Pharmacological Reports
2011, 63, 139–148
ISSN 1734-1140

Enhanced TRAIL-mediated apoptosis in prostate cancer cells by the bioactive compounds neobavaisoflavone and psoralidin isolated from *Psoralea corylifolia*

Ewelina Szliszka[1], Zenon P. Czuba[1], Łukasz Sędek[2], Andrzej Paradysz[3], Wojciech Król[1]

[1]Chair and Department of Microbiology and Immunology, [2]Chair and Department of Pediatric Hematology and Oncology, [3]Chair and Department of Urology, Medical University of Silesia in Katowice, Poniatowskiego 15, PL 40-006 Katowice, Poland

Correspondence: Wojciech Król, e-mail: wkrol@sum.edu.pl

Abstract:
Numerous compounds detected in medical plants and dietary components or supplements possess chemopreventive, antitumor and immunomodulatory properties. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is an important endogenous anticancer factor that induces apoptosis selectively in cancer cells. However, some tumor cells are resistant to TRAIL-mediated apoptosis. Naturally occurring agents could sensitize TRAIL-resistant cancer cells and augment their apoptotic activity. We examined the cytotoxic and apoptotic effects of neobavaisoflavone and psoralidin in combination with TRAIL on LNCaP prostate cancer cells. The cytotoxicity was evaluated by MTT and LDH assays. The apoptosis was detected using Annexin V-FITC by flow cytometry and fluorescence microscopy. The LNCaP cells were shown to be resistant to TRAIL-induced apoptosis. Our study demonstrated that neobavaisoflavone and psoralidin sensitized TRAIL-resistant cells and markedly augmented TRAIL-mediated apoptosis and cytotoxicity in prostate cancer cells. Cotreatment of LNCaP cells with 100 ng/ml TRAIL and 50 μM neobavaisoflavone or 50 μM psoralidin increased the percentage of the apoptotic cells to 77.5 ± 0.5% or 64.4 ± 0.5%, respectively. The data indicate the potential role of the bioactive compounds isolated from the medicinal plant *Psoralea corylifolia* (neobavaisoflavone and psoralidin) in prostate cancer chemoprevention through enhancement of TRAIL-mediated apoptosis.

Key words:
neobavaisoflavone, psoralidin, TRAIL, apoptosis, prostate cancer

Abbreviations: Akt/PKB – protein kinase B, DMSO – dimethyl sulfoxide, DR – death receptor, EGCG – epigallocatechin 3-gallate, IAP – inhibitor of apoptosis proteins, LDH – lactate dehydrogenase, LNCaP – prostate cancer cell line, MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, NFκB – nuclear factor κB, PBS – phosphate buffered saline solution, PI – propidium iodide, PS – phosphatidylserine, TRAIL – tumor necrosis factor-related apoptosis-inducing ligand

Introduction

Psoralea corylifolia (Leguminosae) is one of the most popular and multi-purpose medicinal plants and is widely distributed in Southeastern Asian countries. *Psoralea corylifolia* has been used in complementary

and alternative medicine for the treatment of pollakiuria, nephritis, osteoporosis, hypertension and cardiovascular diseases [18, 38]. The mature or dry fruits of this plant are a well known health supplement ingredient [37]. *In vitro* and *in vivo* studies have demonstrated that the active fraction from the seeds and roots of *Psoralea corylifolia* exhibits antimicrobial, antioxidative, immunomodulatory and anticancer properties [18, 36, 38]. *Psoralea corylifolia* extract contains a number of bioactive compounds that are the molecular basis of its action, including flavonoids (neobavaisoflavone, isobavachalcone, bavachalcone, bavachinin, bavachin, corylin, corylifol, corylifolin and 6-prenylnaringenin), coumarins (psoralidin, psoralen, isopsoralen and angelicin) and meroterpenes (bakuchiol and 3-hydroxybakuchiol) [38].

The use of naturally occurring dietary agents is becoming increasingly appreciated as an effective strategy of prostate cancer chemoprevention [15, 27]. Cancer chemoprevention is a means of cancer control in which malignancy is prevented or reversed by nutritional or pharmacological intervention with natural or synthetic substances [25].

Prostate cancer is one of the most commonly diagnosed cancers in man and the second leading cause of cancer-related deaths in the European Union and the United States [6, 11]. Prostate cancer represents an ideal disease for chemopreventive intervention due to its long latency, late age of onset, relatively slower rate of growth and progression, high incidence, tumor marker availability, identifiable preneoplastic lesions and risk group [3, 35].

Tumor necrosis factor related apoptosis inducing ligand (TRAIL), a member of the TNF superfamily, selectively induces apoptosis in cancer cells with no toxicity against normal tissues [2, 5]. TRAIL molecules are expressed on the cell surface of T lymphocytes, natural killer cells, dendritic cells, neutrophils, monocytes or macrophages and can be cleaved into a soluble, secreted form. TRAIL plays an important role in immune surveillance and defence mechanisms against tumor cells [2, 34]. TRAIL is a promising antitumor agent capable of killing cancer cells *via* receptor-mediated programmed death through its interaction with the death receptor TRAIL-R1 (DR4) and/or TRAIL-R2 (DR5) [5, 34]. However, some tumor cells are resistant to TRAIL-mediated cytotoxicity. The decreased expression of death receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5) or other proapoptotic proteins as well as the increased expression of antiapoptotic proteins in cancer cells are involved in TRAIL-resistance [34]. We and others have shown that TRAIL-resistant prostate cancer cells can be sensitized by chemotherapeutic agents, ionizing radiation or dietary phytochemicals [1, 5, 7–10, 12, 16, 17, 20–24, 28, 29, 33].

