Progress in studies on ABC transport inhibition

Book of Abstracts

April 21st, 2010

Chemical Research Center
Hungarian Academy of Sciences

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Chairman

György Hajós
Chemical Research Center
Hungarian Academy of Sciences

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Welcome

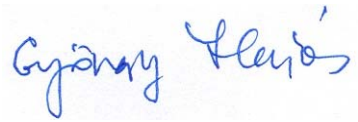
A warm welcome to you at this symposium! For you, as an active scientific contributor to the field, it is needless to emphasize how important it is to find new ways to combat resistance and, thus, to enhance the survival chances of more and more patients suffering in serious diseases like cancer, tuberculosis, malaria, etc.

A previous Cost program (B16, “Reversal of bacterial resistance”, terminated in 2006) and its more specific, actively running continuation (BM 0701, ATENS, started in 2008) decided to bring together scientists in Europe from different areas (biology, medicine, physics, chemistry, biochemistry) and to concentrate their efforts in order to initiate elaboration of new diagnostics, recognition of new lead molecules for inhibition of resistance.

The present mini symposium has been realized under the auspices of ATENS Cost program on the occasion that, fortunately, several scientists of this Cost action are coincidently in Hungary at this time. The participants decided to inform each other on the stand of their own research and to carry out thorough discussions on the importance and most promising approaches of research of MDR-inhibition.

As director of the Institute of Biomolecular Chemistry, Chemical Research Center of the Hungarian Academy of Sciences, it is my pleasure to offer the Lecture hall of the Center for the venue of this symposium. I wish you a substantial participance with inspiring lectures and discussions, and a pleasant stay in Budapest!

I cordially thank the National Office for Research and Technology for financial support



György Hajós
chairman

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General Information

Venue

Chemical Research Center of the Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59. Main Lecture Hall, Building IV, 2. floor.

Registration

the participation is free

Projection

Speakers are kindly asked to hand over their disks or USB sticks to the technical staff well before the beginning of the symposium.

Scientific program

10:00 G. Hajós: Opening of the Symposium

10:10 L. Amaral: Efflux Pumps that Bestow Multi-Drug Resistance to Bacteria and Cancer Cells.

10:50 G. Spengler: Screening of Efflux Pump Modulators by Real-Time Fluorometry in Bacteria and Cancer Cells

11:10 A. Martins: Influence of Calcium and pH in the Accumulation and Efflux of EB

11:30 – 11:50 Coffee break

11:50 G. Hajós: New heterocyclic molecules in the service of research on MDR inhibition

12:10 J. Molnár: Inhibition of Drug Resistance of Bacteria and Cancer Cells.

12:40 J. Sherly: Comparison of the Antitumour Activities of Selected Steroidal Compounds on Mdr and A2780cis Cell Lines

12:50 Z. G. Varga: Quorum sensing inhibition by phenothiazines and essential oils

13:00 – 13:40 Lunch

13:40 M. Pascu: Laser and Optical Techniques Used to Modify Molecular Structures of Medicines

14:20 L. Vereczkey: Culture of Primary Hepatocytes: a Useful Model for Studying Abc Transporter Activities

14:50 A. Zalatnai: *In vivo* Effects of Mdr-Revertant Organosilicon Compounds (Sila-409 And Sila-421) on Human Pancreatic Cancer Xenografts

15:20 L. Homolya: The Enigma of Multidrug Transporters: Direct Drug Extrusion from the Plasma Membrane

15:40 G. Szakács: P-glycoprotein is the Achilles' Heel of Multidrug Resistant Cancer Cells

16:00 J. Kristiansen: Antimicrobial Activities of Neurotropics *in vitro* and *in vivo*

16:30 L. Amaral: Closing words

Content

EFFLUX PUMPS THAT BESTOW MULTI-DRUG RESISTANCE TO BACTERIA AND CANCER CELLS

Leonard Amaral and Joseph Molnar

Unit of Mycobacteriology, Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa.

