Poster presentations

P1

Human adipose tissue pretreatment method development for tocopherols' and tocotrienols' targeted metabolomics in breast cancer disease

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The "vitamin E" family consists primarily of four naturally occurring tocopherols (alpha-, beta-, gamma- and delta-) and four unsaturated corresponding tocotrienols, which differ in their structure by the composition of methyl groups attached to the chromanol ring. Apart from their potent antioxidant activity, it is hypothesized that some of these compounds might play protective role against cancer diseases, for instance breast cancer and prostate cancer. In the field of cancer prevention, developing metabolomic approach is one to be considered in potential biomarkers' research. For this reason, targeted metabolomic analysis is required for the evaluation of tocochromanols' utility in breast cancer prevention.

The aim of this study was development of a selective and rapid method for isolation of tocopherols and tocotrienols from human adipose tissue. After mechanical extraction with isopropanol/ethanol mixture (5:1) with addition of BHT and in the presence of internal standard (deuterated alpha-tocopherol), samples were centrifuged in 4°C and the supernatant was collected. Then samples were diluted with water and solid-phase extraction was performed. For this purpose, polymeric SPE tubes were used (Bond Elut Plexa, 30 mg, 1 ml, Agilent Technologies). After samples application, SPE bed was purified with 80% methanol solution and target analytes were eluted with isopropanol. Finally, extracts were evaporated to dryness and residues were dissolved in 100 µl of 0.04% BHT in ethanol. Tocopherols' and tocotrienols' contents in human adipose tissue were determined with the use of LC-APCI-MS/MS. The chromatographic conditions were as follows: 3.0×100 mm Cosmosil 2.5π-NAP column (Nacalai Tesque, Japan) with the mobile phase methanol/water (9:1, *v*/*v*) in an isocratic flow. Mass spectrometry experiments were performed with multiple reaction monitoring (MRM) in the positive polarization mode. The developed pretreatment method is efficient for tocochromanols' determination in human adipose tissue.

Key words: tocopherols; tocotrienols; breast cancer

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P2

Induction of G2/M phase arrest and apoptosis of human pancreatic cancer BxPC-3 cells by potent antitumor 1-nitroacridine derivative C-1748

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Pancreatic ductal adenocarcinoma (PDA) is among the most lethal human cancers, in part because it is insensitive to many chemotherapeutic drugs. Gemcitabine still remains the best chemotherapeutic agent available for the treatment of advanced pancreatic cancer. However, gemcitabine treatment results in only a marginal survival advantage. Thus, there is a strong need for the continuous development of novel therapeutic agents to improve pancreatic cancer therapy.

The compound C-1748 is the most active derivative of 1-nitroacridine antitumor agents developed in our laboratory. Strong cytotoxic activity against colon cancer cell lines (HCT8 and HT29) and high antitumor activity against xenografts in nude mice of prostate (LnCaP) and colon carcinoma (HCT8), along with low mutagenic potential and slight myelosuppressive properties allowed the selection of C-1748 for phase I clinical trials. The aim of the current study was to investigate and characterize the cellular response of human pancreatic cancer cell line BxPC-3 to C-1748 treatment.

Cell cycle analysis revealed that between 96 h and 192 h of C-1748 treatment, BxPC-3 cells underwent accumulation in the G2/M phase which was preceded by prolonged arrest in the G1 phase. The cell cycle perturbations were accompanied by the appearance of sub-G1 fraction, which can be considered as the apoptotic cells population. C-1748 induced apoptosis was more significant in dose- than time-dependent manner and was confirmed by morphological changes like condensed chromatin and apoptosis-body like structures in DAPI stained cell nuclei. Apoptosis induced by C-1748 was also confirmed by flow cytometry analysis of phosphatydylserine externalization, PARP cleavage and mitochondrial dysfunction.

To sum up, major cellular response triggered by C-1748 in BxPC-3 cells was cell cycle arrest in G2/M phase and induction of apoptosis. These results highlight the therapeutic potential of C-1748 in pancreatic cancer and support rationale for its further investigation towards this type of malignancy.

Key words: C-1748; apoptosis; cell cycle; pancreatic cancer; cancer treatment

P3

Impact of isothiocyanates on normal and cancer cells

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Epidemiological studies revealed that there is an inverse correlation between consumption of cruciferous vegetables and the incidence of certain types of cancer. Isothiocyanates (ITC) are the products of hydrolysis of glucosinolates, compounds naturally present in cruciferous plants. Previous studies performed on animal models as well as cancer cell lines proved chemopreventive and anticancer properties of isothiocyanates. The mechanism of their action involves an inhibition of cancer cell proliferation, G2/M cell cycle arrest and/or apoptosis induction. However, little is known about the influence of these compounds on normal, non-transformed cells.

We investigated the impact of two isothiocyanates: sulforaphane (SFN) and phenethyl isothiocyanate (PEITC) on viability, induction of apoptosis as well as genotoxic stress in the non-transformed human dermal fibroblasts (HDFa) and the prostate cancer cells (PC3). The results obtained showed that both SFN and PEITC efficiently decreased viability of prostate cancer cells, whereas fibroblasts were either completely (PEITC) or partially resistant (SFN) to ITC. Moreover, we observed PARP cleavage, a marker of apoptosis induction, in SFN as well as PEITC treated PC3-cells. The same concentrations of ITC were unable to induce such effect in fibroblasts. As published data indicated that ITC induce DNA damage checkpoint, we compared effect of ITC on the level of DNA double-strand breaks (DSB) in both cell lines. SFN and, to a lesser extent PEITC, treatment resulted in increased H2A.X phosphorylation (a marker of DSB) in PC3 cells, which was observed as soon as 2 hours after ITC addition. Examined compounds had minimal effect on H2A.X phosphorylation in fibroblasts. DNA damage in cancer cells was reversible, as removal of ITC led to drop in H2A.X phosphorylation.

Summarizing, our results indicate that SFN and PEITC are selective for the cancer cells and exert no detrimental effects on normal, non-transformed cells. Because of these properties isothiocyanates might be effective and safe drugs in cancer prevention and therapy.

Key words: isothiocyanates; HDFa; PC3; DNA damage

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P4

Cold plasma induced anticancer properties of saline and RPMI 1640 medium

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Low-temperature plasma reactor declustered water to single molecules measuring 1 nM each. Such water as well as saline were characterized by low viscosity, high diffusivity, and very low density as well as low dielectric constant. They dissolve 35–40% more substances and also raise the solubility of gases and salts. Hydrate bonds in water are probably changed and such water may interact with structures as DNA or proteins. Induction of apoptosis of pancreas cancer cells was observed when electron structure of water was changed.

The main objective of the present study was to evaluate an influence of saline treated with plasma on growing normal fibroblast and cancer cells of different lines. Additionally, DNA synthesis ([3H]-thymidine incorporation) and apoptosis process (flow cytometry) were studied. Moreover, RPMI 1640 medium treated with plasma was used to investigate a growth of leukemia cell lines.

Results obtained clearly showed that plasma treated saline did not change a growth of human skin fibroblasts (CRL-1474) when saline was added to culture during all period of study (120 h), however, colon cancer (DLD-1) and leukemia (MOLT-4) viable cells were markedly reduced. Decreased synthesis of DNA and increased apoptosis in flow cytometry was confirmed by increased number of cells with high activity of caspase 3. In addition, transmission electron micrographs of saline (plasma modified) treated MOLT-4 cells also showed ultrastructural characteristics of apoptosis. An increased number of apoptotic cells of leukemia cell lines: HL-60, HL-60/MX1, HL-60/MX2, CCRF/CEM, CEM/C1, J45 and U266 was observed while growing them on RPMI 1640 medium treated with plasma. In summary, a very promising outcome for cancer prevention was brought in our results as cold plasma modified the properties of saline and culture medium.

Key words: cold plasma; cancer cell lines; apoptosis

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P5

Does the high-intensity circuit training may be considered an anti-stress treatment ?

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Multiple lines of compelling evidence suggest that physical activity reduces the risk of different types of malignances, especially those of the colon, breast, prostate, endometrial, and lungs. Literature data indicate that a long-lasting psychological stress is one of the factors, which may stimulate *carcinogenesis*. Sustained elevated synthesis of the heat shock protein (HSP) is a defensive mechanism against stress. Hence, recent findings reporting Hsp70 as a novel fatigue-signaling factor prompted measurements of Hsp70 and Hsp27 levels.

