CANCER RESEARCH INSTITUTE, SLOVAK ACADEMY OF SCIENCES AND SLOVAK CANCER RESEARCH FOUNDATION

NATURAL COMPOUNDS IN CANCER PREVENTION AND TREATMENT

BOOK OF ABSTRACTS

OCTOBER 13-15, 2009, SMOLENICE CASTLE, SLOVAKIA

INTRODUCTION

The health-beneficial effects of plants or their extracts and chemical structure diversity represent the fount of inspiration for new ways in cancer chemoprevention and treatment. The conference is dedicated to commemorate the 50th anniversary of pioneering work of prof. Drobnica's team in the field of cancerostatic effects of natural and synthetic isothiocyanates, the results of which were published in Neoplasma journal.

The goal of the proposed conference is to bring together European food engineers, epidemiologists, cancer researchers and clinicians to explore new utilization of plant agents in cancer prevention and their possible involvement in future treatment protocols.

SCIENTIFIC AND ORGANIZING COMMITTEE

Sedlak Jan , Slovak Republic
Mithen Richard , United Kingdom
Hunakova Luba, Slovak Republic
Halkier Barbara Ann, Denmark
Gerhaeuser Clarissa, Germany
Duraj Jozef, Slovak Republic
Collins Andrew, Norway

SCHEDULE

TUESDAY 13 OCT 2009

10:00-12:00 Walking City Tour through Old Town of Bratislava
Departing from Hotel Carlton - Hviezdoslavovo nám. 3, 811 02 Bratislava.

12:00-14:00 Bus transfer to conference venue (Smolenice Castle)

12:00 departing from hotel Carlton - Hviezdoslavovo nám. 3, 811 02 Bratislava
12:20 Bus transfer stops at Main Train Station of Bratislava - Predstaničné námestie 1, 811 01 Bratislava
12:40 Bus transfer stops at Main Bus Station Mlynské Nivy - Mlynské Nivy 31, 821 09 Bratislava
13:00 Bus transfer to Smolenice Castle stops at Bratislava International Airport - Letisko M. R. Štefánika, 823 11 Bratislava 22
14:00 Arrival to Smolenice Castle

14:00-16:00 Lunch, registration, check in

16:00-17:30 Opening remarks & The ITC research in Slovakia

- **Coevals of prof. Drobnica**, The History of ITC Research in Slovakia: The Fifties of the Last Century.
- **Jan Sedlak, director of Cancer Research Institute SAS**, The Last 10 years of ITC research in Slovakia.
- **Jana Jakubikova**, Dana-Farber Cancer Institute, Boston, USA, Cancer Research Institute SAS, Bratislava, Slovakia: Isothiocyanates Exert Anti-myeloma Activity and Enhance Therapeutic Cytotoxicity.

17:30-18:00 Coffee break

18:00-19:40 Section V Intervention Studies and Functional Food

- **18:00-18:45** **Richard Mithen**, IFR Norwich, UK: Human Intervention Studies with Broccoli and the Mode of Action of Sulforaphane in Vivo.
- **18:45-19:00** **Dana Cholujova**, Cancer Research Institute SAS, Bratislava, Slovakia: BioBran Modulates the Innate Immune System Functions: Multiple Myeloma Patients' Study.
- **19:00-19:15** **Emil Tomasik**, INFREDPharm, Slovakia: The Perspective Use of the Preparation Mellozan in Oncology.
- **19:15-19:30** **Kean Ashurst**, Sulforaphane (ITC's) Applications for Improved Health - Yesterday! – Today! – Tomorrow!

20:00- Welcome party Smolenice Castle

WEDNESDAY 14 OCT 2009

8:00-9:00 Breakfast

9:00-10:45 Section I Chemoprevention, chemoprotection

- **9:00-9:45** **Clarissa Gerhauser**, German Cancer Research Center, Heidelberg, Germany: Prostate Cancer Preventive Potential of Cruciferous Vegetables.
- **9:45-10:00** **Yongping Bao**, University of East Anglia, Norwich, UK: Novel Targets of Dietary Isothiocyanates in Cancer Prevention.
- **10:00-10:15** **Katarzyna Skupinska**, National Medicines Institute, Warsaw, Poland: Isothiocyanates as Chemopreventive Agents Against Polycyclic Aromatic Hydrocarbons.
- **10:15-10:30** **Monika Kassayova**, Melatonin in the Prevention of Experimental Mammary Carcinogenesis.
- **10:30-10:45** **Rudolf Štětina**, The Influence of Antioxidants (Ellagic Acid, Epigallocatechin Gallate) on the Removal of DNA Damage Induced With Different Chemical Mutagens.

9:45-11:15 Coffee break

11:15-12:45 Section III Biomarkers and Epidemiology

- **11:15-12:00** **Andrew Collins**, Department of Nutrition, University of Oslo, Norway: Non-antioxidant Effects of Antioxidants.
- **12:00-12:15** **Jian-Min Yuan**, University of Minnesota, MN, USA: Isothiocyanates and Cancer Prevention – are we ready to move forward from observational studies to large clinical trials?
- **12:15-12:30** **Kristin Moy**, University of Minnesota, MN, USA: A Prospective Study of Urinary Biomarker for Isothiocyanates and Risk of Gastric Cancer in Shanghai, China.
- **12:30-12:45** **Katarina Volkovova**, Oxidative Damage and Antioxidant Protection in Relation to Ageing.

13:00-14:00 Lunch

14:00-15:30 Section IV Plant Biochemistry/Biotechnology and Organic Chemistry

- **14:00-14:45** **Michael Dalgaard Mikkelsen**, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark: Engineering Benzylglucosinolate into Tobacco.
- **14:45-15:00** **Eva Cellarova**, Faculty of Science, UPJS, Kosice, Slovakia: Biotechnological Alternative in Production of Bioactive Substances in the Genus Hypericum.

- **15:00-15:15** **Jan Imrich**, Institute of Chemistry, Faculty of Science, UPJS, Kosice, Slovakia: Synthesis and Antitumor Activity of Compounds Based on 9-Isothiocyanatoacridine.
- **15:15-15:30** **Vladimir Frecer**, Cancer Research Institute SAS, Bratislava, Slovakia: Computer-assisted Combinatorial Drug Design.

15:45 **Departure of sightseeing trip to Červený Kameň Castle (Red Stone Castle)**

16:30-18:00 **Visit of the Červený Kameň Castle**

18:00 **Departing from Červený Kameň Castle to Fugger Manor in Častá Village**

18:30-19:00 **Wine tasting in vine cellars of Fugger Manor**

19:00-21:00 **Farewell dinner in vine cellars of Fugger Manor House**

21:00 **Departure to Smolenice Castle**

21:30 **Poster session**

THURSDAY 15 OCT 2009

8:00-9:00 **Breakfast, check out**

9:00-10:00 **Section II Treatment and Signaling Pathways**

- **9:00-9:30** **Jan Sedlak**, Cancer Research Institute SAS, Bratislava, Slovakia: Exploiting ITC-modulated Signaling Pathways in Cancer Therapy.
- **9:30-9:45** **Peter Ferenc**, Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: Down-regulation of BCL-2 and AKT Induced by Combination of Photoactivated Hypericin and Genistein in Human Breast Cancer Cells.
- **9:45-10:00** **Jozef Duraj**, Cancer Research Institute SAS, Bratislava, Slovakia: Administration of Isothiocyanate (E-4IB) and Cisplatin Leads to Altered Signalling and Lysosomal Export in Human Ovarian Carcinoma Sensitive and Cisplatin-Resistant Cells.

10:00-10:30 **Coffee break**

10:30-12:00 **Section II Treatment and Signaling Pathways (Cont.)**

- **10:30-10:45** **Antonietta Melchini**, University of Messina, Italy: Selective Antiproliferative Activity in Vitro of Structurally Related Isothiocyanates, Erucin and Sulforaphane, on Human Prostate Cells.
- **10:45-11:00** **Eva Horvathova**, Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute SAS, Bratislava, Slovakia: Effects of Carvacrol and Rosemary Oil Supplementation on Oxidative DNA Damage Induced in Primary Rat Hepatocytes by 2,3-dimethoxy-1,4-naphthoquinone (DMNQ).
- **11:00-11:15** **Veronika Sackova**, Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: Manumycin A as Agent Sensitizing Adenocarcinoma Cell to Photodynamic Treatment.
- **11:15-11:30** **Peter Solar**, Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: HSP90 Protein as a Target for Improving Photodynamic Therapy.
- **11:30-11:45** **Jaromir Mikes**, Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: The Role of Protein P53 in Photodynamic Therapy with Hypericin.
- **11:45-12:00** **Martin Kello**, Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: Potentiation of Photodynamic Therapy with Hypericin by Exogenous Polyunsaturated Fatty Acids.

12:00 **Closing remarks**

13:00-14:00 **Lunch**

14:00-15:50 **Bus transfer departure from Smolenice Castle to Bratislava**

LECTURES

- **L1 Jana Jakubikova**, Dana-Farber Cancer Institute, Boston, USA, Cancer Research Institute SAS, Bratislava, Slovakia: Isothiocyanates Exert Anti-myeloma Activity and Enhance Therapeutic Cytotoxicity.
- **L2 Richard Mithen,** IFR Norwich, UK: Human Intervention Studies with Broccoli and the Mode of Action Of Sulforaphane in Vivo.
- **L3 Dana Cholujova,** Cancer Research Institute SAS, Bratislava, Slovakia: BioBran Modulates the Innate Immune System Functions: Multiple Myeloma Patients' Study.
- **L4 Emil Tomasik,** INFREDPharm, Slovakia: The Perspective Use of the Preparation Mellozan in Oncology.
- **L5 Kean Ashurst,** Sulforaphane (ITC's) Applications for Improved Health - Yesterday! – Today! – Tomorrow!
- **L6 Clarissa Gerhauser,** German Cancer Research Center, Heidelberg, Germany: Prostate Cancer Preventive Potential of Cruciferous Vegetables
- **L7 Yongping Bao,** University of East Anglia, Norwich, UK : Novel Targets Of Dietary Isothiocyanates in Cancer Prevention
- **L8 Katarzyna Skupinska,** National Medicines Institute, Warsaw, Poland: Isothiocyanates as Chemopreventive Agents Against Polycyclic Aromatic Hydrocarbons.
- **L9 Monika Kassayova,** Melatonin in the Prevention of Experimental Mammary Carcinogenesis.
- **L10 Rudolf Štětina,** The Influence of Antioxidants (Ellagic Acid, Epigallocatechin Gallate) on the Removal of DNA Damage Induced With Different Chemical Mutagens.
- **L11 Andrew Collins,** Department of Nutrition, University of Oslo, Norway: Non-antioxidant Effects of Antioxidants.
- **L12 Jian-Min Yuan,** University of Minnesota, MN, USA: Isothiocyanates and Cancer Prevention – Are We Ready to Move Forward from Observational Studies to Large Clinical Trials?
- **L13 Kristin Moy,** University of Minnesota, MN, USA: A Prospective Study of Urinary Biomarker For Isothiocyanates and Risk of Gastric Cancer in Shanghai, China.
- **L14 Katarina Volkovova,** Oxidative Damage and Antioxidant Protection in Relation to Ageing.
- **L15 Michael Dalgaard Mikkelsen,** Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark: Engineering Benzylglucosinolate into tobacco.
- **L16 Eva Cellarova,** Faculty of Science, UPJS, Kosice, Slovakia: Biotechnological Alternative in Production of Bioactive Substances in the Genus Hypericum.
- **L17 Jan Imrich,** Institute of Chemistry, Faculty of Science, UPJS, Kosice, Slovakia: Synthesis and Antitumor Activity of Compounds Based on 9-Isothiocyanatoacridine.
- **L18 Vladimir Frecer,** Cancer Research Institute SAS, Bratislava, Slovakia: Computer-assisted Combinatorial Drug Design.

- **L19 Jan Sedlak,** Cancer Research Institute SAS, Bratislava, Slovakia: Exploiting ITC-modulated Signaling Pathways in Cancer Therapy.
- **L20 Peter Ferenc,** Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: Down-regulation of BCL-2 and AKT Induced by Combination of Photoactivated Hypericin and Genistein in Human Breast Cancer Cells.
- **L21 Jozef Duraj,** Cancer Research Institute SAS, Bratislava, Slovakia: Administration of Isothiocyanate (E-4IB) and Cisplatin Leads to Altered Signalling And Lysosomal Export In Human Ovarian Carcinoma Sensitive and Cisplatin-Resistant Cells.
- **L22 Antonietta Melchini,** University of Messina, Italy: Selective Antiproliferative Activity in Vitro of Structurally Related Isothiocyanates, Erucin and Sulforaphane, on Human Prostate Cells.
- **L23 Eva Horvathova,** Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute SAS, Bratislava, Slovakia: Effects of Carvacrol and Rosemary oil Supplementation on Oxidative DNA Damage Induced in Primary Rat Hepatocytes By 2,3-dimethoxy-1,4-naphthoquinone (DMNQ).
- **L24 Veronika Sackova,** Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: Manumycin A as Agent Sensitizing Adenocarcinoma Cell to Photodynamic Treatment.
- **L25 Peter Solar,** Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: HSP90 Protein as a Target for Improving Photodynamic Therapy.
- **L26 Jaromir Mikes,** Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: The Role of Protein P53 in Photodynamic Therapy with Hypericin.
- **L27 Martin Kello,** Institute of Biology and Ecology, Faculty of Science, UPJS, Kosice, Slovakia: Potentiation of Photodynamic Therapy with Hypericin by Exogenous Polyunsaturated Fatty Acids.