In this work we investigated the apoptotic and/or cytotoxic effects of two bioactive compounds isolated from *Psoralea corylifolia* in combination with TRAIL on LNCaP prostate cancer cells. We showed for the first time that neobavaisoflavone and psoralidin markedly augment TRAIL-mediated apoptosis in prostate cancer cells and sensitize LNCaP cells to TRAIL-induced apoptosis. Figure 1 presents the structures of neobavaisoflavone and psoralidin. In our opinion the enhancement of TRAIL-mediated apoptosis in prostate cancer cells by the tested compounds indicate the emergency and suitableness of further studies on their chemopreventive activities.

Materials and Methods

Cell line and cell culture

The human hormone-sensitive prostate cancer LNCaP cell line was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). LNCaP cells were maintained in RPMI

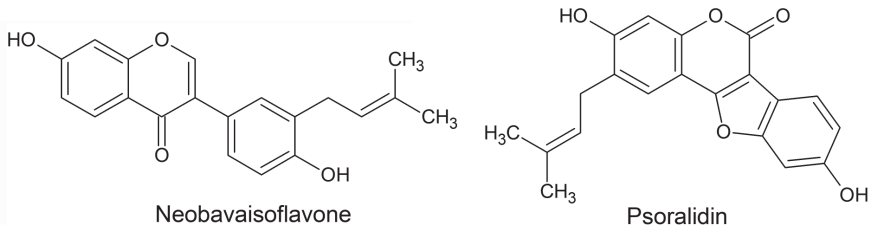


Fig. 1. Chemical structures of the studied bioactive compounds identified in *Psoralea corylifolia*

1640 medium with 10% fetal bovine serum, 4 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin and were grown in monolayer cultures at 37°C and atmosphere containing 5% CO_2 [28, 29, 32]. Reagents for cells culture were purchased from PAA, The Cell Culture Company (Pasching, Austria).

Chemicals and reagents

Neobavaisoflavone and psoralidin were obtained from Alexis Biochemicals (San Diego, CA, USA). The tested compounds were dissolved in dimethyl sulfoxide (DMSO) (neobavaisoflavone and psoralidin – 50 mM) to obtain the working concentrations. The final concentration of DMSO in the culture medium was controlled at 0.1% (*v/v*). Soluble recombinant human TRAIL (rhsTRAIL) was purchased from PeproTech Inc. (Rocky Hill, NJ, USA).

Cytotoxicity assay

The cytotoxicity was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay as described [14, 19, 28]. The MTT assay is based on the cleavage of the tetrazolium salt MTT to form blue formazan dye by viable cells. The LNCaP cells (2×10^5/ml) were seeded 48 h before the experiments in a 96-well plate. Neobavaisoflavone or psoralidin (20–50 μM) with or without TRAIL (50–200 ng/ml) were added to the cells. After 48 h the medium was removed, and 20 μl MTT solutions (5 mg/ml) (Sigma Chemical Co., St. Louis MO, USA) were added to each well for 4 h. The resulting blue formazan crystals were dissolved in DMSO. Controls included native cells and medium alone. The spectrophotometric absorbance at 550 nm was measured using a microplate reader (ELx 800, Bio-Tek Instruments Inc., Winooski, VT, USA). The percent cytotoxicity was calculated by the formula: percent cytotoxicity (cell death) = (1 – [absorbance of experimental wells/absorbance of control wells]) × 100%.

Lactate dehydrogenase release assay

Lactate dehydrogenase (LDH) is a stable cytosolic enzyme that is released upon membrane damage in necrotic cells. LDH activity was measured using a commercial cytotoxicity assay kit (Roche Diagnostics GmbH, Mannheim, Germany), in which LDH released in culture supernatants is measured with a coupled enzymatic assay, resulting in conversion of a tetrazolium salt into a red formazan product. The LNCaP cells were treated with various concentrations of neobavaisoflavone and psoralidin (20–50 μM) alone and in combination with TRAIL (50–200 ng/ml) for the indicated period of time. The sample solution (supernatant) was removed, and the LDH released from the cells into culture medium was measured. The maximal release was obtained after treating control cells with 1% Triton X-100 (Sigma Chemical Co., St. Louis, MO) for 10 min at room temperature [28– 32]. The necrotic percentage was expressed using the formula: (sample value/maximal release) × 100%.

Detection of apoptosis by flow cytometry

Apoptosis was measured using flow cytometry to quantify the levels of phosphatidylserine (PS) on the outer membrane of apoptotic cells. Externalized PS on the outer surface of the cytoplasmic membrane becomes labelled by Annexin V-FITC, which has a high affinity for PS-containing phospholipids bilayers. The Annexin V assay was performed using the Apoptotest-FITC Kit (Dako, Glostrup, Denmark). Briefly, prostate cancer cell line LNCaP (2×10^5/ml) cells were seeded in 24-well plates for 48 h and then exposed to neobavaisoflavone and psoralidin (20–50 μM) and/or TRAIL (50–200 ng/ml) for 48 h. After this time cancer cells were washed twice with PBS (phosphate buffered saline solution) and resuspended in 1 ml of binding buffer. The cell suspension (500 μl) was then incubated with 5 μl of Annexin V-FITC and 10 μl of propidium iodide (PI) for 10 min at room temperature in the dark. The population of Annexin V-positive cells was evaluated by flow cytometry (BD FACScan, Becton Dickinson Immnunocytometry Systems, San Jose, CA, USA) [29, 32].