Consequently, the aim of the study was to investigate the influence of a high-intensity circuit training (HICT) on blood HSP70 and HSP27 concentrations among middle age women. Fourteen women completed a single unit of HICT according to the ACSM recommendations. Based on their status, students or working, the participants were allocated into groups: student group (SG, n=7, 22.7±3 year old) or working group (WG, n=7, 38±7 year old). Prior to the intervention, body composition, aerobic capacity and daily activities' assessments were performed. Throughout the intervention, blood samples were collected at rest, 1 h and 24 h after training to evaluate the concentrations of HSPs as well as the pro-and anti- inflammatory cytokines. Statistical analysis was performed using the Magnitude Based Interference (MBI). Effects were calculated within and between groups using mix modeling.

The data obtained demonstrated that response to the HICT was distinct in two groups. Values of HSP27 recorded 1 h after training in WG exhibited a significant drop (effect very likely), whereas in SG the descendant effect was small. A similar tendency for WG was noted in values of HSP70 recorded 24 h after training (effect most likely). The statistical analysis indicated a large decreasing effect in WG (effect most likely), whereas in SG the drop effect was again small. In addition, the drop in HSP70 and HSP27 in WG was accompanied by an elevated synthesis of the anti-inflammatory cytokine IL-10 1 h after training (effect large very likely), maintained also 24 h later (effect moderate very likely).

Basing on the obtained data, we concluded that HICT could be recommended as an anti-inflammatory treatment, especially for middle age women.

Key words: cytokine; exercise; anti-inflammation

P6

Method of Au nanoparticle preparation for gas sensors

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The non-toxic method for Au nanoparticles generation in solution was proposed. The product formation was observed by Atomic Force Microscopy. As a source of gold precursor, gold (III) chloro-trihydrate was used, while the reductor was L-ascorbic acid. The nanoparticles were originally designed for gas sensing in combination with graphene or polymers to be applied as biosensors in health and environmental monitoring. However, the method enabled the obtainment of non-toxic nanoparticles in solution that can be used in medicine, e.g. as drug delivery system. For instance, the nanoparticles can be generated in a deep eutectic solvent - choline chloride and urea and be used as a delivery system of the former. Choline chloride, (2-hydroxyethyl)trimethylammonium chloride, is widely known as vitamin B4. Vitamin B4 is responsible for the development and maintenance of cellular structure and for control of muscles, respiration system, heart beating and memory. Former methods of auric nanoparticles generation gave products posing toxicological hazard.

Key words: auric nanoparticles; gas sensing; non-toxic

P7

Phytoecdysteroid containing plants – a source of bioactive compounds with potential anticarcinogenic effects

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Ecdysteroids are a group of steroids synthesized in invertebrates as molting hormones and also in some plants as secondary metabolites acting as a defense against parasites. These compounds do not naturally occur in mammals and are not structurally related to mammalian steroid hormones, consequently do not activate mammalian hormone receptors. Ecdysteroids exhibit very low toxicity to mammals and to date no serious side effects after intake of these compounds have been reported. Plant extracts containing ecdysteroids (i.e. 20-hydroxyecdysone, α-ecdysone, inokosterone, makisterone) have been used as remedies in folk medicine for centuries. The most popular extracts are from *Leuzea carthamoides* and are believed to exhibit such therapeutic effects as: tonic, roborant, adaptogenic, antidepressive, anticancerogenic and body strengthening after physical and mental efforts. The unique medicinal properties of *L. carthamoides* result from its multicomponent and complex composition. These plants contain bioactive compounds belonging to other classes such as phytosterols, flavones, steroids, flavonoids, phenolic acids, triterpernes, diterpenoids, sesquiterpene lactones, thiophens, antocyanins and tannins, but the exact composition and the mode of action of these compounds, individual or in combination, is still little known. Over past few years, many possible uses of ecdysteroid containing extracts in healthcare have been suggested. The most interesting approach is their application in gene-switch therapy and in gene expression regulation. Ecdysteroids employed in gene therapy have been shown to allow a rapid, precise and reversible induction or suppression of therapeutic genes. This may open the new perspectives in treatment of genetically predisposed diseases and also in cancer prevention, especially when deregulation of particular gene activity is a risk factor.

Key words: *Leuzea carthamoides*; ecdysteroids; gene-switch therapy

P8

Physiological and pharmacological effects of 2-methoxyestradiol

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2-methoxyestradiol (2-ME) is one of the principal physiological 17β-estradiol derivatives, believed to be novel and potentially active anticancer agent evaluated in ongoing advanced clinical trials. Possible anticancer mechanisms of 2-ME action seem to be directly associated with the inhibition of angiogenesis and induction of apoptosis in tumorous and proliferating cells.

The aim of the study was to determine physiological and pharmacological anticancer, neurotoxic and genotoxic properties of 2-methoxyestradiol.

We used highly metastatic osteosarcoma 143B, hippocampal HT22, neuroblastoma SHSY5Y cell lines. The cells were treated with physiological and pharmacological concentrations of 2-ME. Cell death was measured by PI/Annexin V staining, inhibition of cell cycle, level of reactive oxygen and nitrogen species and neuronal nitric oxide synthase localization were determined by imaging cytometry and flow cytometry. Comet assay was performed in order to determine DNA damage.

Herein, we evidenced that 2-ME exerts anticancer properties not only at pharmacological but also physiological concentrations. Moreover, it exerts neurotoxic and genotoxic effects when used at physiologically and pharmacologically relevant concentrations.

Key words: 2-methoxyestradiol; osteosarcoma; cell death

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P9

Oxidative stress effects are altered by plant foods

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Rotenone is a pesticide capable to induce oxidative stress in cells and to cause damage to the macromolecules. Oxidative damage to DNA triggered by rotenone in the mitochondrial and the nuclear genomes were described. The deleterious effects of oxidative stress can be attenuated by the enhancement of antioxidant defense provided by diet. These are mainly fruit and vegetable products rich in polyphenols and other phytochemicals showing antioxidant activities. The aim of the study was to evaluate the effects of 5 week administration of pomegranate juice and rotenone on the selected parameters of the antioxidant defense and DNA damage in rats.

In animals receiving rotenone (1 mg mg/kg b.w., s.c. daily) a significant decrease in the hepatic level of reduced glutathione was demonstrated. Surprisingly, the same effect was found in animals receiving orally concentrated pomegranate juice alone (daily 500 mg/kg b.w.). In rats which were administered with pomegranate juice and rotenone no protective effects were observed. Pomegranate juice caused significant increase in paraoxonase-1 (PON1) activity in the rat plasma and the liver homogenate. Rotenone significantly reduced PON1 in the plasma and increased its activity in the liver. In animals receiving pomegranate juice and rotenone an increase in PON1 in the plasma and in the liver was observed, suggesting protective potential of pomegranate phytochemicals.

Rotenone caused DNA damage (measured in comet assay) in the liver and in the whole blood leukocytes. Pomegranate induced non-significant damage to DNA. In the animals treated with pomegranate juice and rotenone the protection of DNA was observed. Plasma Total Antioxidant Status (reduction of ABTS radical) increased due to the pomegranate administration, however, rotenone treatment also caused a rise in the reducing power of plasma. Our findings might suggest the toxic and adaptive response of the rats to rotenone, and the protective effect of pomegranate juice against oxidative insult.

Key words: rotenone; pomegranate; DNA damage

P10

7-methyljuglone suppresses NF-κB signaling through IKKα inhibition in breast cancer cells

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The NF-κB transcription factor is upregulated in certain types of breast cancers, in particular HER2-overexpressing breast tumors. HER2 is overexpressed in approximately 30% of breast cancer occurrences and is associated with increased tumor aggressiveness and poor clinical outcome. Although therapies targeting the HER2 receptor have proven effective, resistance towards therapy is often acquired. Recently anti-HER2 drug resistance has been correlated with the upregulation of NF-κB, in particular the expression of NF-κB-regulated anti-apoptotic genes. Thus the search for effective agents that target NF-κB is warranted in order to achieve long-term inhibition of HER2-overexpressing breast tumors.

7-methyjuglone, a naphthoquinone constituent present in plants from the *Droseraceae* family, displays a wide range of biological activities, including cytotoxic activity. Our previous research demonstrated high anti-proliferative activity of 7-methyljuglone towards breast cancer cells. Therefore, the aim of the present research was to evaluate the anti-proliferative activity of 7-methyjuglone towards HER2-overexpressing breast cancer cells and define the mode of cell death induced by 7-methyljuglone in these cells.