POSTERS

- **P1 Rebeka Tomasin,** Laboratory of Nutrition and Cancer, Institute of Biology, State University of Campinas, UNICAMP, Campinas, Brazil: Aloe Vera and Honey Reduce Tumour Growth by Decreasing its Cell Proliferative Capacity in Tumour-bearing Rats.
- **P2 Katarzyna Lubelska,** National Medicines Institute, Warsaw, Poland: Sulforaphane and Alyssin as Chemopreventive Agents.
- **P3 Luba Hunakova,** Cancer Research Institute SAS, Bratislava, Slovakia: Modulation of Markers Associated with Aggressive Phenotype in MDA-MB-231 Breast Carcinoma Cells by Sulforaphane.
- **P4 Peter Kutschy,** Institute of Chemical Sciences, Faculty of Science, UPJS, Kosice, Slovakia: 1-Methoxyindole Phytoalexins: Synthesis and Anticancer Activity.
- **P5 Monia Lenzi,** Department of Pharmacology, University of Bologna, Italy: Combination of Doxorubicin and Sulforaphane for Reversing Doxorubicin-resistant Phenotype in Mice Fibroblasts with p53(Ser220) Mutation.
- **P6 Magdalena Milczarek,** National Medicines Institute, Warsaw, Poland: Analysis of Interaction of Isothiocyanates with 5-Fluorouracil in Chinese Hamster Lung Fibroblast Cell Line.
- **P7 Petr Benes,** Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic: Wedelolactone Reduces Growth and Induces Differentiation and Apoptosis of Breast Cancer Cells.
- **P8 Alexandra Hudecova,** Comet Assay: A Useful Method For Studying Papaver Rhoeas and Gentiana Asclepiadea Flower Extracts.
- **P9 Anna Smiechowska,** The Impact of Cultivation Conditions on Phytocomlex Composition and Chemopreventive Properties of White Cabbage.
- **P10 Patricia Maimone,** Antiproliferative Effects of Juniperus Communis l. Var. Communis Berry Extract in the Human Hepatocellular Carcinoma Cell Lin.
- **P11 Jung Hyun Kim,** Increased Histone Acetylation by Kale Sprout Intervention in a Human Prostate Cancer Xenograft Mode.
- **P12 Michal Pastorek,** Regulation Of Hypoxic Pathway By Natural Isothiocyanate Sulforaphane In Drug-resistant Ovarian Carcinoma Cell Lines.

L1

ISOTHIOCYANATES EXERT ANTI-MYELOMA ACTIVITY AND ENHANCE THERAPEUTIC CYTOTOXICITY

Jakubikova, J. [1,2,3], Cervi, D.[1,2], Ooi, M.[1,2], Cholujova, D.[3], Daley, J. F.[1,2], Klippel, S.[1,2], Leiba, M.[1,2], Blotta, S.[1,2], Richardson, P. G.[1,2], Mitsiades, C. S.[1,2], Sedlak, J.[3] and Anderson, K. C.[1,2]

[1]Dana Farber Cancer Institute, Department of Medical Oncology, Boston MA 02115, USA
[2]Department of Medicine, Harvard Medical School, Boston MA 02115, USA
[3]Cancer Research Institute, Department of Tumor Immunology, Vlarska 7, 83391 Bratislava, Slovak Republic

jana_jakubikova@dfci.harvard.edu

Multiple myeloma (MM), a plasma cell malignancy, remains largely incurable with 3 to 5 years survival, despite the use of conventional and novel chemotherapies. Isothiocyanates (ITCs), a family of phytochemicals found in cruciferous vegetables, and provide chemopreventive effects associated with a reduced risk of several types of cancer. It has also been reported that ITCs enhance the chemosensitivity of diverse types of tumor cells. We therefore evaluated the anti-myeloma activity of the ITCs, sulforaphane (SFN) and phenylethyl isothiocyanate (PEITC), on a panel of human MM cell lines, sensitive and resistant to conventional and novel anti-myeloma drugs. ITCs induced apoptotic death of MM cells; depletion of mitochondrial membrane potential; cleavage of PARP and caspases-3 and -9; and down-regulation of Mcl-1, X-IAP, c-IAP and survivin. ITCs also induced G2/M cell cycle arrest and mitotic phosphorylation of histone H3, accompanied by decreased protein expression of cyclin B1 and p-cdc2. Multiplex analysis of phoshorylation of diverse components of signaling cascades revealed changes in MAPK activation; increased phosphorylation of c-jun and HSP27; and decreased phoshorylation of Akt, GSK3α/β and p53.

In addition to the cytotoxic effect of ITCs as single agents, synergy with established anti-MM agents has been studied. Our study revealed that SFN exhibited synergistic effects with established anti-MM drugs such as bortezomib, dexamethasone, doxorubicin, and melphalan; whereas PEITC had synergistic effect combined with thalidomide derivative lenalidomide, bortezomib and melphalan. Bone marrow microenvironment has been essential for tumor cell growth and survival in vivo and critical for maintenance of disease. Importantly, ITCs treatment, both alone and in combination with the aforementioned agents, also significantly suppressed proliferation of MM cell lines co-cultured with the human bone stromal cell line HS-5. These results indicate that SFN and PEITC can suppress growth and promote death of MM, both alone and in combination with currently established anti-MM drugs, suggesting their therapeutic potential in MM.

L2

HUMAN INTERVENTION STUDIES WITH BROCCOLI AND THE MODE OF ACTION OF SULFORAPHANE IN VIVO

Mithen, R., and Traka, M.

Institute of Food Research, Colney Lane, Norwich UK

richard.mithen@bbsrc.ac.uk

Epidemiological studies provide evidence that consuming a moderate amount of broccoli per week can reduce the risk of incidence and progression of prostate cancer, and of cancer at several other sites. Isothiocyanates, derived from glucosinolates that accumulate in broccoli are widely considered to be likely candidates that mediate the protective effect of broccoli. Cell and animal models have been extensively used to explore the potential biological activity of isothiocyanates, but the majority of these studies have used concentrations of ITCs that are far in excess of that obtained through the diet. In this paper, we summarize results from a series of acute and long term human intervention studies and from cell and animal models that suggest that sulforaphane – the major ITCs obtained from broccoli consumption - acts in vivo to down regulate PI3K/AKT mediated cell signaling probably, probably due to its activity as a non specific receptor tyrosine kinase inhibitor.

L3

BIOBRAN MODULATES THE INNATE IMMUNE SYSTEM FUNCTIONS: MULTIPLE MYELOMA PATIENTS` STUDY

Cholujova, D., Jakubikova, J., Sulikova, G., Chovancova, J., Hunakova, L., Duraj, J., and Sedlak, J.

Cancer Research Institute SAS, Laboratory of Tumor Immunology

dana.cholujova@savba.sk

Multiple myeloma (MM) is incurable malignancy of plasma cells that accumulate at multiple sites in the bone marrow. Patients with MM suffer from humoral and cellular immunodeficiencies that can lead to life-threatening infections.

BioBran is a food supplement derived from rice bran hemicellulose hydrolyzed by Shiitake mushroom enzymes with modulatory effects on innate immune cells, as well as on adaptive lymphocytes.

The impact of BioBran consumption on innate immune system of MM patients was evaluated in the placebo-controlled study. We monitored changes in natural killer (NK) cell activity by performing flow-cytometric cytotoxicity assay and the proportion of NK and dendritic cell (DC) subpopulations in peripheral blood by immunophenotypic analysis.
Using multiplex bead array immunoassay we analyzed a panel of 27 cytokines in patients' plasma.

BioBran showed modulatory effects on DC and NK cells, as we observed significant augmentation of NK activity, as well as increased percentage of overall DC population and myeloid DC subpopulation in comparison to placebo group. There was a shift of Th1/Th2 cytokine balance to more active Th2 immunity in MM patients when compared to healthy blood donors. The levels of 10 cytokines were significantly increased after BioBran treatment.

The results of the present study indicate that BioBran consumption could be beneficial as a supplement for maintenance of myeloid DC subpopulation and NK activity.

L4

THE PERSPECTIVE USE OF THE PREPARATION MELLOZAN IN ONCOLOGY

Tomasik E., Mansurova F.,. Zikriyahodzhaev D.Z., and Lanina N.

INFREDpharm, s.r.o. (Slovakia), Research Institute of Gastroenterology of the Academy of Sciences of Tajikistan Republic, the Republican Clinical Centre of the Ministry of Health of Tajikistan Republic

Gnackova.j@infredpharm.eu

Preparation Mellozan® produced by INFREDpharm, s.r.o. (Slovakia) is a sterile 0.4% solution for injections in ampoules of 1 and 3 ml. The pharmaceutical substance is used in manufacturing process. The active substance is a complicated complex of compounds obtained by biotechnological processing of natural raw material – honey, which is ecologically pure (it is confirmed by Certificate of Bioproduct). Synthetic or other additives are not present. The preparation is registered officially in the Republic of Tajikistan under the trade name MELLINOL®, and in the Republics of Moldova and Kyrgyzstan under the trade name MELLOZAN®. The aproved indication of the registered preparation is the treatment of liver diseases of different etiology. The results of the current research determined that preparation Mellozan® has the following pharmacodynamic effects: immunomodulative, cytoprotective, regenerative.

Nonclinical studies showed that Mellozan has a dose-dependent immunoregulatory activity. This is demonstrated by its ability to induce production of different types of cytokines: proinflammatory (IL-1, IL-6, IL-8), anti-inflammatory (IL-10), immunoregulatory (IFN-γ, IL-4).

Clinical studies proved that the preparation increases the number of T-lymphocytes and NK-cells, which corresponds with the enhancement of protective immune reactions in patients with the chronic viral hepatitis.

On the other side, Mellozan decreases the immunopathological activity of B-lymphocytes with its hyperproduction of antibodies of different isotypes. The administration of the drug to patients with the dysfunction of the immune system caused the normalization of the T-lymphocytes number, normalized immunoregulatory index (ratio T-helpers/-cytotoxic lymphocytes), and increased the cytotoxic activity NK cells. It also increases the synthesis of the immunoglobulines by the plasmatic cells, increases the functional activity of mononuclear phagocytes, and decreases the level of the spontaneous apoptosis of the Th-lymphocytes, which improves the antiviral immunity.
Wide spectrum of the pharmacodynamic effects, in particular hepatoprotective and immunomodulative allowed us to anticipate its possible efficacy in oncological therapy, as the adjuvant preparation for a better tolerance of the chemo- and radio-therapy, decrease of the side effects by the cytostatic therapy and normalisation of the immune and haematologic patterns.

The clinical studies of Mellozan® in the completion therapy of 82 patients with the different types of malignity, were performed in the Republic Clinical Oncology Centre and Tajik State Medical University (Dushanbe, Tajikistan), in the 2005-2006. The patients underwent the therapy by Mellozan – 10 injections of 3 ml every other day intramusculary (monotherapy) and in combination with chemotherapy, either before or after the radiotherapy. The mode of the treatment was determined according to the activity of tumorous process, the patient status and previous therapy. More than 70% of the patients were patients with primary disease without the previous antineoplastic therapy.

Proper antineoplastic effect of the preparation was assessed only in 7 patients with breast cancer who refused the chemotherapy. Uneasiness feelings, depressions, general weakness and other general symptoms were the most occurred symptoms noticed by the patients before the treatment in the hospital. These patients went through the course of Mellozan therapy before the surgical intervention. The somatic status of all these patients showed the improvement after this therapy. It was possible to see escaping of the depression, improvement of the apetite and increase of the activity. In 6 cases was determined the diminution of the tumor size up to 40-44%

The results of the clinical studies showed that the use of the complex therapy by Mellozan increased the efficacy of the cytostatic drugs, activity of immune system (92% patients) independently from the dissemination of the process. Mellozan showed the regulative impact on the processes of the lymphocytes differentiation and activated antitumorous immune reactions. The increase of the terminal differentiated lymphocytes number (expressing Fas-receptor for induction of the apoptosis), expressing of the receptors to the IL-2 and transferrin - which is the sign of the active proliferation of lymphocytes was possible to see after the first phase of the therapy.

The results of the clinical studies confirmed that use of Mellozan increases the resistance to the chemo- and radio-therapy. The hepatotoxic effect of the cytostatic drugs decreased. The main indicators of the liver function restored to the normal values. Administration of Mellozan corrected the haemopoiesis in the red and white component, improved the indicators of the immune system destroyed by the chemo- and radio-therapy.

All patients treated by complex therapy with Mellozan were able go through full scheme of antitumor therapy without a necessary interruptions what improved the results of the treatment.

The achieved results of the clinical studies showed that Mellozan is a perspective drug in the oncological therapy. Mellozan is able to increase the efficacy of the therapy, the tolerance to the side effects of the radio and chemotherapy and improves the quality of life.

L6

PROSTATE CANCER PREVENTIVE POTENTIAL OF CRUCIFEROUS VEGETABLES

Kim, J. H.[1], Bähr, M.[1], Wagner, J.[2], De Nicola, G[3], Bub, A[4], Rüfer, C[4], Jung, M[2], Iori, R[3], Gerhäuser, C.[1]

[1]German Cancer Research Center Heidelberg, Germany
[2]Universtiy of Freiburg, Germany
[3]Research Center for Industrial Crops, Agriculture Research Council, Bologna, Italy
[4]Max Rubner-Institute, Karlsruhe, Germany

c.gerhauser@dkfz-heidelberg.de

Cruciferous vegetables are rich in glucosinolates, which are converted to isothiocyanates (ITCs) and indole-based compounds by the enzymatic activity of myrosinase. High intake of cruciferous vegetables has been associated with a low incidence of prostate cancer in various case control studies. However, many other epidemiologic studies reported no or negative correlation between risk of prostate cancer and cruciferous vegetable consumption. Mechanisms of chemopreventive activities of selected compounds derived from cruciferous vegetables, such as the isothiocyanates sulforaphane and the indole based compounds indole-3-carbinol and 3,3'-diindolylmethane, include modulation of detoxifying enzymes, anti-inflammatory activity (NF-κB inhibition), anti-angiogenic properties, induction of cell cycle arrest, apoptosis and autophagy, and modulation of signal transduction pathways. Tumor-growth inhibitory efficacy in animal models has been demonstrated at various organ sites including the prostate. However, except the induction of detoxifying enzymes, relatively little is known on mechanisms affected by dietary intervention with cruciferous vegetables, which represent a mixture of various constituents potentially enhancing or blocking their activities (Pappa et al., Carcinogenesis 2007).