Detection of apoptosis by fluorescence microscopy

Apoptotic cells were quantified by fluorescence microscopy method using Apoptotic & Necrotic & Healthy Cells Quantification Kit from Biotium Inc. (Hayward, CA, USA) according to the manufacturer's instructions [31]. The LNCaP cells (2.5×10^5/ml) were seeded 24 h before the experiments in a 24-well plate. Neobavaisoflavone or psoralidin (50 μM) with or without TRAIL (50–100 ng/ml) were added to the cancer cells, and 48 h later, the cells were washed with PBS

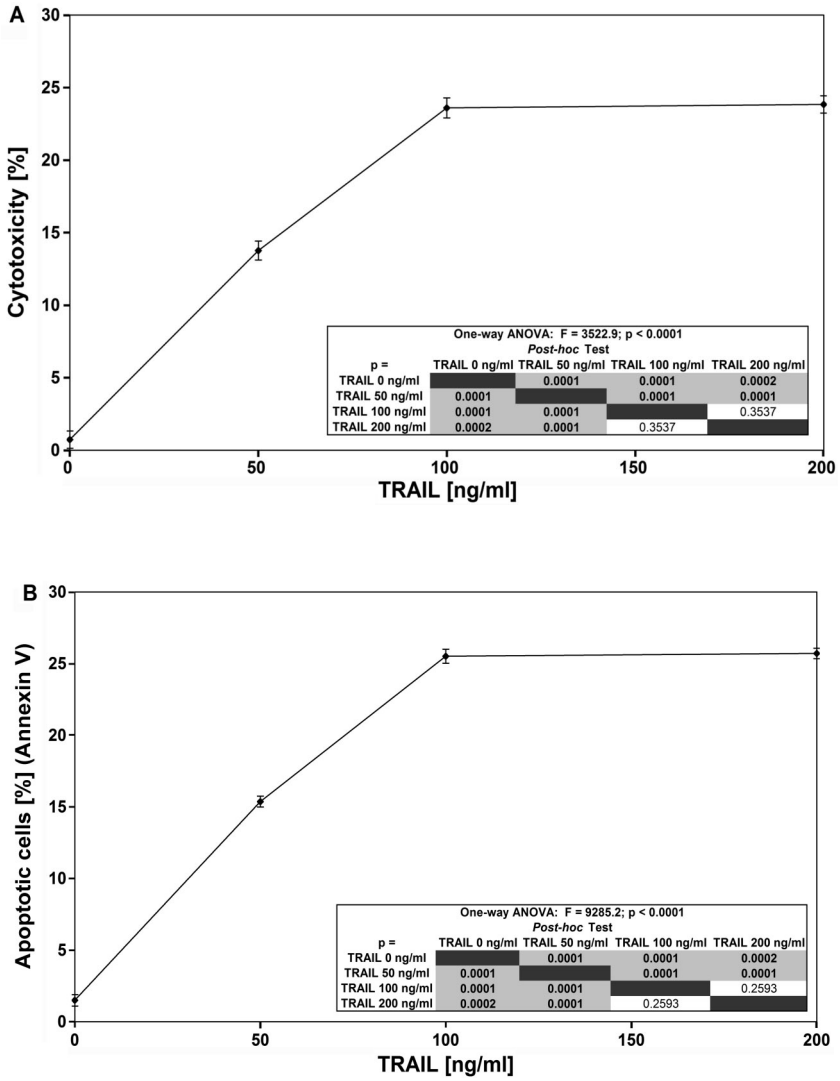


Fig. 2. Cytotoxic and apoptotic effects of TRAIL on LNCaP prostate cancer cells. The cancer cells were incubated for 48 h with TRAIL at concentrations of 50–200 ng/ml. The values represent the mean ± SD of three independent experiments performed in quadruplicate (n = 12) for cytotoxicity, or in duplicate (n = 6) for apoptosis ($p < 0.05$). (**A**) Cytotoxic activity of TRAIL in LNCaP cells. The percentage of cell death was measured by MTT cytotoxicity assay. (**B**) TRAIL-induced apoptosis in LNCaP cells. Apoptotic cell death was detected by Annexin V-FITC staining using flow cytometry

and detached from the cell culture wells by trypsin. Next, the LNCaP cells were centrifuged to discard the supernatant, washed with PBS and resuspended in binding buffer (100 µl/sample). A combination of 5 µl of FITC-Annexin V, 5 µl of ethidium homodimer III and 5 µl of Hoechst 33342 solutions was added to each tube. The samples were incubated at room temperature for 15 min in the dark. After staining, the cancer cells were washed with binding buffer, placed on a glass slide and covered with a glass coverslip. The stained cells were observed under a fluorescence inverted microscope IX51 (Olympus, Tokyo, Japan) using filter sets for FITC, TRITC and DAPI. The healthy cells (stained with Hoechst 33342) emitted blue fluorescence, apoptotic cells (stained with FITC-Annexin V and Hoechst 33342) emitted green and blue fluorescence and necrotic cells (stained with ethidium homodimer III and Hoechst 33342) emitted red and blue fluorescence. Cancer cells stained with blue, red and green, were dead cells progressing from the apoptotic cell population. The cells were counted and the apoptotic cells were expressed as percentage of total cells.

Statistical analysis

The results are expressed as the means ± SD obtained from three independent experiments performed in quadruplicate (n = 12) for cytotoxicity or duplicate (n = 6) for apoptosis. Statistical significance was evaluated using Levene's test and analysis of variance (ANOVA); p-values < 0.05 were considered significant.