The results of our research demonstrated high anti-proliferative activity of 7-methyljuglone towards HER2-overexpressing breast cancer cells, BT474 and SKBR3. The mode of cell death induction was through apoptosis, as determined by cytometric analysis of caspase activation and Annexin V-PE staining. The measurement of NF-κB transcriptional activity, with a NF-κB-driven luciferase reporter assay, revealed that 7-methyjuglone inhibits NF-κB activity in BT474 and SKBR3 cells. Decreased NF-κB activity correlated with a reduction in phosphorylated NF-κB, a decrease in IKKa levels and an increase in the levels of the NF-κB inhibitor, IkBa. Moreover, 7-methyljuglone reduced the levels of NF-κB-regulated anti-apoptotic gene products such as Bcl-2, Bcl-xL and survivin in HER2-overexpressing breast cancer cells. Thus, these findings indicate that 7-methyljuglone induces apoptosis in HER2-overexpressing breast cancer cells through IKKα-mediated NF-κB inhibition.

Key words: HER2-overexpression; 7-methyljuglone; NF-κB

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P11

Antitumor activity of newly synthesized aliphatic *N*-substituted ebselen derivatives

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The search for new drugs in cancer treatment has been justified for many decades and yet our progress in curing tumor has been marginal. However, there is relatively enough data indicating that organoselenium compounds could fulfill this need of drugs with versatile target action. Organoselenium compounds (benzizoselenazolone analogs) have been shown to have a variety of biological activities including anticancer capacity and anti-inflammatory activity. Ebselen derivatives as mimetics of GPx can exhibit high antioxidant activity.

Moreover, our data demonstrated that all of the studied selenocompounds possessed high antioxidant activity but only few of them had anticancer activity. The anticancer activity of these compounds was evaluated on prostate cancer cell lines characterized by different genetic background. The effects of the benzizoselenazolone analogs on cell viability and Akt signaling pathway were evaluated. We observed that among twenty structurally different ebselen derivatives, four of them demonstrated antiproliferative activity at 40 μM concentration. Three of them were more cytotoxic to DU145 cell lines than to PC-3 and these data correlate with basal Akt activity, which is higher in PC-3 cells. On the other hand the cytotoxicity of N-butyl-1,2-benzisoselenazol-3(2*H*)-one was similar in both cell lines indicating different mode of action compared to other three selenocompounds. Moreover our preliminary data indicate that some of the newly synthesized compounds inhibit prostate cancer cell proliferation through p66Shc signaling pathways. In conclusion, our results show the anticancer effectiveness of newly synthesized benzizoselenazolone analogs.

Key words: prostate cancer; benzizoselenazolone analogs; kinase Akt

P12

Characterization of the interaction between CD160 and HVEM proteins, involved in immune response inhibition in melanoma

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Melanoma is the most serious type of skin cancer. The number of cases worldwide has doubled in the past twenty years [1]. Patients with melanoma often have increased numbers of tumor antigen specific T cells that can be beneficial for patients [2]. Indeed, one of the most promising methods to treat melanoma is immunotherapy that supports activation and function of the patient's T cells. The CD160 protein was identified as a co-inhibitory molecule that binds to the herpesvirus entry mediator (HVEM), a TNF receptor superfamily member. CD160 is expressed on the surface of immune cells, including T, B and NK cells [3]. The HVEM-CD160 complex inhibits $CD4^+$ T cell activation [4].

Our research is focused on blocking the interaction between CD160 and HVEM proteins to stimulate immune response. Therefore, we characterize the interaction of both proteins by using affinity chromatography and mass spectrometry. Both protein fragments engaged in the interaction will be characterized, based on which we can evaluate various possible strategies to block the interaction.

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Key words: melanoma; CD160 protein; HVEM protein; mass spectrometry; affinity chromatography; peptides

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P13

The effect of sirtuin 6 up-regulation on glycolysis and glutaminolysis in hypopharyngeal (FaDu) carcinoma cells

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Most cancer cells exhibit increased glucose uptake under aerobic conditions and display high expression and activity of several glycolytic enzymes leading to the high rate of glucose catabolism and lactate production, which is called the Warburg effect. Moreover, recent studies revealed the coordinated regulation of glycolysis and glutaminolysis. Both processes are intensified partly as a result of HIF-1 and cMYC up-regulation, respectively. Moreover, nuclear sirtuin SIRT6, NAD^+-dependent histone deacetylase, may be involved in the control of genes involved in energy metabolism by co-repressing these transcription factors.
The aim of this study was to investigate the influence of resveratrol, a natural stilbene, its synthetic derivative DMU-212 as well as small-molecule HIF-1, cMYC, glycolysis and glutaminolysis inhibitors on the expression of SIRT6 and key metabolic enzymes in FaDu hypopharyngeal carcinoma cells.
FaDu cells were treated for 24 h with 10 μM resveratrol, 2 μM DMU-212, 50 μM KG-548 (HIF-1 inhibitor) and 10058-F4 (cMYC inhibitor), 1 or 2 mM 2-deoxyglucose (glycolysis inhibitor) and 0.1 or 0.2 mM aminooxyacetic acid (glutaminolysis inhibitor). RT-PCR analysis was performed to evaluate relative expression of *GLUT1*, *HK2*, *PFKM*, *PKM2*, *LDHA*, *HIF-1alpha*, *SLC1A5*, *GLS*, *GDH*, *cMYC* and *SIRT6*.
Modulation potential of SIRT6 expression was demonstrated for DMU-212, resveratrol, aminooxyacetic acid and KG-548. SIRT6 up-regulation was only partly followed by decrease in metabolic genes expression. 10058-F4 more potently inhibited glycolysis than KG-548. A dose-dependent decrease in analyzed genes expression was also observed after 2-deoxyglucose treatment.
Analysis of the metabolic end-products in culture medium revealed that all of the tested compounds decreased lactate concentration. Ammonia level was significantly decreased only as the result of treatment with cMYC inhibitor.
Overall, the results of this study indicate that although both stilbenes may increase the expression of SIRT6 its effect on glycolysis and related signaling pathways is limited.

Key words: the Warburg effect; sirtuin 6; FaDu cells

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P14

Pro-healthy effect of exercise is related to changes in iron metabolism

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Despite several pro-healthy effects of regular physical training being well-documented, its influence on body iron stores is not fully explored. Body iron accumulation is associated with high risk of several morbidities. Thus, we hypothesized that regular training would result in pro-healthy changes manifested by reduction of body iron stores via shifts in iron metabolism-regulating proteins in both senior and master sportswomen/men .
Study was performed on young men training judo and no trained control group (age 22±1.5 years). Moreover, elderly men and women (age 65±4.0 years) participated in this study and they underwent 32 weeks of the training 3 times/week. Fitness level, blood morphology, CRP, vitamin D, ferritin, hepcidin and soluble hemojuvelin were assessed before and after the training. We observed that young sportsmen are characterized by lower body iron stores and higher blood hepcidin concentration compared to control group. In elderly subjects we observed that the training program caused a significantly drop in ferritin which is a good marker of body iron stores. This decrease was accompanied by improvement of physical cardiorespiratory fitness. The concentration of blood CRP diminished but the changes were non-significant. Furthermore, positive correlation was observed between blood hepcidin and ferritin concentration after the training. Interestingly, the training raised blood hemojuvelin which inversely correlated with vitamin D concentration. Our data clearly indicate that regular exercise training reduced body iron stores in both young and elderly people. Considering that reduction of body iron by phlebotomy reduced risk of cancer, heart attack and diabetes type II, the same can be achieved by physical training.

Key words: iron; hepcidin, inflammation

P15

Relationship between the betalain composition in *Opuntia ficus indica* and *Beta vulgaris* varieties and biological activity of their extracts

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Betalains are a class of pigments that are much less commonly found in nature than anthocyanins or carotenoids. They occur only in a few edible plants such as red beetroot (*Beta vulgaris*), prickly pears (*Opuntia ficus indica),* amaranth (*Amaranthus* sp.) and some mushrooms. This group of pigments can be divided into two major groups: yellow vulgaxanthins and red betanins. Despite their structural similarity to compounds from the group of alkaloids, betalains do not show any toxic properties in human organism, that is why they are highly recommended as healthy alternative to synthetic red food colorants. Moreover, published studies showed that these compounds have antimicrobial and antiviral activity.

The aim of our study was to compare the betalain profiles for three prickly pear varieties (yellow, orange and red) and two beetroot varieties (white and red) to examine their impact on antioxidant and biological activity of tested plant extracts. The composition of betalains and other biologically active phytochemicals was characterized using HPLC-DAD-MS, total antioxidant activity was assessed by spectrophotometric tests (ABTS, DPPH, FC and FRAP), antioxidant profiles were obtained with the aid of chromatographic techniques (TLC and HPLC with post-column derivatization). Biological activity was determined using MTT cytotoxicity assay, Ames test and comet assay. The highest content of betalain was observed in red beetroot, followed by red, orange and yellow opuntia, in white beetroot no betalain were detected. The strong relationship between betalain content and antioxidant activity was observed, while studies on biological activity of tested fruit extracts showed that they do not exhibit cytotoxic, genotoxic or mutagenic effects in cellular models employed.