In the present study, we tested the tumor growth inhibitory potential of kale sprouts in a human prostate cancer xenograft model. LNCaP human prostate cancer cells were injected to male nude Balb/c mice. Mice were fed with either regular rodent chow or chow supplemented with 20% kale sprouts (containing about 60 μmol glucosinolates per 5 g chow consumed daily, together with active myrosinase) from a week before cell injection until sacrifice after 7.5 weeks of intervention.

Compared with normal diet, kale sprout intervention did not significantly inhibit tumor growth. Secreted prostate specific antigen (PSA), which is used as a biomarker for prostate cancer, was highly correlated with tumor size in both intervention groups. Interestingly however, PSA plasma levels in the kale sprout group were 2.5-fold higher ($p<0.05$) than in the control group. mRNA expression of hormone-regulated genes such as androgen receptor, insulin like growth factor-1 and its receptor, and PSA were significantly increased. Although there was no difference in the expression of PCNA as a proliferation

marker, phosphorylated histone H3 staining as a marker of mitotic arrest was enhance in the kale sprout group. Also, we observed an increase in histone H4 acetylation, which we could not link to an inhibition of HDAC activity, but which might be involved in up-regulation of p21 mRNA and protein expression. Interestingly, hemoglobin levels in tumor cell lysate and vessels in tumor sections were significantly reduced by consuming kale sprout powder, indicating anti-angiogenic potential. This was associated with down-regulated VEGF-c expression.

In conclusion, our study indicate that the combination of components in kale sprouts affect both pro-proliferative (hormone signaling) and anti-proliferative mechanisms (histone H4 acetylation, cell cycle arrest, anti-angiogenesis), which may abrogate their effect on tumor growth. These results may contribute to a better understanding of the effects of cruciferous vegetable consumption in cancer prevention.

Pappa et al., Quantitative combination effects between sulforaphane and 3,3'-diindolylmethane on proliferation of human colon cancer cells in vitro. Carcinogenesis 28, 1471-1477 (2007)

L7

NOVEL TARGETS OF DIETARY ISOTHIOCYANATES IN CANCER PREVENTION

Bao, Y.

School of Medicine, Health Policy and Practice,University of East Anglia, UK

y.bao@uea.ac.uk

Epidemiological studies suggest that a diet rich in cruciferous vegetables is associated with a decreased risk of many common cancers. Their protective effects are attributed, at least in part, to isothiocyaniates, the breakdown products of glucosinolates. Many studies have demonstrated that isothiocyanates can modulate multiple cellular targets including phase I carcinogen-activating enzymes, phase II detoxification enzymes, cell cycle arrest and apoptosis.

Recent studies suggest that sulforaphane can upregulate sequestosome p62 and induce autophagy (cellular self-eating). p62 is a common component of protein aggregates associated with protein misfolding diseases and play an important role in the life and death decisions of the cell. Interestingly, we have shown that sulforaphane can induce Nrf2 translocation into nucleus and increase p62 expression in human hepatocytes. Therefore, we speculate that this stimulation of the Nrf2 pathway by sulforaphane and consequent increase in p62 could facilitate targeting of damaged cellular components to autophagosomes and play a role in cancer prevention.

Furthermore, we have shown that sulforaphane can down-regulate COX-2 expression in colon and bladder cancer cells. Moreover, we have established that synergistic interactions occur between isothiocyanates and mineral selenium in the regulation of antioxidant gene expression including thioredoxin reductase (TR1) and gastrointestinal glutathione peroxidase (GI-GPx).

Very recently, we have found that sulforaphane down-regulated the expression of serotonin receptors and serotonin reuptake transporter (SERT), promoting signals for serotonin release by mediating G-proteins and activating neurotransmitter receptors, which are important in the proliferation of colon cancer cells.

In summary, the effect of sulforaphane on the expression of p62, COX-2 and neurotransmitter receptors may provide new insights into the development of strategies that utilize dietary isothiocyanates for cancer prevention.

L8

ISOTHIOCYANATES AS CHEMOPREVENTIVE AGENTS AGAINST POLYCYCLIC AROMATIC HYDROCARBONS

Skupińska, K., Lubelska, K., Misiewicz, I., Kasprzycka-Guttman, T.

National Medicines Institute, 00-725 Warsaw, Poland, Chełmska 30/34

*skupinska@il.waw.p*l

Plants are a good source of chemopreventive compounds. It was shown in epidemiologic studies that diet rich in Brassica vegetables decrease risk of cancer development. Isothiocyanates (ITC), present in these vegetables are responsible for these properties. A well-known isothiocyanate present mainly in broccoli sprouts is sulforaphane (SFN), which inhibits the growth of cancer cells, induces apoptosis and activity of II-phase enzymes and also inhibits I- phase enzymes activity. The literature data indicate that modification of ITC structure can alter its chemopreventive properties. Hence, two sulforaphane analogues were synthesized: isothiocyanate-2-oxohexyl with exchanged sulfur atom into carbon, and alyssin with prolonged aliphatic chain.

Polycyclic aromatic hydrocarbons (PAH) are environmental pollutant. After entering the body they are metabolized by CY P1A1 and CYP1A2 belonging to I-phase enzymes to oxy-derivatives which are able to bind to DNA, what can initiate carcinogenesis. PAHs induce CYP1A1 and CYP1A2 activity via Aryl Hydrocarbon Receptor (AhR) - dependent pathway increasing the rate of its own metabolism to carcinogenic metabolites. Thus, inhibition of CYP1A1 and CYP1A2 enzymes activity is considered as a chemoprevention strategy.

The aim of the study was to determine if sulforaphane and its analogues can inhibit the CYP1A1 and CYP1A2 activity induced by polycyclic aromatic hydrocarbons differing in the structure and carcinogenicity. This study aimed also to check if synthesized analogues possess better chemopreventive properties than SFN. The mechanism of ITC action was also studied.

The research was conducted on two cell lines: HepG2 – a human liver cancer cell line and Mcf7 – human breast cancer cell line. The ability of ITC to inhibit the CYP1A1 and CYP1A2 activity was assessed with EROD and MROD assay. Investigation of ITC action mechanism included: ITC ability to directly inhibit catalytic activity of CYP1A1 and CYP1A2 enzymes, ITC impact on the enzymatic protein level, and ITC ability to block the translocation of Ah receptor to the nucleus induced by PAHs. To study the protein level and AhR localization immunocytochemistry and confocal microscopy were applied.

The results revealed that only in Mcf7 cells these compounds acted as inhibitors. The strongest inhibitory properties were shown by alyssin. The study revealed also that CYP1A1 was inhibited mainly by the direct inhibition of catalytic activity, while CYP1A2 is inhibited by decreasing the enzyme protein level. Above results indicate that studied compounds can be considered as chemopreventive agents, especially in case of estrogen-depending cancers.

L9

MELATONIN IN THE PREVENTION OF EXPERIMENTAL MAMMARY CARCINOGENESIS

Kassayová M., Ahlers I., Orendáš P., Bojková B., Kubatka P., Ahlersová E., Kisková T., Garajová M.

Department of Animal Physiology, Institute of Biology and Ecology, Faculty of Science, P.J. Šafárik University, Moyzesova 11, 041 54 Košice, Slovak Republic

monika.kassayova@upjs.sk

Melatonin (N-acetyl-5-methoxytryptamine, MEL) is a phylogenetically very old, conservative molecule with pleiotropic effects detected in a wide spectrum of bacteria, algae, plants, fungi and various taxa of invertebrates and vertebrates. Well-known is its regulatory function of sleep-wake cycle, circadian and circaannual rhythms. It has also an immunomodulatory, antioxidative and oncostatic properties.

This contribution summarizes the experimental results of our working group during the last 10 years when we have investigated the chemopreventive and antineoplastic properties of MEL (alone or the combination with the other chemopreventive substances) in the model of chemically induced mammary carcinogenesis in female Sprague-Dawley rats.

Mammary carcinogenesis was induced by two chemocarcinogens: a) N-methyl-N-nitrosourea (NMU) administered in two intraperitoneal doses, each of 50 mg/kg b.w. or b) 7,12-dimethylbenz(a)anthracene (DMBA) administered intragastrically in a single dose of 20 mg/animal or in a three doses of 10 mg/animal. Chemocarcinogens were given during the sensitive period of mammary gland development – between 40.-55. Postnatal days. Melatonin was administered as a solution in tap water at a concentration of 20 μg/ml and 4 μg/ml, respectively. The animals were drinking melatonin daily between 3 p.m. and 8 a.m. (from 8 a.m. to 3 p.m. they are drinking tap water). Chemoprevention with MEL began 5-15 days before carcinogen administration and lasted until the end of experiment (20-26 weeks).

Basic tumor growth parameters - tumor incidence, frequency per group and per animal, tumor volume and latency were evaluated. MEL effect was not markedly dependent on the type of chemocarcinogen used or on the MEL concentration. Sporadic decrease of tumor incidence, frequency per group and tumor volume was observed in the groups treated with MEL alone. MEL increased the chemopreventive influence of the other substances used in our experiments: retinyl acetate, raloxifen, indomethacin and celecoxib. Our data suggest rather irregular action of isolated MEL, but the combination with other chemopreventive agents displayed an enhanced effect in mammary carcinogenesis inhibition. Probably a higher dose of MEL will be needed in our future research.

L10

THE INFLUENCE OF ANTIOXIDANTS (ELLAGIC ACID, EPIGALLOCATECHIN GALLATE) ON THE REMOVAL OF DNA DAMAGE INDUCED WITH DIFFERENT CHEMICAL MUTAGENS

Štětina, R.[1] , Polívková, Z.[2], Šmerák, P.[2], Strejčková, L.[3], Flutková,K[3], Tlučhořová,D.[3],Černohorská H.[4], and Brabníková, Z.[4]

[1]Faculty of Military Health Sciences, Hradec Králové
[2]Institute of the General Biology and Genetics; 3rd Medical Faculty, Prague
[3]Pharmaceutical Faculty, Charles University, Hradec králové
[4]University of Pardubice, Faculty of Health Studies

stetina@pmfhk.cz; r.stetina@tiscali.cz

We have followed the influence of several antioxidants (ellagic acid - EA, genistein and epigallocatechin gallate - EGCG) on the mutagenic effect of N-methyl-N-nitrosourea (MNU), aflatoxin B1 and 2-amino-3-metylimidazo(4,5-f)quinoline (IQ). All three antimutagens inhibited the induction of mutations in Salmonella typhimurium assay and the induction of chromosome aberrations in bone marrow mironuclei assay in mice. They also inhibited the induction of DNA breaks in CHO and HepG2 cells treated with MNU or H2O2 in vitro, as we followed using the comet assay. With respect to their capacity to inhibit the induction of DNA damage by different types of mutagens, their effect can be hardly explained only on the basis of antioxidant and scavenging properties. Therefore, we tested also their possible effect on the DNA repair. The removal of DNA breaks induced in HepG2 or AA8 with MNU or H2O2 was faster, when cells were after the treatment with mutagen incubated in the presence of EA, or EGCG.

The effect of EGCG on the repair of oxidised bases induced by H2O2 was studied in more detail. When we followed the kinetics of repair of DNA damage induced with H2O2 we found, that sites cleavable with FPG enzyme (cleaves predominantly 8-oxo-guanies and abasic sites in the DNA) are removed from the DNA significantly slower compared to sites sensitive to endonuclease III (cleaves oxidised pyrimidines and abasic sites), or true DNA breaks. This kinetics was similar both in normal AA8 cells and UV-20 mutant, which is deficient in the step of incision of the nucleotide excision repair. We found, that in the presence of EGCG the repair of FPG sites is slowed down, while the rapidity of the repair of DNA strand breaks and oxidised pyrimidines is normal. Results show, that EGCG may affect some of pathways of base excision repair, which may have an impact to mutagenic effect of particular mutagen. However, effects of tested antioxidants are quite complicated and much more experimental work is necessary to elucidate the nature of their bioprotective properties.

This study was supported by the Grant of Czech Ministry of Health - NR. 8985-3 and by the grant of Czech ministry of Defence no: OVUOFVZ200810

L11

NON-ANTIOXIDANT EFFECTS OF ANTIOXIDANTS

Collins, A. R.1, Azqueta, A1, Brevik, A2, Lorenzo, Y 1,3

1 University of Oslo, Department of Nutrition, Oslo, Norway
2 Norwegian Institute of Public Health, Division of Environmental Medicine, Oslo, Norway
3 Faculty of Medicine, Universidad Autónoma de Madrid, Spain

a.r.collins@medisin.uio.no

Endogenous antioxidant defences, notably the antioxidant enzymes superoxide dismutases and catalase, sulphydryl-rich glutathione and its associated enzymes (peroxidases and transferases), are essential for survival. They maintain the balance between essential oxidative processes and reactive oxygen-mediated cell regulation on the one hand, and an excessive production of free radicals, i.e. oxidative stress, on the other hand. So do we need antioxidants from the diet as well? In excess, could they even upset this balance? The evidence from large-scale intervention studies is that pure antioxidants at high doses may do more harm than good.

Epidemiological evidence still indicates that plant-derived foods can protect against cancer and cardiovascular disease, but this may not be on account of their antioxidant content, as has been widely assumed. There are, of course, many other well-characterised effects of phytochemicals, such as inhibition or activation of phase I or phase II metabolising enzymes, various effects on cell signalling pathways, and – recently – DNA repair.