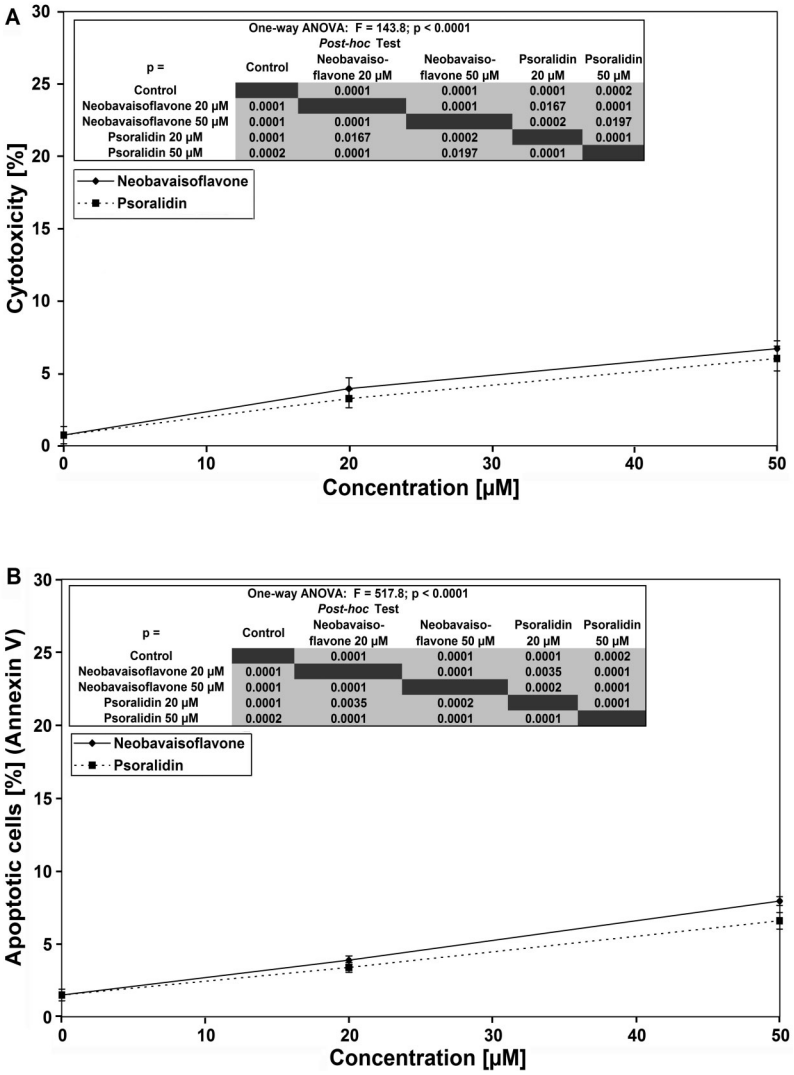


Fig. 3. Cytotoxic and apoptotic effects of bioactive compounds isolated from *Psoralea corylifolia* on LNCaP prostate cancer cells. The cancer cells were incubated for 48 h with neobavaisoflavone and psoralidin at concentrations of 20–50 µM. The values represent the mean ± SD of three independent experiments performed in quadruplicate (n = 12) for cytototoxicity, or in duplicate (n = 6) for apoptosis ($p < 0.05$). (**A**) Cytotoxic activity of bioactive compounds from *Psoralea corylifolia* in LNCaP cells. The percentage of cell death was measured by MTT cytotoxicity assay. (**B**) Bioactive compounds from *Psoralea corylifolia* induced apoptosis in LNCaP cells. Apoptotic cell death was detected by Annexin V-FITC staining using flow cytometry

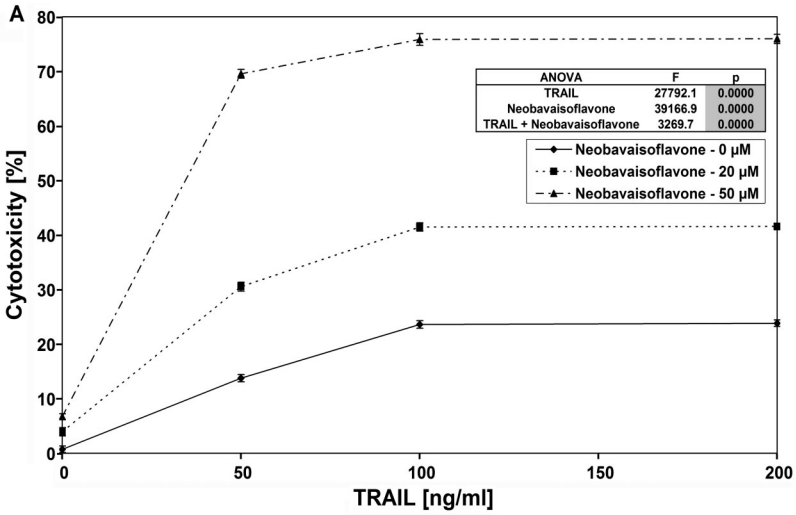
Results

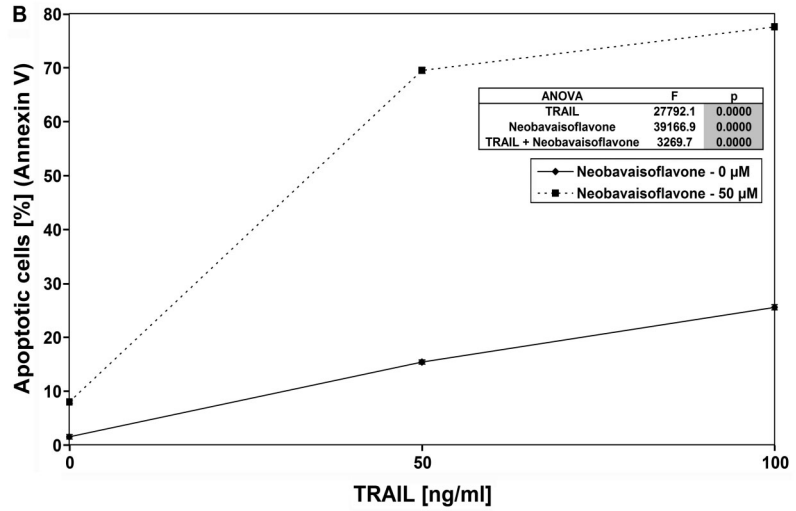
Cytotoxic and apoptotic effect of TRAIL on prostate cancer cells

TRAIL induced cytotoxic and apoptotic effects in LNCaP prostate cancer cells in a dose-dependent manner. The cytotoxicity of TRAIL at the concentrations of 50–200 ng/ml after a 48-h incubation was 13.76 ± 0.66%–23.84 ± 0.60% cell death (Fig. 2A). The necrotic cell death percentage of LNCaP cells examined by lactate dehydrogenase assay and propidium iodide staging was near 0%. Figure 2B presents TRAIL-induced apoptosis in LNCaP cells determined with annexin V staining followed by flow cytometry. The 48 h exposure to TRAIL increased the percentage of apoptotic cells to 15.37 ± 0.38%–25.71 ± 0.36%. The results showed that TRAIL was less active against the tested prostate cancer cell line and confirmed resistance of LNCaP cells to TRAIL-mediated apoptosis and cytotoxicity.