Key words: betalains; cytotoxicity; antioxidants; mutagenicity

P16

Mechanism of TAp73 stabilization upon withaferin A in p53-deficient cancer cells

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p53 protein plays a major role in maintaining the integrity of the genome. It acts as a tumor suppressor, inducing cell cycle arrest or apoptosis, leading to elimination of cancer cells. However, p53 is often lost or mutated in human malignancies. TAp73 protein is a functional and structural homolog of p53. In stress conditions TAp73 can be activated, bind to p53-responsive genetic targets and induce cell death. Therefore, TAp73 can serve as an alternative target for pharmaceutical intervention in cells lacking functional p53.

Plants are a rich source of biologically active compounds that can be used as therapeutic drugs. *Withania somnifera* leaf and root extracts have been used throughout the years in Indian traditional medicine – Ayurveda. Scientific studies on their activity led to identification of a number of compounds, among them anti-cancer steroidal lactone - withaferin A (WA). Further studies proved that WA inhibits tumor progression through inhibiting Akt and NF-κB signaling pathways, altering cytoskeleton proteins, inducing reactive oxygen species (ROS) formation and blocking proteasome activity. These activities were confirmed in studies carried out *in vitro* and *in vivo*.

Our study presents the mechanism of TAp73 stabilization upon WA in cancer cells lacking functional p53 protein. WA induces ROS and launches phase II antioxidant response elements. Immunoprecipitation experiments confirmed that WA promotes TAp73 stabilization through facilitating its binding to NQO1. Furthermore, oxidative stress activates JNK-mediated TAp73 phosphorylation, enhancing TAp73-driven apoptotic response. Knocking down *TAP73* gene expression confirmed that TAp73 activity enables more robust apoptotic response upon WA.

Presented data demonstrate the mode of action of 20S proteasome inhibitor WA, that stabilizes and activates TAp73, causing apoptosis of cells lacking functional p53.

Key words: withaferin; oxidative stress; p73

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P17

Usnic acid modulates the Nrf2-ARE pathway in FaDu hypopharyngeal carcinoma cells

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Head and neck squamous cell carcinoma (HNSCC) is the sixth leading cancer in humans. The survival rates in HNSCC patients remain poor especially in more advanced disease stages when the risk of recurrence is higher. Epidemiological studies have suggested that the consumption of natural products reduces the risk of malignancies including HNSCC. Naturally occurring phytochemicals such as bergenin, arctigenin, usnic acid and xanthohumol were suggested to act as chemopreventive compounds. The aim of this study was the investigation of the influence of these phytochemicals on the expression of the enzymes controlled by the Nrf2/ARE pathway activation in human FaDu hypopharyngeal carcinoma cells.
FaDu cells were treated with either 2 or 10 μM bergenin, usnic acid, arctigenin, xanthohumol.
Western blot analysis detected the presence of Nrf2 both in cytosol and nucleus in all the tested samples however enhanced nuclear/cytosol Nrf2 ratio was observed only after treatment with usnic acid suggesting that it stimulates Nrf2 translocation. Accordingly, usnic acid increased the transcript and protein levels of GSTP, NQO1, SOD, which are controlled by Nrf2.
Since the activation of Nrf2 is accompanied by phosphorylation of GSK-3β, we also measured the expression and level of GSK-3β in cytosolic fraction. Interestingly, usnic acid decreased both *GSK-3β* mRNA and protein, what has been shown to attenuate the transcriptional activity of Nrf2. Other phytochemicals did not affect *GSK-3β*.
To further explore the possible protective effect of the studied compounds, their effect on the level of pro-apoptotic Bax and p53 proteins was additionally analyzed. All the tested compounds decreased *Bax* mRNA but did not change the level of Bax protein. On the other hand significant induction of *p53* was observed only after treatment with usnic acid.
The results of this study indicate that usnic acid may induce cytoprotective response via activation of Nrf2 pathway in FaDu cells.

P18

Oxidative stress induced in response to PDT employing 1,4-Bis[2-(morpholin-4-yl) ethoxy]phthalocyaninato Zinc(II) an in vitro evaluation of mechanism of action

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Introduction: Photodynamic therapy (PDT) is a minimally invasive therapeutic procedure employing photosensitizer, oxygen and light. Individually, these components are not toxic, but when combined may initiate the photochemical reactions which can lead to generation of a highly reactive singlet oxygen and/or reactive oxygen species. Phthalocyanines (Pcs) constitute the group of photosensitizers, which demonstrate favorable photosensitizing and photochemical properties. However, the application of Pcs is limited due to their low solubility in aqueous solutions. Photosensitizer M2TG3, presented herein is substituted with two morpholine groups, which determine its amphiphilic character. Previously, we have reported that M2TG3 possesses strong cytotoxic and pro-apoptotic activity. The aim of this study was to determine the oxidative stress based mechanism of action of M2TG3.
Methods: The detailed studies aiming to determine the mechanism of action were performed using human prostate cancer cell line LNCaP. The oxidative stress after PDT was detected using fluorescence microscopy; glutathione level was measured using fluorescence-based method and superoxide generation in mitochondria using MitoSOX by flow cytometry. Moreover, the influence of diethyldithiocarbamate (ZnCuSOD inhibitor), 2-methoxyestradiol (MnSOD inhibitor), L-buthionine sulfoximine (glutathione synthesis inhibitor) and 3-amino-1,2,4-triazole (catalase inhibitor) in M2TG3 cytotoxicity was assessed. To determine the potential of tested compound in induction of the cellular DNA damage after PDT, the experiments assessing an increase in the 8-hydroxy-2'-deoxyguanosine level using ELISA method were performed.
Conclusions: The oxidative stress induced by M2TG3 mediated PDT was connected with superoxide production and loss of cellular reduced glutathione. The experiments employing antioxidative enzymes inhibitors showed that the cytotoxic effect of PDT-mediated TG3 may be modulated by these inhibitors. Moreover, PDT-mediated M2G3 did not cause DNA oxidative damage at effective doses.

Key words: photodynamic therapy; oxidative stress; phthalocyanines

P19

Cost-optimal screening timelines for colorectal cancer

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While colorectal cancer (CRC) is the second most common cause of US cancer deaths, the path colon cells take to becoming cancer distinguish CRC as a prime target for pre-symptomatic screening methods. CRC develops, in most cases, from polyps. These growths are detectable and removable via colonoscopy. In order to supply a strong argument for the efficacy of pre-symptomatic colonoscopy-based screening, our approach utilizes the multi-stage clonal expansion model of CRC development paired with a framework for the systematic calculation of cost-benefit metrics.

Key words: Colorectal cancer; multistage clonal expansion; colonoscopy; screening; incidence

P20

Antiproliferative activity of 8-hydroksy-quinaldic acid in colorectal adenocarcinoma cells *in vitro*

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Introduction: 8-hydroksy-quinaldic acid is a tryptophan metabolite and constitutes one of the end metabolites of kynurenine pathway. 8-hydroksy-quinaldic acid is a structural isomer of kynurenic acid, another tryptophan metabolite with proved antiproliferative activity towards several cancer cell types *in vitro*. However, biological activity of 8-hydroksy-quinaldic acid in the human organism is broadly unknown.
Aim of the study: The aim of the study was to evaluate the influence of 8-hydroxy-quinaldic acid on growth of colon cancer cells *in vitro*.
Methods: Two colon cancer cell lines (HT-29, LS180) were used in the study and cells were grown in DMEM/F12HAM (1:1) medium supplemented with 10% foetal bovine serum and 100 units/ml penicillin and 100 μg/ml streptomycin. MTT and BrdU tests were applied in order to test cells proliferation/viability upon 8-hydroksy-quinaldic acid treatment for 96 or 48 hours, respectively. Western blot analyses were used to show the expression of several proteins within tested cancer cells.
Results: 8-hydroksy-quinaldic acid suppressed the growth of both HT-29 and LS180 cells. Moreover, it induced remarkable changes in the expression of proliferation and cell cycle regulatory proteins.
Conclusion: Our results show antiproliferative activity of 8-hydroksy-quinaldic acid towards colon cancer cells suggesting chemopreventive potential of tested compound.