Using the comet assay, we have studied DNA repair in two ways: following the time course of removal of lesions in specifically damaged cells, and measuring the repair activity of a cell extract in vitro on a substrate of DNA containing defined lesions. Supplementing the diet with kiwifruit, or eating a high fruit and vegetable diet, enhances lymphocyte BER in the in vitro assay; intriguingly, we found a decrease in NER after the high fruit and vegetable diet. In cell culture, the carotenoid β-cryptoxanthin accelerated the repair of strand breaks and oxidised bases.

It seems that the emphasis on dietary antioxidants is misplaced; phytochemicals have other important protective roles to play.

L12

ISOTHIOCYANATES AND CANCER PREVENTION – ARE WE READY TO MOVE FORWARD FROM OBSERVATIONAL STUDIES TO LARGE CLINICAL TRIALS

Yuan, J. M.

Masonic Cancer Center and Division of Epidemiology and Community Health, School of Public Health, University of Minnesota

jyuan@umn.edu

Humans are exposed to isothiocyanates (ITC) through consumption of cruciferous vegetables (Brassica). ITC is biologically active against chemical carcinogenesis due to its ability to induce phase II conjugating enzymes. ITC also is believed to confer protection against the development of cancer by stimulating apoptosis. Experimental studies have shown that ITC, in particular phenethyl isothiocyanate (PEITC), inhibits the tobacco carcinogen induced lung cancer in animals. Indole-3-carbinol (I3C), another compound present in cruciferous vegetables, is also shown to be chemopreventive in experimental animals. Epidemiological studies have consistently showed that subjects with high consumption of cruciferous vegetables or ITCs are associated with reduced risk of lung, colorectal and gastric cancers. The protection is primarily seen in individuals genetically deficient in phase II conjugating enzymes that metabolize ITC. There are limited studies on I3C in relation to the risk of developing cancer in humans. Given the high correlation between dietary ITC and I3C, observational studies would have limited power to separate the chemoprotective effect of I3C from ITC. Randomized, placebo-controlled phase II clinical trials are required to separately examine the potential chemoprotective effect and to shed light on the biological mechanism(s) of these compounds in cancer protection. The phase II trials will provide clinical data for making the evidence-based decision on launching large randomized phase III clinical trials.

L13

A PROSPECTIVE STUDY OF URINARY BIOMARKER FOR ISOTHIOCYANATES AND RISK OF GASTRIC CANCER IN SHANGHAI, CHINA

Moy, K. A., and Yuan, J. M.

The Masonic Cancer Center and Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis

moyx0022@umn.edu

Epidemiologic data suggests an inverse association between consumption of cruciferous vegetables and risk of human cancers. However, data on specific compounds such as isothiocyanate (ITC) present in cruciferous vegetables and cancer risk are sparse.

We conducted a nested case-control study of gastric cancer within the Shanghai Cohort Study, a prospective cohort of 18,244 middle-aged men in Shanghai, China, to examine the associations between urinary total ITC and the risk of developing gastric cancer. The study included 307 incident gastric cancer cases, and more than 2000 cancer-free individually matched control subjects. In addition to quantification of urinary total ITC, information on lifestyle, dietary, and other environmental exposures was collected through in-person interviews. Overall, high urinary total ITC was associated with reduced risk of gastric cancer. Compared with the lowest quartile, the highest quartile of ITC was associated with a statistically significant approximate 40% reduced risk of gastric cancer.

The risk reduction was more apparent in individuals carrying one or both null genotypes of glutathione S-transferase (GST) M1 and GSTT1. The inverse association between urinary total ITC and cancer risk became stronger with longer duration from the collection of baseline urine for ITC measurement to the diagnosis of cancer. This study provided direct evidence in support of natural compound ITC present in cruciferous vegetables for protection against the development of gastric cancer in humans.

L14

OXIADATIVE DAMAGE AND ANTIOXIDANT PROTECTION IN RELATION TO AGEING

Volkovová, K., and Dušinská, M.

Slovak Medical University in Bratislava

katarina.volkovova @szu.sk

Endogenous oxidative damage to proteins, lipids and DNA is thought to be important etiologic factor in ageing and the development of chronic diseases in elderly. Epidemiological evidence suggests that increased consumption of fruit and vegetable can substantially enhance the protection against many common types of cancer in humans. Higher levels of vitamins E and C in serum have been shown to correlate with lower levels of lipid peroxides.

In a small molecular epidemiological study we investigated altogether 291 healthy subjects, 151 young (78 men and 73 women, 20 to 25 years old) and 140 elderly (39 men and 101 women, 65 to 70 years old).

We measured levels of vitamin C, α-tocopherol, γ-tocopherol, β-carotene, retinol, lycopene and xantophyl in plasma by HPLC. Lipid peroxidation was determined by HPLC as levels of malondialdehyde (MDA) in plasma. Oxidative DNA damage and DNA repair were measured in isolated lymphocytes by the comet assay. Chromosomal aberrations (ABC) and number of micronuclei (NMN) were measured in stimulated lymphocytes. Total antioxidant capacity of plasma (FRAP) was measured spectrophotometrically.

Comparison of biomarkers of oxidative damage and antioxidant protection between young and old groups indicated several significant differences. Oxidative DNA damage (net FPG) was significantly higher in lymphocytes of young people compared to elderly ($p=0.011$), as well as higher in young women compared to elderly women ($p=0.005$).

Women had significantly higher repair rate of oxidative DNA damage compared to men ($p=0.001$). We found significantly more micronuclei in women's lymphocytes ($p=0.001$) compared to men. Elderly people ($p=0.001$) had more micronuclei than young. Similarly, we detected more chromosomal aberrations in elderly group compared to young ($p=0.001$).

There were significant differences in the total antioxidant capacity of plasma. All elderly had higher levels than young ($p=0.001$). The same differences appeared in elderly women vs. young women ($p=0.001$), as well as elderly men vs. young men ($p=0.001$). We found significant differences in plasma concentrations of vitamins between groups.

Vitamin C levels inversely correlated with DNA damage (netAlka) in all group ($p=0.002$), and also in the young ($p=0.01$) and elderly people ($p=0.03$). Retinol inversely correlated with netFPG in the all persons ($p=0.03$), however b-carotene positively correlated with net FPG in all group ($p=0.05$) and in the young ($p=0.001$). Repair rate of oxidative DNA damage positively correlated with levels of lycopene ($p=0.013$) in all groups and negatively with levels of g-tocopherol in all groups ($p=0.03$) and in elderly ($p=0.05$).

Our results suggest that higher levels of antioxidant vitamins in plasma and total antioxidant protection of organism might have protective effect on oxidative DNA damage, thus being beneficial in cancer prevention. However, it is necessary to confirm this result in other studies.

This project was supported by the Slovak grant agency for Science APVT - 21 013202, and by the EU project GOCE 037019.

ENGINEERING BENZYLGLUCOSINOLATE INTO TOBACCO

Mikkelsen, M. D., Geu-Flores, F., Nielsen, M. T., Nafisi, M., Møldrup, M. E., Olsen, C. E., Motawia, M. S., and Halkier, B. A.

Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen

bah@life.ku.dk

The link between regular consumption of cruciferous vegetables and a reduced risk of developing cancer is well established. This cancer-preventive activity has been attributed to glucosinolates, which are defence compounds characteristic of cruciferous plants. We report engineering the production of the bioactive benzylglucosinolate in the non-cruciferous plant Nicotiana benthamiana. The study includes identification of a biosynthetic enzyme, γ-glutamyl peptidase 1 (GGP1), which substantially increases benzylglucosinolate production. GGP1 was shown to metabolize a glutathione conjugate that otherwise accumulates to large extents. Its discovery elucidates an uncharacterized step in the biosynthesis of glucosinolates and expands the reaction repertoire of enzymes containing glutamine amidotransferase domains. The ability to engineer benzylglucosinolate provides the basis for heterologous production of glucosinolates aimed at utilization in cancer-prevention. The engineering strategy developed has great potential as a generally applicable technological platform for gene discovery and proof-of-concept engineering of complex biosynthetic pathways leading to valuable phytochemicals.

BIOTECHNOLOGICAL ALTERNATIVE IN PRODUCTION OF BIOACTIVE SUBSTANCES IN THE GENUS HYPERICUM

Čellarova, E.

P. J. Šafárik University in Košice, Faculty of Science, Institute of Biology and Ecology/Genetics

eva.cellarova@upjs.sk

Medicinal plants from the genus Hypericum, especially H. perforatum that is the most studied one from about 450 species, are attracting considerable interest for centuries. Modern pharmacological studies demonstrate high efficacy of some metabolites, namely naphthodianthrones and acylphloroglucinols with a wide range of activities and sometimes with insufficiently defined pharmaceutical function. Knowledge on biosynthesis of bioactive substances in the representatives of the genus Hypericum and genes encoding for key enzymes in their biosynthetic pathways is very limited. This all suggests for further detail biological and genetic studies of the genus/species. The inconsistent chemical profiles are a result of genetics related to secondary metabolite biosynthesis, physiology and plant-environmental interactions.

In early nineties of the last century when new activities of a dianthrone hypericin, such as anticancer and antiviral have been reported, H. perforatum L. as a natural source of hypericin has developed into a model species for biotechnological and genetic studies in our laboratory.

The biotechnological approach in study/production of desirable secondary metabolites by the representatives of the genus Hypericum comprises:

i) development of an effective regeneration system in vitro by either direct organo genesis or somatic embryogenesis for high-producing genotypes/species;
ii) widening of genetic variation via indirect regeneration from callus;
iii) development of an effective transformation system for studying the gene function and/or for production purposes;
iv) studying the candidate genes in biosynthesis of bioactive compounds and affecting their expression;
v) design of small-scale cultivation of explants in bioreactor under controlled conditions;
vi) development of an effective cryopreservation protocols for establishment of gene bank.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal

L17

SYNTHESIS AND ANTITUMOR ACTIVITY OF COMPOUNDS BASED ON 9-ISOTHIOCYANATOACRIDINE

Imrich, J., Balentová, E., Géci, I., Vilková, M., Tomaščiková, J., Ungvarský, J., Drajna, L.,and Kristian, P.

P. J. Šafárik University, Faculty of Science, Institute of Chemistry, Moyzesova 11, 04167 Košice, Slovak Republic

An original synthon, 9-isothiocyanatoacridine, prepared by Kristian in 1961, has been broadly utilized in our laboratory for numerous syntheses of various classes of new acyclic and heterocyclic compounds possessing moderate antitumor activity in past fifteen years.

The sets of newly prepared compounds comprised acridinyl and 9,10-dihydroacridinyl thioureas, ureas, thiocarbamates, dithiocarbamates, thiosemicarbazides and hydrazones as intermediates and spirodihydroacridines and acridinyl thiazolidines, imidazolines, thiazines, isooxazolines, and oxadiazoles as final heterocyclic structures.

This synthetic variability inspired our search for new synthetic analogues, where structures based on 3,6-disothiocyanatoacridine, 9-isothiocyanato-1,2,3,4-tetrahydroacridine and sugar isothiocyanates proved to be promising synthons, fluorogens, and/or biomarkers.

Due to the presence of a planar acridine skeleton, intercalating properties have been studied by spectral methods and antitumor activity has been proved for many target compounds in collaborations with several laboratories in Slovakia and abroad.

L18

COMPUTER-ASSISTED COMBINATORIAL DRUG DESIGN

Frecer, V.

Cancer Research Institute, Slovak Academy of Sciences, SK-83391 Bratislava, Slovakia

vladimir.frecer@savba.sk

The last decades has witnessed a technological revolution in molecular design, material science and nanotechnology. Combinatorial chemistry, parallel synthesis and high-throughput screening as well as computational design techniques have been embraced by numerous industry sectors as the means to accelerate the discovery of new molecules and materials with the desired properties. The central aim of the computer-assisted molecular and material design techniques is to help the scientists to make the discovery process faster, cheaper and safer and consequently to foster the industrial research and innovation.

We now have the complete genome sequences of more than 100 organisms and can employ the tools of bioinformatics to identify genes and proteins as potential drug targets. The breadth of innovative techniques ranging from fragment-based, analog-based, structure-based and combinatorial library design allows us to design, synthesize, or screen for molecules acting on the chosen drug targets more efficiently.

Principles and selected examples of the use of computer-assisted combinatorial design techniques in the pharmaceutical industry will be presented and discussed. Virtual library design, focusing, virtual screening as well as pharmacokinetic properties prediction, will be illustrated on an example of analog-based design of antiviral compounds.