Cytotoxic and apoptotic effect of the bioactive compounds, neobavaisoflavone and psoralidin, isolated from *Psoralea corylifolia*, on prostate cancer cells

The prostate cancer cells were incubated for 48 h with neobavaisoflavone and psoralidin at concentrations of 20–50 µM. We demonstrated that treatment of LNCaP cells with neobavaisoflavone or psoralidin inhibits cell proliferation by inducing cytotoxicity and apoptosis in a dose-dependent manner. The cytotoxic effect





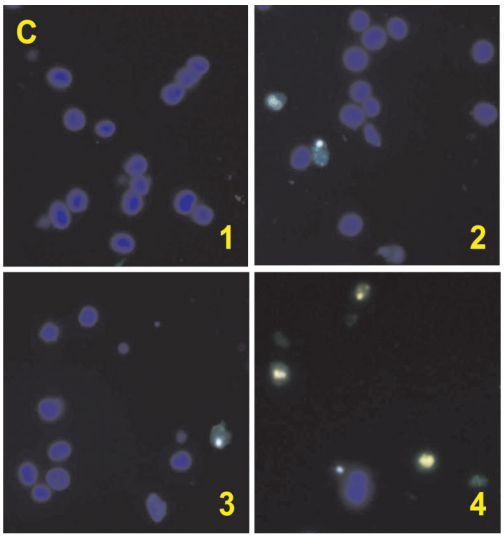
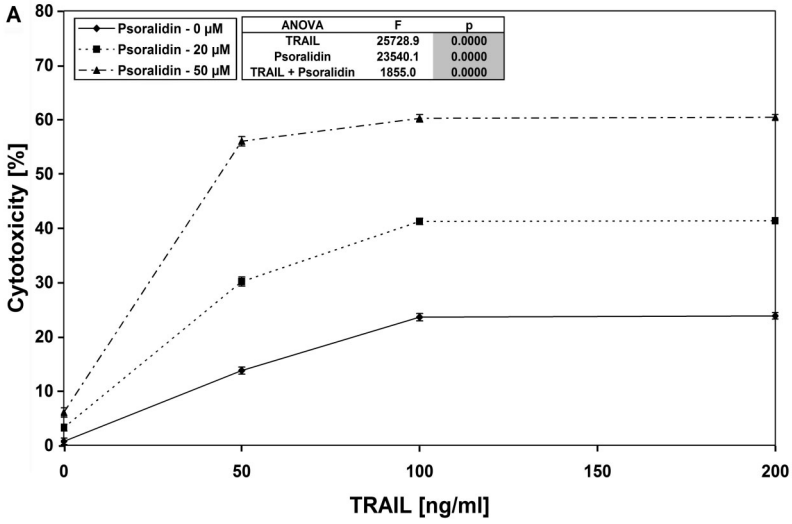
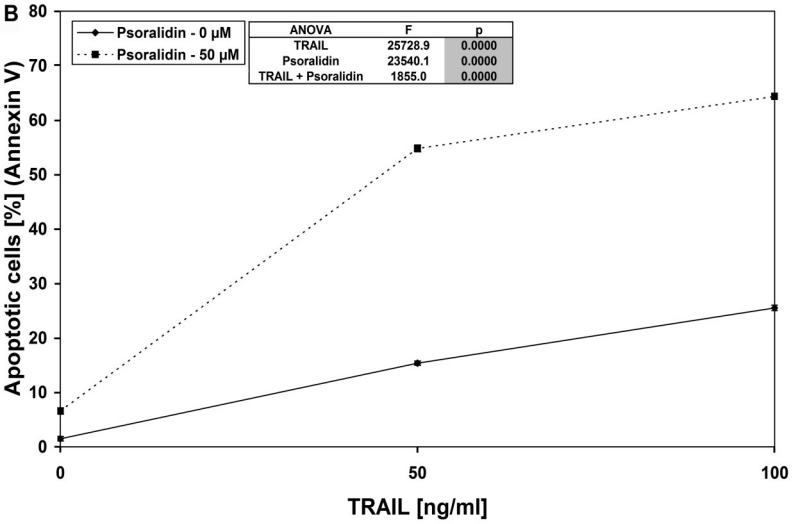


Fig. 4. Cytotoxic and apoptotic effects of TRAIL in combination with neobavaisoflavone on LNCaP prostate cancer cells. The cancer cells were incubated for 48 h with TRAIL at concentrations of 50 ng/ml–200 ng/ml and neobavaisoflavone at concentrations of 20 µM and 50 µM. The values represent the mean ± SD of three independent experiments performed in quadruplicate (n = 12) for cytotoxicity or in duplicate (n = 6) for apoptosis ($p < 0.05$). (**A**) Cytotoxic activity of TRAIL in combination with neobavaisoflavone in LNCaP cells. The percentage of cell death was measured by MTT cytotoxicity assay. (**B**) TRAIL-induced apoptosis in combination with neobavaisoflavone in LNCaP prostate cancer cells. Apoptotic cell death was detected by Annexin V-FITC staining using flow cytometry. (**C**) TRAIL-induced apoptosis in combination with neobavaisoflavone in LNCaP prostate cancer cells: (**1**) control cells, (**2**) cells incubated with TRAIL (100 ng/ml), (**3**) cells incubated with neobavaisoflavone (50 µM), and (**4**) cells incubated with TRAIL (100 ng/ml) and neobavaisoflavone (50 µM). Apoptotic cell death was detected by Annexin V-FITC staining using fluorescence microscopy. The healthy cells (stained with Hoechst 33342) emitted blue fluorescence and apoptotic cells (stained with FITC-Annexin V and Hoechst 33342) emitted green and blue fluorescence