Key words: 8-hydroxy-quinaldic acid; colon cancer; proliferation; cell cycle

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P21

Cantharellus cibarius polysaccharides induce cell cycle arrest in human colon cancer cells

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The anticancer use of biologically active compounds isolated from edible mushrooms raises global interest. Chemopreventive properties are mainly attributed to polysaccharides. In spite of the fact that *Cantharellus cibarius* is one of the most popular edible mushroom, anticancer polysaccharides isolated from them have not been exactly defined and studied so far. The presented studies are an attempt to extend this knowledge.
The mushrooms polysaccharides were extracted with hot water and purified by anion-exchange chromatography and fractionated. The antiproliferative activity in human colon carcinoma cells (LS180) was screened by means of MTT and BrdU assays. At the same time fractions cytotoxicity was examined in the human colon epithelial cells (CCD841 CoTr) by means of LDH assay. Flow cytometry and Western Blotting were applied to cell cycle analysis and protein expression involved in selected polysaccharide anticancer activity. siRNA technique together with BrdU assay were used to confirm discovered molecular targets, modulation of which are crucial for tested fraction anticancer action.
Chemical characterization revealed the presence of polisaccharides in all investigated fractions. *In vitro* studies (LDH, MTT, BrdU tests) have shown that five of six fractions isolated from *C. cibarius* were not toxic against CCD841 CoTr cells and elicited an antiproliferative effect on LS180 cells. The best properties revealed fraction CC5. Flow cytometry and Western Blotting have shown the CC5 induces cell cycle arrest in G0/G1-phase in LS180 cells by modulation of expression of cell cycle regulatory proteins (p21, p53, Cyclin D1, CDK4, CDK6, P-pRb). Alterations in cell cycle progression were primarily a result of enhancement of p53 and p21 expression, which was confirmed by silencing of genes coding above mentioned proteins.
Our results indicate that a polysaccharide fraction from *C. cibarius* possess an anticancer potential and may provide a new chemopreventive option against colon cancer.

Key words: *Cantharellus cibarius*; polysaccharides; cell cycle arrest; colon cancer cells

P22

Methoxy-stilbenes modulate the Ah receptor and cytochromes P450 expression in breast epithelial cells MCF10A

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Chemopreventive activity of resveratrol is limited because of its poor bioavailability. The variety of resveratrol analogues have been synthesized in order to improve its pharmacological parameters. Our earlier as well as the other authors` studies have shown that methylated analogues of resveratrol, particularly, 3,4,5,4'-*trans*-tetramethoxystilbene has the ability to exert stronger antiproliferative and proapoptotic activity than the parent compound. Estrogens are considered major risk factors in breast carcinogenesis partly as result of their metabolic activation by cytochromes P450.
The aim of this study was to evaluate the effect of three synthetic methoxy-stilbenes: 3,4,2'-trimethoxy-*trans*-stilbene (A), 3,4,2',4'-tetramethoxy-*trans*-stilbene (B), and 3,4,2',4',6'-pentametoxy-*trans*-stilbene (C) on the expression of Ah receptor and CYP1A1 and CYP1B1 involved in the estrogen metabolism in breast epithelial cells MCF10A. All three derivatives similarly to parent compound (resveratrol), showed the capacity to modulate AhR, CYP1A1, and 1B1 expression. The most significant changes were found in the case of AhR, which mRNA and protein levels, were significantly decreased as a result of treatment with resveratrol methoxy derivatives, while the highest dose of resveratrol significantly diminished only the AhR protein level. Compounds B and C in the two doses tested and resveratrol in the highest dose reduced also mRNA level of CYP1B1. Resveratrol in the dose of 5 μM and compound C in the dose of 1 μM decreased protein level of both tested CYPs which are controlled by AhR. These results indicate that there is no clear relationship between the level of AhR expression and the induction of CYPs 1A1 and 1B1. Similarly the number of methoxy groups introduced to stilbene structure is only partly related to the modulation of the evaluated parameters. Further studies, especially with breast cancer cell lines, are necessary to elucidate the precise molecular mechanism of methoxy-stilbene derivatives activity.

Key words: methoxy-stilbenes; AhR; CYP1A1; CYP1B1

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P23

Synthesis of novel piroxicam derivatives with anti-inflammatory activity as potent colorectal cancer chemopreventive agents

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Colorectal cancer (CRC) is one of the most common human malignancies in the Western World. Since chronic inflammation is now recognized as a potential risk factor for tumor development, targeting inflammatory pathways has proven effective in preventing formation of colonic tumors [1]. What is more, the prevention of cancer initiation may contribute not only to the downregulation of chronic inflammatory responses but also to the production of reactive oxygen species [2].
The trials of aspirin and COX-2 inhibitors showed that those medicines reduce the risk of CRC. Unfortunately, neither former nor latter may be used in chemoprevention because of an increased risk of vascular events (COX-2 inhibitors), or the greater risk of bleeding complications (aspirin) [3]. That is why other nonsteroidal anti-inflammatory drugs (NSAIDs) are examined for inhibiting the occurrence of CRC. Among the many NSAIDs studied as chemopreventive agents, there is a distinguished group of oxicams (e.g. piroxicam, meloxicam). We designed new piroxicam derivatives as potential multitarget drugs which would be an analgesic, anti-inflammatory and, at the same time, chemopreventive in cancer. The former preliminary experiments carried out on the few newly synthesized piroxicam derivatives revealed, that they reduce the level of the expression of COX-2, Bcl-2 protein and ABCG2 multidrug transporter in human colorectal adenocarcinoma cell line LoVo [4].
In the present work we describe the synthesis and the results of *in vitro* scavenging activity against DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl), as well as *in vivo* anti-inflammatory tests in mice, of three newly synthesized oxicame derivatives named BS17, BS20 and BS22. The results of our work revealed analgesic and anti-inflammatory activity of investigated compounds in the formalin test carried out on mice, as well as their antioxidant activity – which is also one of potential mechanism of chemoprevention – conducted via radical scavenging method measured by electron spin resonance (ESR).

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Key words: chemoprevention; anti-inflammatory; antioxidant; colorectal cancer; piroxicam

P24

Targeting cytochromes P450 family 1 by *trans*-resveratrol analogs – molecular modeling and docking studies

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Cytochrome P450 family 1 (CYP1) includes the isoforms: CYP1A1, CYP1A2 and CYP1B1, that metabolise endogenous and exogenous substrates. The enzymes are responsible for the phase I metabolism of procarcinogens, e.g. polycyclic aromatic hydrocarbons and aromatic and heterocyclic amines. Inhibition of their activities by natural compounds present in human diet constitutes one of the chemopreventive strategies, while in cancer therapy it may be helpful in avoiding drug resistance caused by the metabolism of chemotherapeutics catalysed by CYPs. *Trans*-resveratrol (3,4',5-trihydroxy-trans-stilbene) is a polyphenol found in grapes, berries and peanuts, showing well-characterized beneficial bioactivities. It efficiently inhibits CYP1B1 activity responsible for metabolism of 17-alpha-estradiol (E2) to highly mutagenic 4-hydroxy-E2.
We designed and synthesized a series of *trans*-stilbene derivatives possessing methoxy and methylthio functional groups attached in different positions to the *trans*-stilbene skeleton. The effects of synthesized compounds on the activities of human recombinant CYP1A1, CYP1A2 and CYP1B1 were determined. To explain the variation of inhibitory potency of methoxystilbene derivatives and their methyltio analogs computational analysis was employed. The compounds were docked to CYP1A1, CYP1A2 and CYP1B1 binding sites with the use of Accelrys Discovery Studio 4.0 by the CDOCKER procedure. Preferred orientations of ligands in enzyme active centers were determined. All compounds were relatively poor inhibitors of CYP1A2 that possess the most narrow and flat enzyme cavity among CYP1s. Affinity of 2,3',4'-trimethoxy-*trans*-stilbene, the most potent CYP1B1 inhibitor, was 100 times weaker for CYP1A1, however this significant inhibitory activity did not correlate with calculated binding energy to the active sites of enzymes. For the most active CYP1A1 inhibitors: 2-methytio-3',4'-dimethoxy-*trans*-stilbene and 2-methoxy-4'-methythio-*trans*-stilben the high number of molecular interactions was observed, however the interaction energies were not distinctive. Computational molecular docking studies are aimed on the further improvement of the virtual methods which may be used in the development of anti-cancer drugs targeting CYP enzymes.