L19

EXPLOITING ITC-MODULATED SIGNALING PATHWAYS IN CANCER THERAPY

Sedlak, J.[1,2], Jakubikova, J.[2,3], Bodo, J.[2], Sedlakova, O.[4], Cholujova, D.[2], Pastorek, M.[2], Balaz, P.[5], Hunakova, L.[1,2], and Duraj, J.[1,2]

[1]Cancer Research Institute SAS, Centre of Excellence for Signalosome Research, Bratislava, Slovakia
[2]Cancer Research Institute SAS, Laboratory of Tumor Immunology, Bratislava, Slovak Republic
[3]Dana-Farber Cancer Institute, Department of Medical Oncology, Boston MA, USA
[4]Institute of Virology SAS, Bratislava, Slovak Republic
[5]Institute of Geotechnics SAS, Kosice, Slovak Republic

jan.sedlak@savba.sk

Glucosinolates (beta-thioglucoside-N-hydroxysulfates), the precursors of isothiocyanates (ITCs) have been found in families of angiosperms, many of which are edible and could provide substantial amount of chemopreventive, dietary or pharmacological agents. Since the middle of the last century the systematic investigation of ITCs revealed a number of mechanisms that may be involved in their chemopreventive/anticancer effects. Initially demonstrated as potent inducers of phase II enzymes activation and effective indirect antioxidants, ITCs are known to affect many others signaling/regulatory pathways. The in vitro models of primary or permanent cell lines help to dissect the molecular mechanisms influenced by ITC treatment many of which were confirmed in animal models such as activation of apoptosis, modulation of intracellular redox state or inhibition of HDAC activity. Anti-inflammatory, -angiogenic, -neoplastic activities of ITCs in in vivo models may stem from the fact that they modulate epithelial-mesenchymal transition and target population of tumor-initiating cells as well as phoshorylation signaling pathways and epigenetic regulation. The promising perspective arises from observations that ITCs behave synergistically with chemotherapeutic drugs to achieve cytotoxic effect. These small, multi-target reactive molecules represent the plentiful source of interesting compounds modulating the essential cellular pathways.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal

L20

DOWN-REGULATION OF BCL-2 AND AKT INDUCED BY COMBINATION OF PHOTOACTIVATED HYPERICIN AND GENISTEIN IN HUMAN BREAST CANCER CELLS

Ferenc, P., Solár, P., Kleban, J., Mikeš, J., and Fedoročko, P.

Institute of Biology and Ecology, Faculty of Science, P.J. Šafárik University

ferenc000@yahoo.com

The combined efect of photodynamic therapy (PDT) and genistein (G) on MCF-7 and MDA-MB-231 human breast cancer cell lines was studied. We investigated the effect of G on the response of estrogen-responsive MCF-7 and estrogen-unresponsive MDA-MB-231 cells to PDT, both identified in our conditions as PDT-hypericin (PDT-H) resistant. For combined therapy, low toxic concentrations of both agents were selected based on MTT assay. The apoptotic process was monitored using cell cycle, nuclear morphology and Western blot analyses supported by caspase-7 activity assay. PDT-H combined with G revealed significant increase in the percentage of apoptotic cells and PARP cleavage, which correlated with depletion of Bcl-2 and increase of Bax protein, as well as with accumulation of MCF-7 and MDA-MB-231 cells in the G2 phase of the cell cycle specifically for the combination of PDT-H + G. Furthermore, G eliminated PDT-H induced activation of Akt and Erk1/2 phosphorylation status in MCF-7 cells. The only decline in Erk1/2 phosphorylation was observed in MDA-MB-231 cells after combined therapy. Our results indicate that combined treatment with PDT-H and tyrosine kinase inhibitor G may significantly improve the effectiveness of PDT therapy by increasing the sensitivity of MCF-7 as well as MDA-MB-231 cells to PDT-H.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal

L21

ADMINISTRATION OF ISOTHIOCYANATE (E-4IB) AND CISPLATIN LEADS TO ALTERED SIGNALLING AND LYSOSOMAL EXPORT IN HUMAN OVARIAN CARCINOMA SENSITIVE AND CISPLATIN-RESISTANT CELLS

Duraj, J., Gronesova, P., Hunakova, L., Cholujova, D., Bodo, J., and Sedlak, J.

Cancer Research Institute, Slovak Academy of Sciences,
SK-83391 Bratislava, Slovak Republic

Jozef.Duraj@savba.sk

The aim of this study was to compare the effect of a new synthetic isothiocyanate derivative, ethyl 4-isothiocyanatobutanoate E-4IB and cisplatin (CDDP) in CDDP-sensitive human ovarian carcinoma cell line (A2780) and its resistant subline (A2780/CP). In parental cells, in comparison to untreated cells, sequential administration of both compounds led to higher exosomal dye (LysoTracker Green DND-26) retention and to alterations of mitogen-activated protein kinases (MAPKs), JNK, ERK and p38, or Akt, accompanied by changes in several anti- and pro-apoptotic molecules and lysosomal protein LAMP-1, as detected by Western blotting. On the contrary, variant A2780/CP cells were resistant to CDDP- or to combined sensitizer (E-4IB)/inducer (CDDP)-related apoptosis induction and exerted minor changes in the levels of these molecules.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal

L22

SELECTIVE ANTIPROLIFERATIVE ACTIVITY IN VITRO OF STRUCTURALLY RELATED ISOTHIOCYANATES, ERUCIN AND SULFORAPHANE, ON HUMAN PROSTATE CELLS

Melchini, A. [1,2], Costa, Ch. [3], Catania, S. [3], Taviano,M. F.[4], Miceli, N.[4], Mithen, R. [2], Traka, M. [2]

[1]Foundation "Prof. Antonio Imbesi", University of Messina, Italy
[2]Institute of Food Research, Norwich Research Park, Norwich, United Kingdom
[3]Interdepartmental Centre of Experimental, Environmental and Occupational Toxicology, University of Messina, Italy
[4]Pharmaco-Biological Department, School of Pharmacy, University of Messina, Italy

amelchini@pharma.unime.it

Cruciferous vegetables are a rich source of isothiocyanates (ITCs) that are recently identified as promising dietary constituents able to protect against the most common cancer types, such as lung, prostate, breast and colon cancers. A primary goal in the anticancer drugs research is the discovery and development of functional molecules characterized by selective cytotoxicity against cancer cells and low toxicity for healthy cells. Literature data show the relevant antiproliferative activity of ITCs in various cancer cell lines. However, their effect in normal cells has not been fully investigated. Previous studies have provided evidence that the 4- (methylthio) butyl isothiocyanate (erucin, ER) is selective in its effects, inducing a strong antiproliferative effect on human leukemia cells, but not in non-transformed human peripheral T lymphocytes.

Recently, the selectivity of β- phenylethyl isothiocyanate (PEITC) against oncogenically transformed ovarian epithelial cells, including cisplatin- resistant cells, has been reported. The aim of this study was to evaluate the differential sensitivity between normal, hyperplasic and cancer cells from human prostate to ER and its oxidized analog, the 4-(methylsulfinyl) butyl isothiocyanate (sulforaphane, SF).

The effect of increasing concentrations of SF and ER on three different human prostatic cell lines, normal prostate epithelium (PNT1A), benign prostatic hyperplasia (BPH-1) and prostate adenocarcinoma cells (PC3) was determinate using in vitro proliferation assays.

Data obtained showed a comparable dose-dependent decrease in PC3 cell viability after ER and SF treatment. Both ITCs were very effective in inhibiting proliferation of PC3 cells already at concentrations that may be achievable in humans (1-2 μM) after consumption of cruciferous vegetables. Cell viability was reduced by around 90% after 75 μM ER and 50 μM SF treatment.

Interestingly, PNT1A and BPH-1 cells were less sensitive to both ITCs, and the

trend for reduction in cell viability was different compared to PC3 cells. In these cell lines, ITCs started exhibiting significant cytotoxicity at higher concentrations (≥50 μM ER, SF) and reduced cell viability by more than 70% after 125 μM ER and 75 μM SF. Our preliminary data demonstrate that these structurally related ITCs exhibit a stronger cytotoxicity against cancer cells compared to non malignant cells. Thus, understanding the specific action of ITCs on oncogenic signalling pathways may be necessary to make the potential selectivity of dietary ITCs a significant therapeutic application.

L23

EFFECTS OF CARVACROL AND ROSEMARY OIL SUPPLEMENTATION ON OXIDATIVE DNA DAMAGE INDUCED IN PRIMARY RAT HEPATOCYTES BY 2,3-DIMETHOXY-1,4-NAPHTHOQUINONE (DMNQ)

Horváthová, E., and Slameňová, D.

Cancer Research Institute, Slovak Academy of Sciences, Laboratory of Mutagenesisand Carcinogenesis, Vlárska 7, 833 91 Bratislava, e-mail: Eva.Horvathova@savba.sk

eva.horvathova@savba.sk

Free radicals can alter the structure of biomacromolecules and induce cancer as well as heart, eye and neurological diseases. The detrimental effects of oxidation caused by radicals can be diminished by synthetic or natural compounds that can bind these free radicals. However, the use of synthetic preparations has caused some problems due to their highly volatile nature, instability at high temperatures and strict law restrictions. Consequently, it is necessary to use safer and more reliable chemicals to prevent oxidation reactions. Recently, there has been a growing interest in plants as natural sources of compounds appropriate for the prevention of oxidation, controlling pathogens and/or toxin-producing microorganisms in food, and for the treatment of several diseases. Although plant volatiles are common ingredients of the diet and are known especially for their positive action, the increased human exposure requires a careful re-assessment of their positive and negative characteristics in different experimental systems.

The aim of this study was to determine in ex vivo experiments the potential protective effects of carvacrol, a component,- or rosemary oil, a whole plant essential compound,- supplementation on the level of oxidative DNA damage induced in primary rat hepatocytes by 2,3-dimethoxy-1,4-naphthoquinone (DMNQ).

Hepatocytes were isolated either from control rats or rats to which carvacrol in two concentration (30 or 60 mg/kg/day) for 7 days or rosemary oil in three concentrations (0.125; 0.25 or 0.5‰) for 14 days were given as a drinking component. Primary rat hepatocytes were then exposed to DMNQ. The levels of oxidative DNA lesions in control and treated cells were measured by the modified single cell gel electrophoresis, which includes digestion with specific repair enzyme mixture of endonuclease III (EndoIII) and formamidopyrimidine-DNA glycosylase (Fpg).

Our results showed that carvacrol- or rosemary oil-supplementation for 7 or 14 days, respectively, did not induce any negative effect on the health condition of animals or on the level of DNA damage in isolated hepatocytes. On the other hand, we found out that supplementation of rats with carvacrol or rosemary oil efficiently decreased the level of DMNQ-induced oxidative DNA lesions in primary rat hepatocytes.

This study was supported by the Slovak Research and Development Agency grant APVV 51-015404 and by Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Academy of Sciences grant VEGA 2/0072/09.

L24

MANUMYCIN A AS AGENT SENZITIZING ADENOCARCINOMA CELLS TO PHOTODYNAMIC TREATMENT

Sačková, V., Mikeš, J., Jendželovský, R., and Fedoročko, P.

Institute of Biology and Ecology, Department of Cellular Biology, Faculty of Science, Pavol Jozef Šafárik University, Moyzesova 11, 040 01 Košice, Slovak Republic

veronika.sackova@upjs.sk

Several photosensitizers and farnesyltransferase inhibitors are extensively studied as promising anticancer drugs. In this study, hypericin, a photoactive component of Hypericum perforatum, was used as photosensitive drug and manumycin A, a natural product of Streptomyces parvulus, was used as selective farnesyltransferase inhibitor. Our experiments were designed to investigate the apoptotic machinery and the possible participation of caspase-independent pathway activated by hypericin mediated photodynamic therapy, alone and in combination with manumycin, on HT-29 adenocarcinoma cells. Viability, survival, clonogenic and fluorogenic caspase activity assays and western blot analysis of selected proteins in whole and fractionated cell lysates were performed. Photoactivated hypericin and manumycin, used separately, reduced cell viability and proliferation, caused activation of caspases and altered expression of apoptotic proteins. Pre-treatment of cells with non-cytotoxic concentrations of manumycin prior to hypericin light activation, sensitized cells to effect of photoactivated hypericin. This combined therapy increased inhibition of clonogenic tumor growth and resulted in an enhanced apoptotic response of HT-29 adenocarcinoma cells (elevated caspase activity, massive PARP cleavage, complete Bax cleavage, Akt cleavage) compared with the effects on cells treated with hypericin and manumycin alone. Based on these results, the effective anticancer activity of photosensitive drug and farnesyltransferase inhibitor combination could represent a new modality approach for treatment of the cancer.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal

L25

HSP90 PROTEIN AS A TARGET FOR IMPROVING PHOTODYNAMIC THERAPY

Solár, P., Chytilová, M., Ferenc, P., Solárová, Z.*, and Fedoročko, P.

Laboratory of Cell Biology, Institute of Biology and Ecology, Faculty of Sciences, P. J. Šafárik University, Košice, Slovak Republic
**Geriatric Nursing Clinic, Faculty of Medicine, P. J. Šafárik University, Košice, Slovak Republic*

peter.solar@upjs.sk

Our results show that photodynamic therapy (PDT) of hypericin induces the phosphorylation of Erk1,2 proteins and increases the level of survivin, which is the inhibitor of apoptosis protein. Survivin is a client protein for the 90 kDa heat shock protein (Hsp90), and the binding of survivin to Hsp90 assists in the maturation, proper folding, assembly, and transport of this protein.
17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) is a benzoquinone ansamycin antibiotic with destabilizing effect on different Hsp90 client proteins, such as Akt, HER-2, Raf kinase, survivin and others. In contrast to other geldanamycin (GA) analogues, 17-DMAG has several potential advantages, e.g. water solubility, higher oral bioavailability and/or less toxic metabolites.

In our study we were focusing on the determination of the 17-DMAG effect on SKBR3 breast cancer cells (overexpressing HER-2 oncogene) as well as on the response of SKBR3 cells on PDT with hypericin when modulated by 17-DMAG administration. Low concentration of 17-DMAG without the effect on survival of SKBR3 cells significantly reduced metabolic activity (MTT assay), viability and cellularity of SKBR3 cells induced 24 and 48 h after PDT by hypericin. Moreover, we observed a significant decrease of SKBR3 cells in S phase as well as an increase in G1 phase of the cell cycle after 24h treatment with IC10 concentration of 17-DMAG and an increase in G2 phase of SKBR3 cells when 17-DMAG was combined with PDT. Furthermore, IC50 concentration of 17-DMAG induced decrease in HER-2, Akt, phosporylated Erk1,2 and survivin protein level in SKBR3 cells short time after its application. In this regard, the level of phosphorylated Erk1,2 and survivin induced 24 after PDT stayed decreased when SKBR3 cell were pretreated with 17-DMAG. Interestingly, IC10 concentration of 17-DMAG led to total depletion of Akt, P-Erk1,2 proteins and to decrease of survivin level in analyzed cells after 48 h. On the other hand, 17-DMAG did not change HER-2 relative expression (mRNA level) in SKBR3 cells, but caused a significant decrease of HER-2 mRNA in MCF.7 cells characterized with lower HER-2 expression.