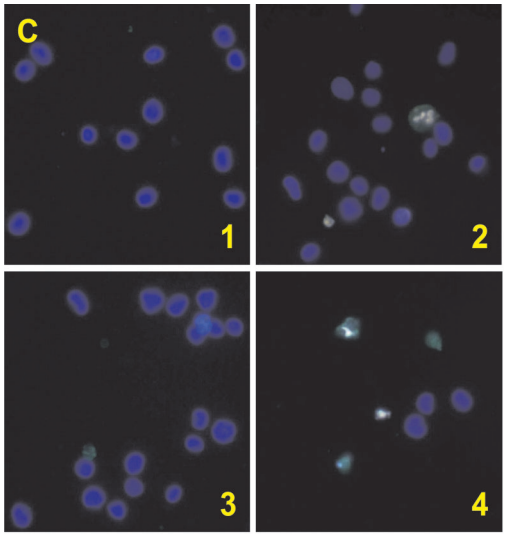


Fig. 5. Cytotoxic and apoptotic effects of TRAIL in combination with psoralidin on LNCaP prostate cancer cells. The cancer cells were incubated for 48 h with TRAIL at concentrations of 50 ng/ml–200 ng/ml and psoralidin at concentrations of 20 μM and 50 μM. The values represent the mean ± SD of three independent experiments performed in quadruplicate (n = 12) for cytotoxicity or in duplicate (n = 6) for apoptosis ($p < 0.05$). (**A**) Cytotoxic activity of TRAIL in combination with psoralidin in LNCaP cells. The percentage of cell death was measured by MTT cytotoxicity assay. (**B**) TRAIL-induced apoptosis in combination with psoralidin in LNCaP prostate cancer cells. Apoptotic cell death was detected by Annexin V-FITC staining using flow cytometry. (**C**) TRAIL-induced apoptosis in combination with psoralidin in LNCaP prostate cancer cells: (**1**) control cells, (**2**) cells incubated with TRAIL (100 ng/ml), (**3**) cells incubated with psoralidin (50 μM), and (**4**) cells incubated with TRAIL (100 ng/ml) and psoralidin (50 μM). Apoptotic cell death was detected by Annexin V-FITC staining using fluorescence microscopy. The healthy cells (stained with Hoechst 33342) emitted blue fluorescence and apoptotic cells (stained with FITC-Annexin V and Hoechst 33342) emitted green and blue fluorescence

of the tested agents on LNCaP cells was 3.94 ± 0.76% –6.70 ± 0.54% cell death for neobavaisoflavone and 3.25 ± 0.63% – 6.03 ± 0.86% for psoralidin (Fig. 3A). The necrotic cell death percentage of LNCaP cells examined by lactate dehydrogenase assay and propidium iodide staging was near 0%. The Annexin V assay revealed apoptotic cells exposed to neobavaisoflavone and psoralidin (Fig. 3B). These compounds induced the following apoptotic effects, neobavaisoflavone 3.88 ± 0.29%–7.93 ± 0.30% and psoralidin 3.38 ± 0.33%–6.58 ± 0.57%, respectively. The bioactive components exhibited low cytotoxic and apoptotic activity against LNCaP cells.

Cytotoxic and apoptotic effect of TRAIL in combination with neobavaisoflavone or psoralidin on prostate cancer cells

We investigated the cytotoxic and apoptotic effects of TRAIL in combination with neobavaisoflavone or psoralidin on prostate cancer cells. The co-treatment of LNCaP cells with TRAIL at concentrations of 50–100 ng/ml combined with the tested compounds at concentrations of 20–50 μM increased the percentage of cell death to 30.53 ± 0.82% – 75.84 ± 1.07% for neobavaisoflavone and to 30.16 ± 0.85% – 60.22 ± 0.69% for psoralidin. The cytotoxicity measured by MTT assay is shown in Figures 4A and 5A. TRAIL and both components of *Psoralea corylifolia* induced a cytotoxic effect in cancer cells by inducing apoptosis. The necrotic cell death percentage of LNCaP cells examined by Apoptest-FITC and the lactate dehydrogenase assay was near 0%. We found that neobavaisoflavone and psoralidin strongly cooperated with TRAIL to induce apoptosis in prostate cancer cells. The percentage of the apoptotic cells after 48 h exposure to 100 ng/ml TRAIL with 50 μM neobavaisoflavone were elevated at 77.55 ± 0.51% and with 50 μM psoralidin at 64.36 ± 0.52% (Figs. 4B and 5B).

Discussion

Dysregulated apoptotic pathways have an important role in the initiation and progression of prostate cancer [13]. The accumulated data from *in vitro* and *in vivo* studies suggest that TRAIL plays an important role in the maintenance of immune homeostasis, host tumor surveillance and defence against cancer cells [2, 34]. We investigated the cytotoxic and apoptotic effects of TRAIL in combination with the bioactive compounds: neobavaisoflavone and psoralidin, on prostate cancer cells. The recombinant human TRAIL used in this study is a soluble protein based on a natural ligand. TRAIL induces programmed death in various cancer cells *in vitro* and *in vivo* [2]. However, some tumor cells are resistant to TRAIL-mediated cytotoxicity [34]. We and others have demonstrated that the LNCaP cell line was resistant to TRAIL-induced apoptosis. TRAIL-resistant prostate cancer cells can be sensitized by bioactive phytochemicals [7–10, 12, 16, 17, 20–24, 29, 32, 33]. Our results indicate that neobavaisoflavone and psoralidin markedly augment TRAIL-mediated apoptosis in LNCaP prostate cancer cells. Further investigations will be required to explain the cellular signalling pathways by which neobavaisoflavone and psoralidin modulate the TRAIL-induced death. Similar studies have described the synergistic apoptotic effect of TRAIL with naturally occurring bioactive compounds on prostate cancer cells. Flavonoids found in fruits and vegetables can sensitize TRAIL-resistant prostate cancer cells. For example, quercetin enhanced TRAIL-mediated cytotoxicity by activating caspases, inhibiting phosphorylation of serine/threonine kinase Akt (protein kinase B) and decreasing expression of antiapoptotic survivin [16, 17]. Other dietary flavonoids, apigenin and baicalein, increased expression of the death receptor TRAIL-R2 on prostate cancer cell surfaces and augmented TRAIL-mediated apoptosis [12, 33]. The green tea flavonoid EGCG (epigallocatechin 3-gallate) synergized with TRAIL to promote cell death by increasing expression of the death receptor TRAIL-R1 and modulating pro- and antiapoptotic Bcl-2 family proteins in prostate cancer cells [24]. Resveratrol, a chemical in red wine, overcame TRAIL-resistance in prostate cancer cell through upregulation of death receptors TRAIL-R1 and TRAIL-R2, modulation of Bcl-2 or IAP family proteins and activation of effector caspases [21, 23]. The active component of the spice turmeric, derived from the rhizome of *Curcuma longa* named curcumin, increased expression of death receptors TRAIL-R1 and TRAIL-R2, induced activation of caspases and release of cytochrome c from mitochondria, modulated the expression of Bcl-2 or IAP family proteins, suppressed phosphorylation of Akt and inhibited activation of NFκB sensitizing prostate cancer cells to TRAIL-mediated apoptosis [7–10, 23].

