account the extent of performed experiments it is necessary to conduct further research regarding a molecular aspect of observed effects in order to explain the mechanism of taxane effects on separate pathways transducing TRAIL-induced apoptosis signals in cancer cells and to determine the reason why prostate cancer cells become hormone-refractory.

CONCLUSIONS

Studied prostate cancer cells: LNCaP, DU145 and PC3 were resistant to the cytotoxic effects of TRAIL.

The activity of taxanes: paclitaxel and docetaxel towards the studied prostate cancer cells was similar and directly proportional to the concentration of the studied substance. The studied taxanes exerted their cytotoxic effects towards hormone-sensitive (LNCaP) and hormone-resistant (DU145, PC3) prostate cancer cells.

Augmentation of the cytotoxic effects of TRAIL on prostate cancer cells following taxane (paclitaxel or docetaxel) use has been observed for hormone-resistant (DU145, PC3) and hormone-sensitive (LNCaP) prostate cancer cells. Both cytostatics exerted similar potentializing effects on the TRAIL activity on prostate cancer cells. Taxanes augmented TRAIL cytotoxicity by 46-48% on average when compared to the action of the ligand alone.

Administration of taxanes combined with TRAIL resulted in a desired cytotoxic effect also for lower levels of these substances. Such measures may make it possible to decrease the toxic effects of these substances in the *in vivo* setting.

Paclitaxel and docetaxel intensified the cytotoxic effects of TRAIL on the studied prostate cancer cells.

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