These results show that targeting Hsp90 client proteins increases the efficiency of antineoplastic effect of PDT in vitro.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal

L26

ROLE OF PROTEIN p53 IN PHOTODYNAMIC THERAPY WITH HYPERICIN

Mikeš, J, Jedležovský, R., Koval', J., Kello, M., Sačková, V., Kulíková, L., Šemerláková, M., and Fedoročko, P.

Laboratory of Cell Biology, Institute of Biology and Ecology, Faculty of Sciences, P. J. Šafárik University, Košice, Slovak Republic
**Geriatric Nursing Clinic, Faculty of Medicine, P. J. Šafárik University, Košice, Slovak Republic*

jaromirmikes@yahoo.com

Photodynamic therapy with hypericin (HY-PDT) known as an efficient modality for treatment of various cancerous and non-cancerous diseases is based on administration of a non- or weakly-toxic photosensitizer and its activation with light of appropriate wavelength. Although the role of p53 protein in cell death signaling is well established, relatively little is known of its impact on the efficiency of HY-PDT.

In our experiments we found prevalence of necrosis accompanied by suppression by caspase-3 activation as the principal response to HY-PDT despite different PDT doses and the absence of anti-apoptotic Bcl-2 expression in HT-29 cells; even if the same condition induced caspase-3 activity at similar toxicity in HeLa cells. Introduction of Bcl-2 into HT-29 cells invoked caspase-3 activation, changed Bcl-XL expression pattern, increased the apoptosis ratio with no effect on overall toxicity, and supported arrest in the G2/M-phase of cell cycle. Since it is known that Bcl-2 suppression in HT-29 is reversible and linked to the over-expression of mutated p53 and also considering our data, we suggested that the mutation in p53 and events linked to this feature might play role in cell death signaling in colon cancer cells. To prove the concept, comparison of sensitivity and long-term survival of p53-null versus wt-p53 expressing HCT-116 cells was accomplished in addition. We found that the lack of p53 function did not affect cell proliferation or attenuate the initial phases of programmed cell death. However, analyses of apoptosis in the final stages revealed suppression of its incidence and delayed activation of caspase-3 in p53-null cells. Long-term survival regarded as clonogenic ability was significantly enhanced in p53-null cells and especially in hypoxia. Bringing the evidence together, we prove that despite insignificant impact of overall toxicity, expression and status of p53 affects finalization of apoptotic process and the long-therm survival of tumor cells. Since destruction of tumor tissue and its vascular system by PDT tends to lead to hypoxia, superior survivalof p53-defficient cells under given conditions might result in recurrence of cncer diseases and therefore p53-status might be considered as a bad prognosis factor in p53-mutated tumors.

This work was supported by the Slovak Research and Development Agency under the contract No. VVCE-0001-07, by the Science and Technology Assistance Agency under contract No. APVV 0321-07, by the Scientific Grant Agency of the Ministry of Education of the Slovac Republic No. VEGA 1/0240/08 and by Internal grant system of P.J.Šafárik University in Košice under contract No. VVGS 26/09-10.

L27

POTENTIATION OF PHOTODYNAMIC THERAPY WITH HYPERICIN BY EXOGENOUS POLYUNSATURATED FATTY ACIDS

Kello, M., Mikeš, J., Jendželovský, R., Koval', J., and Fedoročko, P.

Institute of Biology and Ecology, Faculty of Science, PJ. Šafárik University in Košice, Moyzesova 11, 040 01 Košice, Slovak Republic

kellomartin@yahoo.com

Extensive research over the past several decades has identified numerous dietary and botanical natural compounds that have beneficial effect in prevention and cure of oncological patients. Exploitation of lipid emulsions in nutrition diet has become a new promising modality in cancer therapy. Lipid emulsions containing polyunsaturated fatty acids (PUFAs) as essential components should been considered not only as a source of energy but also as a tool for specific pharmacological targeting. PUFAs are important in several physiological cell functions, such as signal transduction, cell growth, differentiation and viability. As components of membrane phospholipids, PUFAs change membrane fluidity and remodel membrane structure, which influence other membrane-mediated cellular functions. Dietary therapy of oncological patients employing specific fatty acids (n-3, n-6) decreases tumour growth as a single therapy and increases therapeutic response of tumour to broad spectrum of chemotherapeutics.

Hypericin, as photochemical extract from St. Johns Wort (Hypericum perforatum) and related species, has been shown to have potent, broad spectrum of activity including proapoptotic. Photodynamic therapy (PDT) based on hypericin is currently being used as an alternative treatment modality for some types of cancers. Combination of hypericin-based PDT with nutrition therapy by PUFAs could bring considerable asset in tumour therapy. This particular approach in tumour therapy is not examined properly these days.

This work was supported by the Slovak Research and Development Agency under the contract No. VVCE-0001-07, by the Science and Technology Assistance Agency under contract No. APVV-0321-07, by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic No. VEGA 1/0240/08.

P1

ALOE VERA AND HONEY REDUCE TUMOR GROWTH BY DECREASING ITS CELL PROLIFERATIVE CAPACITY IN TUMOUR-BEARING RATS

Tomasin, R., Cintra, M. C. and Gomes Marcondes, G.

Laboratory of Nutrition and Cancer, Department of Physiology and Biophysics, Institute of Biology, State University of Campinas, UNICAMP, Campinas, Brazil

rebekatomasin@hotmail.com

Cancer is responsible for around 8 million of deaths and 11 million new cases are diagnosed every year. Researches show the importance of co-adjuvant therapies in cancer control. Aloe vera can reduce tumoral mass and metastasis, while honey inhibits tumour growth. This research verified the influence of Aloe vera and honey on tumour growth, accessing tumoral size and cell proliferative rate after 7, 14 and 20 days of subcutaneous Walker 256 carcinoma implantation in adult rats. WA group received gavage of Aloe vera and honey solution daily, while the CW group was gavaged with 0.9% NaCl solution. The effect of Aloe vera and honey against tumour growth could be seen comparing CW and WA tumour growth evolution, (relative weight (%)), since the difference between CW and WA increased especially after 20th day of tumour evolution (CW 7d=0.79±0.32; WA 7d=0.68±0.43; CW 14d=4.14±2.08; WA 14d=3.17±1.38; CW 20d=7.57±2.98; WA 20d=5.16±2.46). In order to justify the less tumoral growth evolution, the tumour cell proliferative rates were obtained by imunohistochemestry assay for Ki-67 (a nuclear protein current during G-1, S, G-2 and mitosis phases). The number of stained nuclei (%) in WA tumours evidently decreased in all accessed days (CW 7d=71.0±10.9; WA 7d=51.4±18.1; CW 14d=69.6±13.5; WA 14d=37.2±16.4; CW 20d=59.1±22.7; WA 20d=32.0±3.3). These data suggest that Aloe vera and honey can modulate tumour growth (reducing the cell proliferation), helping the less tumor evolution (tumour weight reduction). Certainly a large and complex number of mechanisms are involved in this process which Aloe vera and honey can be related, including the main point to manage and modulate the tumour proliferative capacity.

P2

SULFORAPHANE AND ALYSSIN AS CHEMOPREVENTIVE AGENTS

Lubelska, K., Skupińska, K., Misiewicz, I., and Kasprzycka-Guttman, T.

National Medicines Institute, 00-725 Warsaw, Poland, Chełmska 30/34

klubelska@il.waw.pl

Isothiocyanates (ITC) are the group of chemical compounds containing chemopreventive agents. They are found in vegetables from the family Cruciferous. Mainly they occur in vegetables such as broccoli, cabbage, brussels sprouts, cauliflower etc. The results of epidemiological studies indicate a correlation between high consumption of Brassicas containing ITC and lower risk of human cancers. ITC are strong inducers of phase 2 detoxification enzymes like glutathione S-transferases, NADPH:quinine oxidoreductase 1 (QR), etc. This gens - encoding detoxification and antioxidant proteins are regulated by nuclear factor-erythroid 2 p45-related factor 2 (Nrf2). Under homeostatic conditions, Nrf2 is mainly sequestered in the cytoplasm by protein – Keap1. Oxidants and electrophiles caused that Nrf2 is released from Keap1 protection and translocates to the nucleus. It has been shown that Nrf2 null mice are highly sensitive to carcinogen and oxidative stress.

In this study we evaluated chemopreventive activity of ITC: sulforaphane (SFN) and alyssin, which was synthesized in order to search most efficient chemopreventive agents. Each of them are naturally present in family Brassicaceae. The study was carried out in human intestinal Caco-2 cells. In order to evaluate chemopreventive activity of ITC we studied dose and time–dependent changes in viability and QR activity. We performed a MTT - cytotoxicity test, which is a quantitative colorimetric method for mammalian cell survival and cell proliferation. The assay measures the amount of formazon produced witch proportional to the number of living cells present in the culture. The absorbance was measured with Shimadzu UVmini 1240 spectrophotometer at 570 nm. The QR activity was determined by measuring the NADPH-dependent menadiol-mediated reduction of MTT. The absorption was set at the level of 11300 M-1 cm-1 at λ = 610 nm. The QR activity was normalized per mg of total protein and expressed in mU/mg protein. Total protein content was assessed by the Bradford assay. In order to study mechanism of QR induction we also examined the time-dependent changes in the subcellular localization of Nrf-2 by immunostaining with anti-Nrf2 antibody and detection the results witch help of confocal microscope (Olympus Fluo View 500 system equipped with Olympus IX70 microscope).

Our results have shown dose–dependent changes in cell viability and time–dependent changes in QR, which indicates that ITC are effective chemopreventive agents for Caco-2 cells.

P3

MODULATION OF MARKERS ASSOCIATED WITH AGGRESSIVE PHENOTYPE IN MDA-MB-231 BREAST CARCINOMA CELLS BY SULFORAPHANE

L. Hunakova, L.[1], Sedlakova, O.[2], Cholujova, D.[1], Gronesova, P.[1], Duraj, J.[1] and Sedlak, J.[1]

[1]Tumor Immunology Laboratory, Cancer Research Institute, Vlarska 7, Bratislava 833 91, Slovak Republic
[2]Institute of Virology, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovak Republic

exonhun@savba.sk

Because of the multiplicity of oncogenic pathways, some tumours may behave extremely aggressively. This aggressivity is dependent on tumor microenvironment, feasibility of neoangiogenesis, degradation of extracellular matrix, disruption of epithelial integrity and release of cells from intercellular interactions, and acquisition of EMT. Sulforaphane (SFN) is a naturally occurring cancer chemopreventive isothiocyanate found in cruciferous vegetables, consumption of which has been associated with reduced risk of cancer. In this study, we describe effect of SFN on various aspects determining invasive behavior of MDA-MB-231 human breast carcinoma cells.

We studied modulation of molecules associated with epithelial to mesenchymal transition (EMT), hypoxic marker CA IX and mitochondrially located peripheral benzodiazepine receptor (PBR) using flow cytometry, gene expression of MMP1, 3, 7, 9, 14, POU5F1 and Twist1 mRNA by RT PCR, and cytokine production by multiplex bead assay. SFN downregulated PBR and vimentin expression in a dose dependent manner, but did not significantly affect CA IX protein expression. Among studied MMP's, MMP14 mRNA was downregulated and MMP7 significantly decreased while MMP1, 3 and 9 remained unchanged. We observed marked down regulation of Twist1 and POU5F1, transcription factors that mediate EMT and the self-renewal of undifferentiated embryonic stem cells. SFN reduced also the production of pro-inflammatory cytokines IL-1b, IL-6, TNF-alpha, IFN-gamma, immunomodulating cytokine IL-4 and growth factors involved in angiogenesis PDGF and VEGF.

Our results indicate that SFN efficacy is associated with the reversal of several biological characteristics connected with EMT or implicated in the matrix degradation and extracellular proteolysis, as well as with reduced production of pro-inflammatory cytokines and pro-angiogenic growth factors, but not with CA IX expression in MDA-MB-231 cells.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal and Vega grant 02/0119/08.

P4

1-METHOXYINDOLE PHYTOALEXINS: SYNTHESIS AND ANTICANCER ACTIVITY

Kutschy, P.[1], Čurillová, Z.[1], Salayová, A.[1], Repovská, M.[1], Mezencev, R.[2], Mojžiš, J.[3], and Pilátová, M.[3]

[1]P.J. Šafárik University, Faculty of Science, Institute of Chemical Sciences, Department of Organic Chemistry, Moyzesova 11, 040 01 Košice, Slovak Republic
[2]Georgia Institute of Technology, College of Science, School of Biology, 315 Ferst Drive, Atlanta, GA 30332, USA
[3]P.J. Šafárik University, Faculty of Medicine, Department of Pharmacology, SNP 1, 040 66 Košice, Slovak Republic

peter.kutschy@upjs.sk

Plants produce defensive secondary metabolites in response to various forms of stress, including microbial infection, physical or chemical stress. Since the 1940, when professor Műller defined these substances as phytoalexins (plants protecting compounds), many natural products of this group have been identified, isolated and studied from the point of view of their biological activities. Among the others, indole-derived sulfur-containing phytoalexins isolated from the plant family Brassicaceae (syn. Cruciferae) appeared to be attractive chemopreventive and potentially anticancer (antiproliferative) agents. We have found that suitably designed analogs of cruciferous 1-methoxyindoline phytoalexins may exhibit a higher cytotoxic activity against human cancer cell lines, compared to natural leads. In this contribution the syntheses and in vitro anticancer activity of selected cruciferous phytoalexins and their analogs will be presented. Although mechanism of action of these compounds is not yet elucidated, glutathione depletion and topoisomerase II inhibition have been suggested as a part of their anticancer mode of action. Remarkable possibilities of structural variations of the analogs of indole and indoline phytoalexins are not much explored and new analogs with improved cytotoxicity and selectivity toward cancer cell lines could be designed and synthesized.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0514-06

P5

COMBINATION OF DOXORUBICIN AND SULFORAPHANE FOR REVERSING DOXORUBICIN-RESISTANT PHENOTYPE IN MICE FIBROBLASTS WITH p53Ser220 MUTATION

Lenzi, M., Fimognari, C., Cantelli-Forti, G., and Hrelia, P.