The role of natural dietary products in prevention of prostate cancer has been confirmed in numerous preclinical and epidemiological studies [15, 26]. Although highly attractive from a theoretical point of view, this approach to treating cancer is practically limited by difficulties in the discovery and development of novel and effective chemopreventive agents. The cytotoxic and apoptotic effects of the bioactive compounds isolated from *Psoralea corylifolia* in combination with TRAIL on prostate cancer cells were examined for the first time in our study. The previous investigations demonstrated that neobavaisoflavone augmented TRAIL-mediated apoptosis in HeLa cancer cells [4]. There is, however, no evidence of antitumor activity of neobavaisoflavone and psoralidin against prostate cancer cells. Our study showed that neobavaisoflavone and psoralidin enhanced the apoptosis-inducing potential of TRAIL and sensitized TRAIL-resistant LNCaP prostate cancer cells. The strong cytotoxic and apoptotic effects of these compounds isolated from *Psoralea corylifolia* in combination with TRAIL against prostate cancer cells suggest their potential use in chemoprevention strategies.

Acknowledgment:
This project was supported by a research grant KNW-1-060/09 from the Medical University of Silesia in Katowice (Poland).

References:

1. Andrzejewski T, Deeb D, Gao X, Danyluk AL, Arbab AS, Dulchavsky SA, Gautam SC: Therapeutic efficacy of curcumin/TRAIL combination regiment for hormone-refractory prostate cancer. Oncol Res, 2008, 17, 257–267.
2. Almasan A, Ashkenazi A: Apo2L/TRAIL: apoptosis signaling, biology and potential for cancer therapy. Cytokine Growth Factor Rev, 2003, 14, 337–348.
3. Barqawi A, Thompson IM, Crawford ED: Prostate cancer chemoprevention: an overview of United States trials. J Urol, 2004, 171, 5–9.
4. Bronikowka J, Szliszka E, Czuba ZP, Zwolinski D, Szmydki D, Krol W: The combination of TRAIL and isoflavones enhances apoptosis in cancer cells. Molecules, 2010, 15, 2000–2015.
5. Bucur O, Ray S, Bucur MC, Almasan A: Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand in prostate cancer therapy. Front Biosci, 2006, 11, 1549–1568.
6. Crawford ED: Understanding the epidemiology, natural history, and key pathways involved in prostate cancer. Urology, 2009, 73, 4–10.
7. Deeb D, Jiang H, Gao X, Al-Holou S, Danyluk AL, Dulchavsky SA, Gautam SC: Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)1-6-heptadine-3,5-dione $C_{21}H_{20}O_6$] sensitizes human prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand/Apo2L-induced apoptosis by suppressing nuclear factor-κB via inhibition of prosurvival Akt signaling pathway. J Pharmacol Exp Ther, 2007, 321, 616–625.
8. Deeb D, Jiang H, Gao X, Divine G, Chapman RA, Dulchavsky SA, Gautam SC: Chemosensitization of hormone-refractory prostate cancer cells by curcumin to TRAIL-induced apoptosis. J Exp Ther Oncol, 2005, 5, 81–91.
9. Deeb D, Jiang H, Gao X, Hafner MS, Wong H, Divine G, Chapman RA et al.: Curcumin sensitizes prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand/Apo2L by inhibiting nuclear factor-κB through supression of IκBα phosphorylation. Mol Cancer Ther, 2004, 3, 803–812.
10. Deeb D, Xu YX, Jiang H, Gao X, Janakiraman N, Chapman RA, Gautam SC: Curcumin (diferuloylmethane) enhances tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in LNCaP cancer cells. Mol Cancer Ther, 2003, 2, 95–103.
11. Heidenreich A, Aus G, Bolla M, Joniau S, Matveev VB, Schmid HP, Zattoni F, European Association of Urology: EAU guidelines on prostate cancer. Eur Urol, 2008, 53, 68–80.
12. Horinaka M, Yoshida T, Shiraishi T, Nakata S, Wakada M, Sakai T: The dietary flavonoid apigenin sensitizes malignant tumor cells to tumor necrosis factor-related apoptosis-inducing ligand. Mol Cancer Ther, 2006, 5, 945–951.
13. Huang X, Zhang X, Farahvash B, Olumi AF: Novel targeted pro-apoptotic agents for the treatment of prostate cancer. J Urol, 2007, 178, 1846–1854.
14. Jun-Ying Y, Cun-Shuan X: Antitumor effects of a selenium heteropoly complex in K562 cells. Pharmacol Rep, 2009, 61, 288–295.
15. Khan N, Adhami VM, Mukhtar H: Apoptosis by dietary agents for prevention and treatment of prostate cancer. Endocr Relat Cancer, 2010, 17, R39–52.
16. Kim YH, Lee YJ: TRAIL apoptosis is enhanced by quercetin through AKT dephosphorylation. J Cell Biochem, 2007, 100, 998–1009.
17. Kim YH, Lee DH, Jeong JH, Guo ZS, Lee YJ: Quercetin augments TRAIL-induced apoptotic death: involvement of the ERK signal transduction pathway. Biochem Pharmacol, 2008, 75, 1946–1958.
18. Latha PG, Evans DA, Panikkar K, Jayavardhanan KK: Immunomodulatory and antitumor properties of *Psoralea corylifolia* seeds. Fitoterapia, 2000, 71, 223–231.
19. Pilarski R, Poczekaj-Kostrzewska M, Ciesiołka D, Szyfter K, Gulewicz K: Antiproliferative activity of various *Uncaria tomentosa* preparations on HL-60 promyelocytic leukemia cells. Pharmacol Rep, 2007, 59, 565–572.
20. Shankar S, Chen Q, Sarva K, Siddiqui I, Srivastava RK: Curcumin enhances the apoptosis-inducing potential of TRAIL in prostate cancer: molecular mechanisms of apoptosis, migration and angiogenesis. J Mol Signal, 2007, 2, 1–14.
21. Shankar S, Chen Q, Siddiqui I, Sarva K, Srivastava RK: Sensitization of TRAIL-resistant LNCaP cells by res-