Key words: cytochromes P450 family 1; *trans*-resveratrol analogs, enzyme inhibition

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P25

Metabolism of antitumour agent 1-nitroacridine derivative, C-1748 in pancreatic cancer cell lines

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Pancreatic cancer has the highest mortality rate of all major cancers because of limited treatment options. Surgical removal of the tumour is possible only in its early stage, nevertheless the asymptomatic development very often makes unable an accurate diagnose. In the case of metastatic pancreatic cancer only chemotherapy, mainly with gemcitabine, can be offered to patients. However common resistance towards gemcitabine imposes the research for new active compounds.
The compound 9-(2'-hydroxyethylamino)-4-methyl-1-nitroacridine, C-1748 is the most potent antitumour derivative among 1-nitroacridines developed in our department. It possesses strong cytotoxic activity against colon and prostate cancer cells what allowed its selection for preclinical studies. Preliminary results indicate therapeutic potential of C-1748 against pancreatic cancers.
The cytotoxic effect of an antitumour agent often depends on its metabolism leading to the activation or detoxification of the parent compound. The metabolism of C-1748 with human liver microsomes, human P450 reductase and in HepG2 cells was well studied and structures of four metabolites were identified.
The aim of the current study is to investigate the metabolism of C-1748 in pancreatic cancer cell lines: Panc-1, MiaPaCa-2 and BxPC-3, which show different expression of cytochrome P450 isoenzymes. Both: medium and cellular extracts were analyzed by HPLC/UV-Vis after drug treatment of the cells. A few metabolites were accumulated inside the cells whereas two products were found in the medium; but the metabolic profiles depended on the type of pancreatic cell line. One of the metabolite observed in all cancer cells was identified as the acridine derivative with an additional 6-membered ring.
In conclusion, P450 mediated metabolism of C-1748 in pancreatic cancer cell lines strongly influenced its cytotoxicity. Moreover, C-1748 as a substrate for P450 isoenzymes in pancreatic cancer cells and a modulator of UGT activity, could be responsible for drug-drug interactions in the combined therapy.

Key words: pancreatic cancer; antitumour agent; drug metabolism

P26

Edible mushrooms as a source of chemopreventive beta-glucans

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Polysaccharides, including the beta-glucans, are the best known and most potent mushroom derived compounds with chemopreventive activity. These compounds have been reported to demonstrate immunomodulating, anti-inflammatory, antioxidant, hypocholesterolemic and antidiabetic effect. Since the strong relationship has been demonstrated between inflammation and cancer, the role of beta-glucans in reducing the risk of inflammation-associated tumor development is crucial. Mushroom polysaccharides do not attack cancer cells directly, but mostly by activating different immune responses in the host. They are known to stimulate natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses. Beta-glucans have been described as modulators of both humoral and cellular immunity. They were also shown to exert beneficial effects in pre-inflammatory responses, indicating that beta-glucan can be a modulator of the anti-inflammatory response.
The medicinal properties of mushroom glucans depend on their structure, molecular weight, polymeric backbone, sugar composition and their degree of branching. Polysaccharides with antitumor properties have been screened mostly in the fruiting bodies; therefore the aim of our study was the evaluation of the amount of beta- glucans in different edible mushroom species (*Armillaria mellea, Cantharellus cibarius, Rozites caperatus, Suillus bovinus, Sparassis crispa*) growing wild in Poland. The content of beta-glucans in the polysaccharide extracts was determined using the Mushroom and Yeast β-glucan Assay Procedure (Megazyme Int.). Our results indicate that investigated species constitute an interesting source of beta-glucans.
The immunomodulating properties of beta-glucans are especially valuable as a means of prophylaxis, a mild and non-invasive form of treatment, prevention of metastatic tumors, as well as in supporting the treatment of cancer patients submitted to chemotherapy, to improve immunologic status and reduce untoward effects on normal tissues.

Key words: mushrooms; beta-glucans; anti-inflammatory

P27

Phospholipids as potential prevention factor in carcinogenesis

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Phospholipids are a group of complex lipids, which can be used in cancer chemoprevention. Glicerophospholipids are composed of fatty acids esterified to a glycerol backbone, a phosphate group and a hydrophilic residue such as: choline, ethanoloamine, serine or inositol. In the *sn-2* position of glicerophospholipid usually there are unsaturated fatty acids, i.e. linolenic acid, while in the *sn-1* position more typical are saturated fatty acids, i.e. palmitic acid. The second group of phospholipids are sphigophospholipids, in which amino-alcohol is build in instead of glycerol. For these two groups of phospholipids have been described preventive activities of carcinogenic processes. Fatty acids from n-3 family, such as eicosapentaenoic or docosahexaenoic acids, which exhibit anti-inflammatory properties are better transported within human body as glicerophospholipids than as free fatty acids. Phospholipids probably have also inhibitory effect on metastasis of tumor cells. This fact is related to the construction of the cancer cell membrane where lipid rafts occur in high concentration. Cancer patients often take non-steroidal anti-inflammatory drugs to decrease pain. Phospholipids can reduce negative impact of these drugs preventing gastrointestinal side effects. Health-promoting phospholipids can be found in some foodstuffs. High content of these lipids occurs in marine products, egg yolk, soybean products or milk. Phospholipids are now widely marketed in the form of dietary supplements. Because of the still unrecognized mechanism of action, as well as low toxicity and widespread occurrence in food, chemopreventive potential of phospholipids deserve more detailed investigations.

Key words: phospholipids; functional food; cancer prevention

P28

Chemopreventive potential of roses

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Carcinogenesis is a sum of alterations in a number of cellular processes and inefficient activity of repair mechanisms. These irregularities are often accompanied by pathogenic conditions, such as oxidative stress, immune deficiency and chronic inflammation. Many epidemiological studies have shown a reduced risk of incidence of many cancers in individuals with the diet rich in fruits and vegetables. Numerous plant compounds have been reported to demonstrate selective activity, destroying cancer cells without damaging normal cells. Moreover, phytochemicals can exert their potential effects at each stage of carcinogenesis. Therefore, plant secondary metabolites are of unflagging interest in chemopreventive studies.

The genus Rosa comprises approximately 200 shrub species distributed widely throughout the temperate and subtropical habitats. Previous research has revealed the presence of many biologically active compounds in different parts of roses. Many of them act as potential natural antioxidant, cytotoxic, radioprotective and anti-inflammatory agents able to affect different stages of tumor formation. Therefore, we have decided to conduct multidirectional analysis of chemical composition and biological activity of different rose extracts, to determine their potential application as chemopreventive products.

We have evaluated rose extracts for their cytotoxic effect against cervical (HeLa) and breast cancer (T47D) cell lines. Moreover, antiradical activity (with DPPH and ABTS) and ferric reducing antioxidant power was assayed. In phytochemical studies liquid chromatography–electrospray ionization–tandem mass spectrometry and spectrophotometric methods were used. As a result, significant and selective cytotoxic and antiradical properties were demonstrated. High contents of phenolic compounds, particularly of phenolic acids, flavonoid glycosides and tannins were also revealed.

Key words: *Rosa rugosa*; polyphenols; antioxidant

P29

Report on the status of the Programme for disposal of asbestos and asbestos-containing products used in Poland and Programme for Asbestos Abatement in Poland in the area of exposure assessment and health protection

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Asbestos is the generic commercial designation for a group of naturally occurring mineral silicate fibres of the serpentine and amphibole series. Asbestos exposure can cause serious health problems leading to numerous diseases such as asbestosis, plural plaques, lung cancer, mesothelioma (considered to be specific to exposure to asbestos) or other cancers of the esophagus, larynx, oral cavity, stomach, colon and kidney. The use of asbestos-containing products was banned in the European Union and according to Ordinance of the Minister of Economy of 13 December 2010, the usage of asbestos containing products in Poland should be abated by the end of 2032. The abatement process is being led under the auspices of the Ministry of Economy with the implementation of the Programme for Asbestos Abatement in Poland 2009–2032. In Poland, after asbestos was banned, the problem focused on monitoring the health of workers with prior exposure to this substance and those currently involved in the demolition of asbestos-containing buildings and facilities and in asbestos removal tasks. In 2001 the Ministry of Health planned a health surveillance program „Amiantus" for workers previously exposed to asbestos. In 2013, approximately 7000 workers of which 63% were male, previously exposed to asbestos from 28 companies were included in the program. In the period 2004–2013 activities relating to health care, including measurements of asbestos fibres concentration in the air, and assessing health risks for people, were conducted. Together, these years measures were carried out in 949 gminas located in 296 poviats, which accounts for 41% of the country. As a result of the report, the area of the country was divided into zones with varying degrees of risks of asbestos related disease based on registered cases of diseases resulting from exposure to asbestos dust. In the zone, in Mazowieckie, Malopolskie and Lubelskie administrative areas 57% of all diagnosed asbestos related diseases were reported