Department of Pharmacology, Alma Mater Studiorum - University of Bologna

m.lenzi@unibo.it

Traditional cytotoxic chemotherapeutic approaches cannot cure most advanced solid malignancies. The major factor that limits the effectiveness of chemotherapy in patients with advanced cancer is the acquisition of resistance. Chemoresistance is a multifactorial process, which includes alterations in drug accumulation, increased activity of gluthatione S-transferases, loss of function and mutations of p53, etc. One strategy for reversing drug resistance is the concomitant use of chemopreventive agents (i.e. non-cytotoxic agents able to block the progression to invasive cancer) that are by themselves non-toxic but that cause a better response rates than either reagent alone. Sulforaphane is one of the most promising chemopreventive agent. Sulforaphane inhibits cell-cycle progression and induces apoptosis in different tumor cell lines. The pro-apoptotic potential of sulforaphane could be effective either alone or in combination with other therapeutic strategies in reversing chemoresistance.

We firstly investigated the effects of sulforaphane on mouse fibroblasts bearing a different p53 status (wild-type, knock-out, mutated) for understanding whether its activity is prevented by a mutated p53 status. p53-knock-out fibroblasts from newborn mice transfected with the p53Ser220 mutation, observed in different human cancers, were used as a model of mutated p53 status. Moreover, since p53Ser220 mutation fibroblasts showed a doxorubicin-resistant phenotype, we secondly treated the cells with a combination of doxorubicin plus sulforaphane. To clarify the optimal schedule of combination, we studied the effects of simultaneous and sequential exposure to doxorubicin and sulforaphane. Taken together, our results suggest that a mutated p53 status did not prevent the induction of apoptosis by sulforaphane and that sulforaphane was able to reverse the resistance to doxorubicin when administered simultaneously or after doxorubicin. The association sulforaphane-doxorubicin may therefore allow doxorubicin to be administered at lower doses, thereby reducing its potential toxicity.

P6

ANALYSIS OF INTERACTION OF ISOTHIOCYANATES WITH 5-FLUOROURACIL IN CHINESE HAMSTER LUNG FIBROBLAST CELL LINE

Milczarek, M., Misiewicz, I., Skupińska, K, Lubelska, K., Kasprzycka-Guttman, T.

National Medicines Institute, 00-725 Warsaw, Poland, Chełmska 30/34

gosica61@wp.pl

Sulforaphane is a naturally derived compound that is present in the tissues of cruciferae plants in the form of glucosinolates . It activates phase II enzymes in many types of cells: in hepatic cells, prostate cancer cells, lymphocytes, lymphoblastoid cells. It alters also phase III metabolism, by increased conjugation with GSH and enhanced excretion of conjugates from the cells and also by alteration of activity of ABC-transporters, like Pgp or MRP1 proteins. At higher doses it exhibits cytostatic and cytotoxic activity by influence on cell cycle and induction of apoptosis, what was proven in many types of tissues, mostly in cancer cell lines.

This multipathway activity of sulforaphane makes it one of the most potent chemopreventive compounds among all naturally-derived compounds. The results form in vivo studies also show that sulforaphane inhibits chemically-induced carcinogenesis by the mechanism described above. This formulation is widely available in the form of the dietary supplement. Because of the influence on the enzymes that metabolize xenobiotics the question arises about possible interactions with other drug substances. Sulforaphane was also shown to act synergistically with 5-fluorouracil (5-Fu) in human adenocystic carcinoma at two malignancy It was shown that sulforaphane can act synergistically with doxorubicin what could result in the lower toxicity of doxorubicin in normal cells.

We investigated: antipoliferative, cytotoxic and combined effect of isothiocyanates (ITC):sulforaphane, alyssin, 2-oxohexyl ITC and 5-fluorouracil in Chinese hamster lung fibroblast cell line. The MTT assay was used to test cell viability. The interactions were evaluated in the two schemes of administration: co-administration of 5-Fu with isothiocyanates and pre-treatment with isothiocyanates with the subsequent treatment with 5-Fu. The interaction of tested agents were determined by using a median effect analysis with mutually nonexclusive model as described.

The evaluation of interactions show antagonistic interaction between 5-Fu and sulforaphane and between 5-Fu and 2-oxohehsyl ITC in the two schemes. After co-administration of 5-Fu and alyssin was detected antagonistc effect both compounds but at second scheme of administration was observed additive effect.
We present the first step of our study..

P7

WEDELOLACTONE REDUCES GROWTH AND INDUCES DIFFERENTIATION AND APOPTOSIS OF BREAST CANCER CELLS

Benes, P[1], Pinheiro, D[2], Nemajerova, A[1], Knopfova, L.[1], Smarda, J.[1]

[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2] Faculty of Science, Oporto University, Porto, Portugal

pbenes@sci.muni.cz

Wedelolactone, the naturally occurring coumestan derived from Eclipta prostrate and Wedelia calendulacea, is commonly used in South American medicine as snake antivenom. In traditional Chinese medicine, coumestans are used to treat septic shock, liver diseases and viral infections. Moreover, coumestans reduce cancer risk, potentially due to their structural similarity to phytoestrogens. Recently, it was shown that wedelolactone inhibits growth of prostate and pituitary cancer cells. In this study we tested the effect of wedelolactone on breast cancer cell line MDA-MB-231. We found that 10 and 20 μM wedelolactone inhibits growth of MDA-MB-231 cells by blocking cell cycle progression in the S-phase. Growth inhibitory effect of wedelolactone was associated with cell differentiation as indicated by changes in cell morphology, accumulation of lipid droplets and over-expression of milk fat globule-EGF factor 8. The addition of 30 and 50 μM wedelolactone reduces viability and induces apoptosis of MDA-MB-231 cells documented by chromatin condensation, nuclear fragmentation and poly (ADP ribose) polymerase cleavage. We conclude that wedelolactone possess strong anti-cancer properties and could be considered as potential drug in therapy of breast cancer.

This work was funded by grants IAA501630801 of the Grant Agency of AS CR, MSM0021622415 of the Ministry of Education, Youth ans Sports of the Czech Republic and 204/08/H054 of the Grant Agency of the Czech Republic. Diana Pinheiro was supported by LPP/Erasmus program from the Socrates National Agency.

P8

COMET ASSAY: A USEFUL METHOD FOR STUDYING PAPAVER RHOEAS AND GENTIANA ASCLEPIADEA FLOWER EXTRACTS

Hudecová, A.[1], Hašplová, K.[1], Gálová, E.[1], Miadoková, E[1], Volkovová, K.[2], Dušinská, M.[2,3]

[1]Department of Genetics, Faculty of Natural Sciences, Comenius University, Mlynská dolina B1, 842 15 Bratislava, Slovak Republic
[2]Department of Experimental and Applied Genetics, Slovak Medical University, Limbová 12, 833 03 Bratislava, Slovak Republic
[3]Health Effects Group, CEE, NILU, Kjeller, Norway

hudecovaa@fns.uniba.sk

Plants are universally recognized as a vital part of the world's natural heritage and up to 80% of the populations rely on plants for their primary healthcare. Varieties of medicinal plants are known as a source of natural phytochemicals such as antioxidants that can protect from oxidative stress and from various chronic diseases [5]. The use of traditional medicine is widespread and plants still represent a large source of natural phytochemicals that might serve as leads for the development of novel drugs.

In our studies, methanolic extracts from Papaver rhoeas and Gentiana asclepiadea were evaluated for genotoxic effects using the comet assay. DNA damage was measured in cultured human and monkey cells obtained from different tissues. The method can detect various type of DNA damage induced by genotoxic agents, including radiation, chemicals and agents inducing oxidative stress [3].

Papaveraceae is a family of flowering plants. The flowers of poppy have a long history of medicinal usage, especially for ailments in the elderly and children. Chiefly employed as a mild pain reliever and as a treatment for irritable coughs, it also helps to reduce nervous over-activity [2]. The flowers and petals are anodyne, emollient, expectorant, hypnotic, slightly narcotic and sedatíve. An infusion is taken internally in the treatment of bronchial complaints and coughs, insomnia, poor digestion, nervous digestive disorders and minor painful conditions. The flowers are also used in the treatment of jaundice and –in very small quantities, and under expert supervision – as a sleep-inducing drug [1, 2].

Plants belonging to Gentianaceae are widely used in traditional medicines in many countries. Some of them are used as ingredients in Chinese herbal medicines for stimulation of appetite and gastric secretion, gastro-duodenal protection, liver protection, antifungal treatment, and in some cases for women´s diseases. It is known that family Gentianaceae contains secoiridoid glucosides, flavone C-glucosides, and xanthones as well as their glucosides. It has been reported that gentiopicroside, a compound present in many gentiana species, has free radical scavenging activity.

Experiments were carried out on cell lines: Hep G2 (human hepatocellular liver carcinoma cell line), Caco 2 (human epithelial colorectal adenocarcinoma cell line), Cos 1 (monkey kidney cell lines) and TK 6 (lymphoblast cell line). The cell types were selected as representants of liver, kidney, digestive and lymphatic system (natural food supplements are usually taken perorally).

Cells were treated for 24 hours with 5 different concentrations of plant extract (2,5 %; 0,5 %; 0,25 %; 0,05 % and 0,025 %). Results showed that the highest concentration (2,5 %) of the plant extracts caused DNA damage in most of the cell types. The most sensitive cell lines were TK 6. They showed very strong DNA damage after 24 hour treatment with 2,5% and 0,5 % of plant extract. 100 comets per slide were scored using Perceptive image analysis system Comet assay IV.

Future research will focus on determining the optimal Papaver rhoeas and Gentiana asclepiadea flower extract concentrations to produce these protective effects, and on their mechanisms of action.

This work was supported by the grant UK /203/2008 and the grants VEGA: 2/7033/7 and1/0008/08, SAIA - travel grant, NILUEEA grants (PNRF122) and by COMICS, EC STREP: LSHB-CT-2006-037575.

P9

THE IMPACT OF CULTIVATION CONDITIONS ON PHYTOCOMPLEX COMPOSITION AND CHEMOPREVENTIVE PROPERTIES OF WHITE CABBAGE

Śmiechowska, A., Piasek, A., Szumska, A., Kawecka, A., Kusznierewicz, B., Zemło, D., Szulfer, I., Kielbratowska, M., Konieczka, Namieśnik, J., Bartoszek, A.

Chemical Faculty, Gdańsk University of Technology

anna_smiechowska@o2.pl

The phytochemicals found in white cabbage (Brassica oleracea var. capitata) display an array of biological activities important in cancer chemoprevention. Health promoting properties of this vegetable are determined by the content of bioactive substances, the abundance or even composition of which depend on cultivation conditions. In order to investigate these interrelations we cultivated the seedlings of white cabbage derived from the same source in 10 different locations in Northern Poland from May to late September. The cultivation sites varied as regards ecological settings, environmental conditions, soil quality, insolation and pest abundance.

After harvest, the plant material was used to prepare lyophilisates and juices. In these samples, the following chemical parameters were determined: heavy metal content in vegetables, GLS in extracts from lyophilisates and indole concentration in juices. The antioxidative activity was measured by DPPH, ABTS and FCR test for the extracts and juices. The juices were also used to determine the ability to inhibit the cellular growth and induction of II phase enzymes (DT-diaphorase) in human colon cancer HT29 cells. The analyzed samples turned out to display surprisingly high variability in the case of all parameters determined. This suggests that the cultivation conditions may have crucial influence on chemopreventive value of cabbage.

P10

ANTIPROLIFERATIVE EFFECTS OF JUNIPERUS COMMUNIS L. VAR. COMMUNIS BERRY EXTRACT IN THE HUMAN HEPATOCELLULAR CARCINOMA CELL LINE

Maimone, P.[1], Taviano, M. F.[1], Melchini, A.[1,2], Güvenç, A.[3], De Pasquale, R.[1], Trovato, A.[1], and Miceli. N.[1]

[1]Pharmaco-Biological Department, School of Pharmacy, University of Messina, Italy
[2]Foundation "Prof. Antonio Imbesi", University of Messina, Italy
[3] Department of Pharmaceutical Botany, Ankara University, Turkey

michela.ma@jumpy.it

Plants used in folk medicine continue to be an important source of discovery and development of novel therapeutic agents. Juniperus communis L. var. communis (Jcc) is an evergreen shrub or tree diffused in Europe, Asia and North America, whose berries are known for their biological properties. Literature data suggest that Juniperus L. species have some protective effects against cancer. In vitro and in vivo assays suggest that J. chinensis L. possess antitumor activities with a marked antiproliferative activity in two mammalian cancer cell lines (HeLa, HGC-27). Recent studies show the cytotoxic effects both of J. communis and J. taxifolia in liver and colon carcinoma and in human promyelocytic leukemia HL-60 cells, respectively. The aim of this study was to evaluate in vitro the cytotoxic effects of berries methanol extract of Jcc on human hepatocellular carcinoma (HepG2) cells. The cytotoxic activity of the extract was determined on HepG2 cells after 24 h exposure. Results obtained from the proliferation assay showed a dose-dependent decrease in HepG2 cell viability after treatment with Jcc extract. At the concentration of 40 μg/mL Jcc extract markedly inhibited the growth of HepG2 cells with a reduction of cell viability more than 80% compared to the control cells. The cytotoxic effects observed in human hepatocellular carcinoma cells after exposure to the extract suggest that Juniperus communis L. var. communis could represent a good source of natural compounds potentially important for cancer prevention and treatment.