veratrol (3,4',5-trihydroxystilbene): molecular mechanisms and therapeutic potential. J Mol Signal, 2007, 2, 27–36.
22. Shankar S, Ganapathy S, Chen Q, Srivastava RK: Curcumin sensitizes TRAIL-resistant xenografts: molecular mechanisms of apoptosis, migration and angiogenesis. Mol Cancer, 2008, 7, 1–13.
23. Shankar S, Siddiqui I, Srivastava RK: Molecular mechanisms of resveratrol (3,4',5-trihydroxy-trans-stilbene) and its interaction with TNF-related apoptosis inducing ligand (TRAIL) in androgen-insensitive prostate cancer cells. Mol Cell Biochem, 2007, 304, 273–285.
24. Siddqui IA, Malik A, Adhami VM, Asim M, Hafeez BB, Sarfaraz S, Mukhtar H: Green tea polyphenol EGCG sensitizes human prostate carcinoma LNCaP cells to TRAIL-mediated apoptosis and synergistically inhibits biomarkers associated with angiogenesis and metastasis. Oncogene, 2008, 27, 2055–2063.
25. Singh RP, Agarwal R: Mechanisms of action of novel agents for prostate cancer chemoprevention. Endocr Relat Cancer, 2006, 13, 751–778.
26. Sonn G, Aronson W, Litwin MS: Impact of diet on prostate cancer: review. Prostate Cancer Prostatic Dis, 2005, 8, 304–310.
27. Syed DN, Suh Y, Afag F, Mukhtar H: Dietary agents for chemoprevention of prostate cancer. Cancer Lett, 2008, 265, 167–176.
28. Szliszka E, Bronikowska J, Majcher A, Miszkiewicz J, Krol W: Enhanced sensitivity of hormone-refractory prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated cytotoxicity by taxanes. CEJ Urol, 2009, 62, 29–34.
29. Szliszka E, Czuba ZP, Bronikowska J, Mertas A, Paradysz A, Krol, W: Ethanolic extract of propolis (EEP) augments TRAIL-induced apoptotic death in prostate cancer cells. Evid Based Complement Alternat Med, 2009, doi:10.1093/ecam/nep180.
30. Szliszka E, Czuba ZP, Domino M, Mazur B, Zydowicz G, Krol W: Ethanolic extract of propolis (EEP) enhances the apoptosis-inducing potential of TRAIL in cancer cells. Molecules, 2009, 14, 738–754.
31. Szliszka E, Czuba ZP, Jernas K, Krol W: Dietary flavonoids sensitize HeLa cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Int J Mol Sci, 2008, 9, 56–64.
32. Szliszka E, Czuba ZP, Mazur B, Sedek L, Paradysz A, Krol W: Chalcones enhance TRAIL-induced apoptosis in prostate cancer cells. Int J Mol Sci, 2010, 11, 1–13.
33. Tanaguchi H, Yoshida T, Horinaka M, Yasuda T, Goda AE, Konishi M, Wakada M et al.: Baicalein overcomes tumor necrosis factor-related apoptosis-inducing ligand resistance via two different cell specific pathways in cancer cells but not in normal cells. Cancer Res, 2008, 68, 8918–8927.
34. Thorburn A, Behbakht K, Ford H: TRAIL receptor-targeted therapeutics: resistance mechanisms and strategies to avoid them. Drug Resist Updat, 2008, 11, 17–24.
35. Thorpe JF, Jain S, Marczylo TH, Gescher AJ, Steward WP, Mellon JK: A review of phase III clinical trials of prostate cancer chemoprevention. Ann R Coll Surg Engl, 2007, 89, 207–211.
36. Wang Y, Hong C, Zhou C, Xu D, Qu H, Cheng Y: Screening antitumor compounds psoralen and isopsoralen from *Psoralea corylifolia* L seeds. Evid Based Complement Alternat Med, 2009, 8, 1–7.
37. Zhao L, Wu M, Xiang B: Analysis of *Psoralea corylifolia* L fruits in different regions. Chem Pharm Bull, 2005, 53, 1054–1057.
38. Zhao LH, Huang CY, Shan Z, Xiang BG, Mei LH: Fingerprint analysis of *Psoralea corylifolia* by HLPC and LC-MS. J Chromatogr B, 2005, 821, 67–74.

Received: March 31, 2010; **in the revised form:** August 11, 2010; **accepted:** September 15, 2010.