Key words: asbestos; lung cancer; mesothelioma; asbestos fibres

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P30

Synergistic effect of isothiocyanates and 4-hydroxytamoxifen in breast cancer cells is associated with apoptosis induction

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Breast cancer is the most common cancer in women worldwide. The most commonly used drug for the treatment of both, early and advanced estrogen receptor positive breast cancer in pre- and post-menopausal women, is tamoxifen. The active metabolite of tamoxifen, 4-hydroxytamoxifen, binds to estrogen receptors (ER) causing a blockage of estrogen-stimulated proliferation, migration and invasion of breast cancer cells. However, long term tamoxifen therapy has been associated with the occurrence of serious side effects, such as endometrial cancer, polyps and ovarian cysts. Moreover, the benefits of this therapy are limited due to the development of tamoxifen resistant cells, which is associated with tumor recurrence and poor prognosis.
The purpose of our study was to investigate whether the combination of 4-hydroxytamoxifen with isothiocyanates, such as sulforaphane or erucin, will potentiate apoptosis induced by either agent alone. Numerous studies show that these isothiocyanates remarkably decrease viability of different types of cancer cells, which is associated with apoptosis induction. Our results indicate that investigated isothiocyanates and 4-hydroxytamoxifen in a synergistic way decrease viability of ER-positive breast cancer cell lines (MCF-7 and T47D), as well as their 4-hydroxytamoxifen-resistant derivatives (MCF-7-TAM-R and T47D-TAM-R). The combination of 4-hydroxytamoxifen and sulforaphane or erucin results in an increased PARP cleavage as compared with the single agent treatment. Moreover, intrinsic pathway is involved in co treatment-induced apoptosis, which is associated with the increased levels of pro-apoptotic Bax and decreased levels of anti-apoptotic Bcl-2 and survivin. In addition, combinations of 4-hydroksytamoxifen at low concentration with sulforaphane or erucin efficiently inhibits clonogenic potential of breast cancer cells. In conclusion, our data indicate that sulforaphane or erucin, used in relatively low concentrations, potentiate anticancer activity of 4-hydroxytamoxifen. This strategy might allow for using tamoxifen at lower doses, hence decreasing the level of its toxicity and improving the risk-benefit profile of this agent.

Key words: 4-hydroxytamoxifen; isothiocyanates, breast cancer

P31

The impact of S6 kinases on autophagosomes maturation during the process of autophagy induced by sulforaphane, a chemopreventive and anticancer agent

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Sulforaphane (SFN), an anticancer agent derived from cruciferous plants, induces apoptosis in numerous cancer cell lines. It also induces process of autophagy, which serves to protect cells against death, thus decreases SFN anticancer efficacy.

Autophagy is an evolutionarily conserved process of degradation of long-lived proteins and entire organelles. The main regulator of autophagy is mTORC1 complex. The role of mTORC1 substrates, S6K1 and S6K2 kinases, in autophagy is still controversial.

Our results showed that sulforaphane induces autophagy in prostate cancer cells which is associated with S6K1 inactivation. To specify the role of S6K1/2 kinases in SFN-induced autophagy we used the wild-type (WT) and S6Ks double knock-out (DKO) mouse embryonic fibroblasts (MEFs). Immunodetection of LC3-II protein, a marker of autophagy, revealed that the induction of autophagy after SFN treatment occurs in both cell lines but the level of LC3-II protein is significantly higher in MEF DKO compared to the wild type cells. As one of the reasons could be its diminished degradation by lysosomes we measured autophagy flux in SFN-treated cells. Indeed, in SFN-treated MEFs DKO autophagy flux was impaired, which indicates defects in lysosomal degradation of autophagosomes in cells lacking S6Ks. As literature data showed participation of acetylated tubulin in autophagosomes-lysosomes fusion, we measured its level in WT and DKO MEFs and observed reduction in tubulin acetylation in the MEF DKO. Exogenous expression of S6K1 in DKO MEFs increased the level of acetylated tubulin. To verify the role of acetylated tubulin in autophagy flux in S6K knock-out cells, they were transfected with plasmids encoding the tubulin acetylation-mimicking variant or control vector. Expression of acetylation-mimicking tubulin restored autophagy flux in S6K knock-out cells upon SFN treatment.

Summarizing, obtained results show that S6Ks are necessary for proper lysosomal degradation of autophagosomes during SFN-induced autophagy by regulating acetylation of tubulin.

Key words: autophagy; S6 kinases; sulforaphane

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P32

Design of BTLA inhibitors as new drugs against melanoma

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Metastatic melanoma is responsible for 90% of deaths by skin cancers, because of poor prognosis with less than 10% of patients surviving after 10 years. Recently, immunotherapies against melanoma have demonstrated promising results, but further progress is still necessary. It was shown that BTLA (*B- and T-lymphocyte attenuator*) is involved in the negative regulation of T cell responses in cancer patients and can be targeted by immunotherapy. BTLA binds to a member of the TNF receptor superfamily, herpes virus entry mediator (HVEM) [1].

The main goal of the project is to design efficient and selective BTLA blockers that prevent its interactions with the HVEM protein. In our research we determined whether a fragment of the HVEM protein (14-39) and its variants can inhibit the interaction between BTLA and HVEM. We will describe chemical synthesis of peptides and biological evaluation.

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Key words: melanoma; drug design; BTLA; HVEM; peptides.

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P33

Correlation between central carbon metabolism and DNA replication in human fibroblasts

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DNA replication is an essential processes in every cellular organism. Its precise regulation is crucial for adequate inheritance of the genetic material by daughter cells, and thus, proper functions of cells and organisms. In our research, we aimed to answer the question whether silencing of the TCA (tricarboxylic acid cycle) or PPP (pentose phosphate pathway) genes affect DNA replication in normal cells (HDFa, human dermal fibroblasts isolated from adult skin). Using specific siRNAs, we silenced the aconitase 2 (*ACO2*), isocitrate dehydrogenase 2 (*IDH2*), malate dehydrogenase 2 (*MDH2*), transketolase (*TKT*), hexose-6-phosphate dehydrogenase (*H6PD*), deoxyribose-phosphate aldolase (*DERA*) genes. The level of gene silencing, evaluated by the real time PCR, reached 90–95%. Impact of TCA gene downeregulation on DNA replication was evaluated using flow cytometry. Obtained results showed that silencing of the specific TCA genes results in dysregulation of DNA replication. Depending on the silenced gene, we observed either an increase or a decrease in the fraction of cells reaching the S phase of the cell cycle, relative to the control. These results might be important for studies on cancer cell biology as cancer cell survival strongly depends on carbon metabolism.

Key words: DNA replication; TCA; PPP; tricarboxylic acid cycle; pentose phosphate pathway

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P34

New 1,2-benzothiazine 1,1-dioxide derivatives: synthesis of potential chemopreventive agents with analgesic activity

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Derivatives of 1,2-benzothiazine 1,1-dioxide are known for their wide range of properties, such as antibacterial, antidiabetes, antioxidant, anti-inflammatory, analgesic and neuroprotective [1-5]. The most well-known 1,2-benzothiazines are the *oxicams*, for example *piroxicam*, *meloxicam*, *sudoxicam* or *cinnoxicam* from the group of non-steroidal anti-inflammatory drugs (NSAIDs). Since the discovery of the connection between inflammation and cancer, NSAIDs as anti-inflammatory drugs are extensively studied as chemopreventive agents [6]. However, NSAIDs are not free of strong side effects such as gastrotoxicity, nephrotoxicity or cardiovascular events.

Because of this scientists still are looking for new chemical structures more potent and less toxic. 1,2-benzothiazine derivatives are a promising group of compounds and some of them show strong analgesic, anti-inflammatory and at the same time chemopreventive properties [7].

In our work we have synthesized a group of compounds, 1,2-benzothiazine 1,1-dioxide derivatives, starting from commercially available 1,1-dioxo-1,2-benzothiazol-2-one (saccharine). Saccharine was condensed with corresponding 2-bromoacetophenones in DMF and triethylamine. The next step was *Gabriel-Colman* rearrangement through opening the 1,2-thiazole ring and closing with the formation 1,2-thiazine ring. Target compounds were obtained by condensation corresponding 1,2-benzothiazine with 1-(3-chloropropyl)-4-(*o*/*m*-substituted-phenyl)piperazine in the presence of sodium ethanolate.

New compounds were studied in formalin test on mice and revealed strong analgesic and anti-inflammatory properties. They were 2-4 times more potent than the reference drug – *piroxicam*. What's more, they were active both in the first and the second phase of the formalin test which may suggest mixed – peripheral and central mechanism of action, but this requires further studies. As the next step we plan to study their chemopreventive activity such as inhibition of efflux pomps (P-gp, MRP1 and BCRP), influence on apoptotic proteins (Bax, Bcl-2) and influence on lipid bilayers.