P11

INCREASED HISTONE ACETYLATION BY KALE SPROUT INTERVENTION IN A HHUMAN PROSTATE CANCER XENOGRAFT MODEL

Kim, J. H.[1], Bähr, M.[1], Wagner,J.[2], de Nicola, G[3], Rüfer, C.[4], Jung, M.[2], Iori, R.[3], and Gerhäuser, C.[1]

[1]German Cancer Research Center Heidelberg, Germany
[2]Universtiy of Freiburg, Germany
[3]Research Center for Industrial Crops, Agriculture Research Council, Bologna, Italy
[4]Max Rubner-Institute, Karlsruhe, Germany

j.kim@dkfz.de

Introduction: Prostate cancer is the most commonly diagnosed malignancy in men in industrialized countries. International epidemiological studies have indicated an inverse correlation between prostate cancer and consumption of cruciferous vegetables such as broccoli and kale. Cruciferous vegetables are rich in glucosinolates, which are converted to isothiocyanates and indole-based compounds by the enzymatic activity of myrosinase. Both groups of compounds have cancer preventive potential through various mechanisms including anti-proliferative activities. Isothiocyanate metabolites have been recently identified as inhibitors of histone deacetylates (HDAC) which have the potential to turn on the epigenetically silenced genes, leading to cell cycle arrest and apoptosis.

Purpose: In this study we investigated the effect of kale sprouts commercially available as a food supplement (BS1000) to inhibit the growth of human LNCaP prostate cancer cells in a xenograft model. We were interested whether kale sprout intervention would affect histone acetylation and in studying underlining mechanisms, e.g. HDACs and histone acetyltransferases (HAT).

Treatment and methods: 2x 106 LNCaP cells were injected to the right and left flank of 6-7 old Balb/c male nude mice. Ten mice per group were fed with either regular rodent chow or chow supplemented with 20% kale sprouts (containing about 60 μmol glucosinolates per 5 g chow consumed daily, together with active myrosinase) from a week before cell injection until sacrifice. 7.5 weeks after injection, tumor tissue was collected. HDAC expression and histone H3 and H4 acetylation were detected by Western blotting using antibodies against HDACs 1-5 and acetylated histones H3 and H4. HDAC activity in nuclear extracts of tumor tissue was determined by using ZMAL substrate (Fenic et al., J. Androl. 2008). mRNA expression of selected HATs (HAT, p300, CBP, p300-CBP associated factor (PCAF)) was quantified in tumor tissue by qRT-PCR.

Results: Kale sprout intervention did not significantly inhibit tumor growth in the xenograft model. However, global acetylation levels of histone H4 were increased in the kale sprout-treated group, whereas acetylated histone H3 levels were not significantly changed. The increase of H4 lysine 5, 8, 12 and 16 acetylation was individually confirmed.

Among them, acetylated H4 K12 was most abundant. Unexpectedly, HDAC activity was not significantly inhibited in tumor tissue by kale sprout intervention. Also, the expression of HDAC family members HDAC1-5 was not changed overall, although HDAC 5 expression was reduced while HDAC2 expression was increased. Instead, kale sprout treatment significantly increased mRNA levels of PCAF and CBP by 36% and 23%, respectively, in tumor tissue ($p < 0.001$), which may contribute to increased H4 lysine acetylation.

Conclusion: Taken together, our results indicate that dietary consumption of kale sprouts increased global acetylation in histone H4 in LNCaP prostate cancer xenograft model, possibly by induction of HAT expression rather than inhibition of HDAC activity.

P12

REGULATION OF HYPOXIC PATHWAY BY NATURAL ISOTHIOCYANATE SULFORAPHANE IN DRUG-RESISTANT OVARIAN CARCINOMA CELL LINES

Pastorek, M.[1], Hunakova, L.[1],Barathova, M.[2], and Sedlak, J[1].

[1]Tumor Immunology Laboratory, Cancer Research Institute, Vlarska 7, Bratislava 833 91, Slovak Republic
[2]Institute of Virology, Dubravska cesta 9, Bratislava 842 45, Slovak Republic

exonpast@savba.sk

Isothiocyanate sulforaphane is well known because of its ability to induce apoptosis, detoxification enzymes, cell cycle arrest, and for its chemopreventive property. Development of chemoresistance and hypoxia are often serious problems in modern therapy, therefore we decided to determine the in vitro effect of sulforaphane in ovarian carcinoma cell line A2780 and its two chemoresistant sublines during hypoxic and normoxic conditions. We have confirmed the response of studied cell lines on hypoxia and revealed sulforaphane-induced decrease of promoter activity of CA IX in a concentration-dependent manner. Therefore we have focused on main transcription factor regulating hypoxia and found out that sulforaphane affects hypoxia induced pathways primary by decreasing stability of HIF-1α, although other mechanisms of its regulation can not be excluded.

This project was supported by the Slovak Research and Development Agency under the contract No.VVCE-0001-07 "CeXignal".

LIST OF PARTICIPANTS

1	**Ashurst, Kean**	Caudill Seed Inc, Kentucky (Corporate), USA
2	**Bao, Yongping**	School of Medicine, Health Policy and Practice, University of East Anglia, UK bao@uea.ac.uk
3	**Benes, Petr**	Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic benes@sci.muni.cz
4	**Busiková, Zuzana**	Cancer Research Institute, Slovak Academy of Sciences, Slovak Republic
5	**Collins, Andrew**	University of Oslo, Department of Nutrition, Oslo, Norway a.r.collins@medisin.uio.no
6	**Čellárová, Eva**	P. J. Šafárik University in Košice, Faculty of Science, Institute of Biology and Ecology/Genetics eva.cellarova@upjs.sk
7	**Duraj, Jozef**	Cancer Research Institute, Slovak Academy of Sciences, Slovak Republic Jozef.Duraj@savba.sk
8	**Fedoročko, Peter**	P.J.Šafárik University in Košice/ Institute of Biology and Ecology, Slovak Republic peter.fedorocko@ujps.sk
9	**Ferenc, Peter**	Institute of Biology and Ecology, Faculty of Science, P.J. Šafárik University, Košice ferenc000@yahoo.com
10	**Frecer, Vladimír**	Cancer Research Institute, Slovak Academy of Sciences, SK-83391 Bratislava, Slovak Republic vladimir.frecer@savba.sk

11	**Frecerová, Jana**	Academia Tours / Organizer, Slovak Republic
12	**Gerhaeuser, Clarissa**	German Cancer Research Center Heidelberg, Germany c.gerhauser@dkfz-heidelberg.de
13	**Gronesová, Paulína**	Cancer Research Institute, Slovak Academy of Sciences, Slovak Republic exonpaja@savba.sk
14	**Halkier, Barbara Ann**	Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen bah@life.ku.dk
15	**Hašplová, Katarína**	Comenius University, Faculty of Natural Sciences, Department of Genetics, Slovak Republic
16	**Horváthová, Eva**	Cancer Research Institute, Slovak Academy of Sciences, Laboratory of Mutagenesis and Carcinogenesis, Vlárska 7, 833 91 Bratislava, Slovak Republic Eva.Horvathova@savba.sk
17	**Hudecová, Alexandra**	Department of Genetics, Faculty of Natural Sciences, Comenius University, Mlynská dolina B1, 842 15 Bratislava, Slovak Republic hudecovaa@fns.uniba.sk
18	**Hunáková, Ľuba**	Cancer Research Institute, Slovak Academy of Sciences, Slovakia, Tumor Immunology Laboratory, Cancer Research Institute, Vlarska 7, Bratislava, 833 91, Slovak Republic exonhun@savba.sk
19	**Cholujová, Dana**	Cancer Research Institute, Slovak Academy of Sciences, Laboratory of Tumor Immunology, Slovak Republic dana.cholujova@savba.sk

20	**Imrich, Ján**	P. J. Šafárik University, Faculty of Science, Institute of Chemistry, Moyzesova 11, 04167 Košice, Slovak Republic
21	**Jakubiková, Jana**	Dana Farber Cancer Institute, Department of Medical Oncology, Boston MA 02115, USA jana_jakubikova@dfci.harvard.edu
22	**Kassayová, Monika**	Department of Animal Physiology, Institute of Biology and Ecology, Faculty of Science, P.J. Šafárik University, Moyzesova 11, 041 54 Košice, Slovak Republic monika.kassayova@ujps.sk
23	**Kello, Martin**	Institute of Biology and Ecology, Faculty of Science, PJ. Šafárik University in Košice, Moyzesova 11, 040 01 Košice, Slovak Republic kellomartin@yahoo.com
24	**Kim, Jung Hyun**	German Cancer Research Center Heidelberg, Germany j.kim@dkfz.de
25	**Kutschy, Peter**	P.J. Šafárik University, Faculty of Science, Institute of Chemical Sciences, Department of Organic Chemistry, Moyzesova 11, 040 01 Košice, Slovak Republic peter.kutschy@upjs.sk
26	**Lanina, Natalia**	INFREDpharm (Corporate), Slovak Republic
27	**Lewandowski, Grzegory**	Accompanying person Poland
28	**Lenzi, Monia**	Department of Pharmacology, Alma Mater Studiorum University of Bologna m.lenzi@unibo.it

29	**Lubelska, Katarzyna**	National Medicines Institute, Chełmska 30/34, 00-725 Warsaw, Poland klubelska@il.waw.pl
30	**Maimone, Patrizia**	Pharmaco-Biological Department, School of Pharmacy, University of Messina, Italy michela.ma@jumpy.it
31	**Melchini, Antonietta**	Foundation "Prof. Antonio Imbesi", University of Messina, Italy amelchini@pharma.unime.it
32	**Mikeš, Jaromír**	Laboratory of Cell Biology, P. J. Šafarik University, Košice, Slovak Republic jaromirmikes@yahoo.com
33	**Mikkelsen, Michael D.**	Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen
34	**Milczarek, Małgorzata**	National Medicines Institute, Chełmska 30/34 00-725 Warsaw, Poland gosica61@wp.pl
35	**Mithen, Richard**	Institute of Food Research, Colney Lane, Norwich UK richard.mithen@bbsrc.ac.uk
36	**Moy, Kristin**	The Masonic Cancer Center and Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis moyx0022@umn.edu
37	**Pastorek, Michal**	Tumor Immunology Laboratory, Cancer Research Institute, Vlarska 7, Bratislava, 833 91, Slovak Republic exonpast@savba.sk

38	**Sačková, Veronika**	Institute of Biology and Ecology, Department of Cellular Biology, Faculty of Science, Pavol Jozef Šafárik University, Moyzesova 11, 040 01 Kosice, Slovak Republic veronika.sackova@upjs.sk
39	**Sedlák, Ján**	Cancer Research Institute SAS, Centre of Excellence for Signalosome Research, Bratislava, Slovak Republic jan.sedlak@savba.sk
40	**Skupińska, Katarzyna**	National Medicines Institute, Chełmska 30/34, 00-725 Warsaw, Poland skupinska@il.waw.pl
41	**Smiechowska, Anna**	Chemical Faculty, Gdańsk University of Technology anna_smiechowska@o2.pl
42	**Solar, Peter**	Laboratory of Cell Biology, Institute of Biology and Ecology, Faculty of Sciences, P. J. Šafárik University, Košice, Slovak Republic peter.solar@upjs.sk
43	**Štetina, Rudolf**	Faculty of Military Health Sciences, Hradec Králové stetina@pmfhk.cz; r.stetina@tiscali.cz
44	**Šulíková, Margita**	Cancer Research Institute, Slovak Academy of Sciences, Slovak Republic
45	**Teng, Jin- Hong**	Accompanying person, USA
46	**Tomasik, Emil**	INFREDpharm (Corporate), Slovak Republic
47	**Tomasin, Rebeka**	Laboratory of Nutrition and Cancer, Department of Physiology and Biophysics, Institute of Biology, State University of Campinas, UNICAMP, Campinas, Brazil, rebekatomasin@hotmail.com

48	**Volkovová, Katarína**	Slovak Medical University in Bratislava katarina.volkovova @szu.sk
49	**Wang, Wei**	Accompanying person, UK
50	**Yuan, Jian-Min**	Masonic Cancer Center and Division of Epidemiology and Community Health, School of Public Health, University of Minnesota jyuan@umn.edu

Gold Sponsor

www.krd.cz

KRD is a biotechnology distribution company founded in 1998 with idea to supply the our local research comunities with products for :

1. Genomic research
2. Clinical PCR, RT PCR and real time applications
3. Stem cell research
4. Protein engineering
5. Tissue banking
6. Education in cell and molecular biology

Company has two offices (Prague - Czech republic, Bratislava - Slovakia).

KRD ltd. Pekařská 12 CZ-15500 Prague 5 Czech republic Phone: 00420-2-57013400 Fax: 00420-2-57013405 E-mail: krd@krd.cz www.krd.cz	KRD ltd. Saratovská 26 SK- 841 02 Bratislava Slovakia Phone: 00421-2-645 34 041 Fax: 00421-2-645 34 043 E-mail: krd@krd.sk www.krd.sk

CeXignal

AGENTÚRA
NA PODPORU
VÝSKUMU A VÝVOJA

This conference was supported by the Slovak Research and Development Agency under the contract No. VVCE-0001-07 "CeXignal"

PUBLISHED BY: CANCER RESEARCH INSTITUTE,
SLOVAK ACADEMY OF SCIENCES
AND SLOVAK CANCER RESEARCH FOUNDATION

ISBN 978-80-970128-2-3