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Key words: 1,2-benzothiazine; synthesis; oxicams; cancer; chemoprevention; analgesic

P35

Piroxicam derivatives as inhibitors of human adenocarcinoma cancer cells growth and apoptosis-inducing agents

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Inflammation is considered a hallmark of cancer. Amongst the different mediators of inflammation, the cyclooxygenases (COXs) clearly appear to be implicated in cancer. This family contains three members: ubiquitously expressed COX-1, the inducible COX-2 isoform and COX-3. There is compelling evidence supporting a role for COX-2 in tumour development. COX-2 expression has been shown to be elevated in several human tumours, including colorectal. Nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase (COX)-2 inhibitors, have promise as anticancer agents. Several studies have shown that specific COX-2 inhibitors can prevent or reduce the development of chemoresistance phenotype by downregulation of the expression and function of P-glycoprotein (ABCB1). Pgp which is encoded by MDR1 gene, confers resistance to certain anticancer agents and remained a major reason for failure of cancer therapy.

In our studies novel series of potentially biologically active 1,2-benzothiazine 1,1-dioxides analogs of piroxicam were subjected to evaluation for their ability to modulate MDR mediated by P-glycoprotein. Piroxicam is a non-steroidal anti-inflammatory drug of the oxicam class. It was shown that piroxicam derivatives inhibit adenocarcinoma cells growth and are able to induce apoptosis in a dose-dependent manner. The QSAR methods allowed us to describe electronic, structural and topological parameters and hydrophobicity of new compounds and to correlate these properties with their anti-proliferative and pro-apoptotic activity. Piroxicam derivatives treated cells exhibited cleavage of caspase-3 and modulation of apoptotic proteins, establishing apoptosis as the primary mechanism of cell death. A positive correlation between the expression of COX-2 and P-gp was also shown in our studies.

P36

Effect of sulforaphene on proliferative potential of breast cancer cells

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World epidemiological studies indicate that breast cancer is the most common malignancy among women, taking first position in the global statistics. It is also the most common cause of deaths from cancer among women. The high mortality rate is related to the relatively low efficacy of treatment with radiation therapy and adjuvant systemic therapy. The negative aspects of currently used treatments have contributed to the search for safe compounds of a natural origin which can be used in the prevention and treatment of cancer.

Sulforaphene [4-isothiocyanato-1-methylsulfinylbut-1-ene] is derived from glucoraphanin, which is the main glucosinolate in radish (*Raphanus sativus* L.). Scientific studies indicate that sulforaphane (a structural analog of sulforaphene), shows chemopreventive and anti-tumor activity in *in vivo* and *in vitro* models. Currently, little is known about anticancer activity of sulforaphene. There are many different types of cancer but all share common hallmarks, such as: an insensitivity to growth-inhibitory signals and evasion of programmed cell death. Therefore, the purpose of our study was to investigate whether sulforaphene: i. is a potential growth inhibitor of phenotypically different breast cancer cell lines, ii. induces apoptosis in breast tumor cells, iii. decreases clonogenic potential of two breast cancer lines: triple-negative MDA-MB-231 and SKBR-3 that overexpress HER-2 receptor. The results obtained indicate that sulforaphene decreases viability of both analyzed cell lines in a dose-dependent manner and causes apoptosis-related degradation of PARP polymerase. Moreover, analyzed phytochemical reduces clonogenic potential of these two tumor cell lines. In addition, sulforaphene at low concentrations does not affect the viability of the MCF-10A, non-tumorigenic human breast cells.

In conclusion, sulforaphene exhibits promising characteristics of anticancer and chemopreventive agent.

Key words: isothiocyanate, sulforaphene, apoptosis, breast cancer

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P37

Biochemical characterization of human HtrA3 protease functioning as a tumor suppressor

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HtrA3 belongs to the HtrA (*High temperature requirement A*) family of serine proteases, whose main function is to maintain protein quality control. Generally, the HtrA proteins are composed of the protease (PD) and PDZ domains, the latter functioning in substrate binding and activity regulation. HtrA3 is present in mammalian cells as two isoforms: the long – HtrA3L and the short – HtrA3S, resulting from alternative mRNA splicing. The HtrA3S lacks PDZ domain which is replaced by seven C-terminal amino acid residues, unique for this variant.

HtrA3 is involved in oncogenesis. The *HtrA3* gene expression is reduced in ovarian, endometrial and lung cancers. The decrease in *HtrA3* expression in ovarian cancer is correlated with the degree of malignancy. HtrA3 promotes the induction of the intrinsic, mitochondria-mediated apoptotic pathway. Upon cancer cell treatment with cytotoxic agents, HtrA3 is released from mitochondrium into the cytosol, where it triggers apoptosis *via* its serine protease activity. Thus, it is considered to be a tumor suppressor and a potential therapeutic target in cancer treatment.

We solved the crystal structure of HtrA3L, showing that it forms a trimer with PD similar to those of human HtrA1 and HtrA2. Location of the PDZ domain versus PD is different from that of HtrA1 and HtrA2, and PDZ influences trimer formation. We assayed proteolytic activity of the HtrA3L, HtrA3S and HtrA3ΔPDZ proteins, using β-casein and the fluorogenic peptide Ala(Mca)IRRVSYSF-NH2 as model substrates. The kinetic values of HtrA3 were similar to those observed previously for HtrA2. However, contrary to HtrA2, the HtrA3 kinetics did not indicate the presence of allosteric regulation. Our data indicate that PDZ domain is dispensable for HtrA3 proteolytic activity, and that the unique C-terminal sequence of HtrAS does not significantly influence activity. Our results provide new insights into the structure and function of HtrA3.

Key words: HtrA3; human serine proteases; enzymatic kinetics; apoptosis

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P38

The role of the sewage treatment plant in the removal of genotoxic compounds from the environment

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One reason for increased incidence of various types of cancer are environmental pollutants particularly of anthropogenic origin. All human activities impact the environment, but some have significant strength, just to mention energy industry, mining, transportation, chemical, petrochemical or housing.

Environmental stressors are a large group of compounds in terms of their physicochemical properties, some of which may disturb the biochemical processes taking place in the living organism causing, among others, genotoxic effects.

Surface waters contaminated by urban and industrial effluents are becoming a wide reservoir of these compounds, and the current wastewater treatment technologies are not always able to meet this challenge.

Research conducted in the Department of Analytical Chemistry in collaboration with the Department of Food Chemistry, Technology and Biotechnology were focused on the determination of genotoxic potential of wastewater samples. Samples of influent and effluent wastewater were collected in 2013 from all the major sewage treatment plants across Poland.

Genotoxic potential of the samples was analyzed by comet assay, using a HT29 cell line. Results obtained for the influent samples ranged from 5.91 to 44.44 percent of DNA in tail of the "comet", and for those collected in the effluent form 5.32 to 50.96 percent of DNA.

Comparing the results obtained for samples of wastewater there was an apparent decrease in genotoxicity for effluent samples, however, in a few cases, an increase of genotoxicity was observed for samples collected after the sewage treatment plant. Such cases can attest to the fact that some of the wastewater treatment techniques do not appear to be sufficient to remove compounds that could damage DNA and contribute to many cancer cases.

Key words: genotoxicity; wastewater samples, comet assay

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P39

Changes in iron metabolism in prostate cancer cells treated with diallyl trisulfide

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Iron is responsible for most of cellular processes, including DNA synthesis, cellular respiration, collagen synthesis and many others. In recent years, signaling role of iron became recognized as it can modulate activity of several proteins by generating reactive oxygen species. Among amino acids in the proteins, cysteine has a very high affinity for free iron. Iron bound to cysteine sulfydryl may give rise to hydroxyl radical formation and subsequent reaction with cysteine. Therefore, protein modification and signaling initiated by ROS may be significantly different depending on the availability of chelatable iron.

The main goal of the present study was to evaluate the effects of diallyl trisulfate, an anticancer compound derived from garlic, on iron metabolism in prostate cancer cells. Our data clearly indicate that the rise in labile pool of iron, which is the results of ferritin degradation mediated by JNK/p66Shc/Itch signaling pathway, leads to adaptive response which is manifested by regain of Akt activity and ferritin protein level in PC-3 cell treated with DATS. In conclusion, our data suggest that iron plays important role as a signaling molecule and reveal that this knowledge can be crucial in understanding resistance of cancer cells to chemotherapy.

Key words: iron; ferritin; garlic; Akt