The main cause for multi-drug resistance (MDR) of bacteria and cancer cells is the over-expression of efflux pumps that extrude the antibiotic or anti-cancer agent prior to reaching its intended target. The over-expression of these efflux pumps is due to exposure of the cells to levels of agent that do not kill the cells, and hence, the opportunity for the cell to adapt to the agent takes place. This adaptive mechanism is present in all living cells whether they exist as single free entities such as bacteria, fungi, protozoa as well as in circulating cells such as lymphocytes or macrophages, or tissue fixed cells such as those that line the gastro-intestinal tract, neurological cells of the brain-cerebellum stem, epidermal cells, bone cells or even, muscle cells. The efflux pumps of bacteria may be classified into two main groups that are defined by the source of energy used for their activity. Efflux pumps of bacteria that utilize energy from the hydrolysis of ATP for extrusion of an antibiotic have similar characteristics as those that extrude anti-cancer agents by MDR cancer cells. These efflux pumps are part of the ABC ATP binding cassette family and consist of a single protein that may have multiple domains in the plasma membrane, and in the case of Gram negative bacteria, some of these domains are in the periplasm. The ABC ATP binding transporters have at least two separate distinct sites for the respective binding of ATP and itssubsequent hydrolysis and one for the substrate. Although the binding of the agent to be extruded is independent of metabolic energy, the hydrolysis of ATP generates protons that promote configurational changes in the transporter causing the translocation of the agent from the cytoplasm to exterior of the cell. Bacteria, unlike eukaryotes, have additional transporters that utilize the energy provided by the proton motive force (PMF) for extrusion of the antibiotic. As is the case for ABC ATP binding efflux pumps, the binding of an antibiotic substrate to the transporter is independent of metabolic energy and is modulated by the environmental conditions in which the transporter exists. The function of the PMF is to provide protons that promote the dissociation of the substrate from the transporter-an essential condition for if the substrate is not released, the efflux activity of the pump comes to a halt.

The essence of the lecture will focus on the structure of the transporter components of efflux pumps of MDR bacteria and cancer cells, the environmental conditions that affect the activity of efflux, and exploit these conditions for the design of agents that are to reduce the activity of MDR efflux pumps of bacteria and cancer cells. It is important to note that because the efflux pump targets of bacteria are unique-compounds that bind to the transporter and inhibit irreversibly the activity of the pump are desired, whereas those that are to affect the activity of efflux pumps of the eukaryote should be competitive with the anti-cancer agent. This is essential since if irreversible activity of the transporter takes place, eukaryotic normal cells that utilize the transporter for essential activities would be compromised (cell death).

Lastly, examples where compounds made by Prof George Hajos' group and associates have differential activities against efflux pumps of cancer cells and bacteria will be shown and will be the subject of presentation by others at this symposium. These presentations will provide detailed descriptions of methods that can readily be used for rapid screening of compounds made by the chemical groups of this Institute for Chemistry.

SCREENING OF EFFLUX PUMP MODULATORS BY REAL-TIME FLUOROMETRY IN BACTERIA AND CANCER CELLS

Gabriella Spengler[1,2], Miguel Viveiros[1], Ana Martins[1], Liliana Rodrigues[1,2], József Molnár[3] and Leonard Amaral[1,2]

[1]Unit of Mycobacteriology, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL), Portugal
[2]Unidade de Parasitologia e Microbiologia Médicas (UPMM), Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal
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The efflux pump-based multidrug resistance (MDR) plays an important role in the failure of cancer chemotherapy and the treatment of infectious diseases. MDR efflux pumps utilize cellular energy to extrude antibiotics or biocides actively out of the cell, it is necessary to characterize the efflux activity of MDR bacteria under physiologically relevant conditions. In tumour cell lines, multidrug resistance is often associated with an ATP-dependent decrease in cellular drug accumulation related to the over-expression of the members of the ATP-binding cassette (ABC) transporter superfamily.
The real-time fluorometry uses the fluorochrome ethidium bromide (EB), which is considered a common substrate of bacterial efflux pumps. Ethidium bromide has been shown to be particularly suitable to be used as a probe because it emits weak fluorescence in aqueous solution (outside cells) and becomes strongly fluorescent in non-polar and hydrophobic environments. The methodology is a closer representation of physiological conditions at sites of infection that afford a more defined assessment of overall activity of the efflux pump system. The method has been successfully applied to characterize intrinsic and over-expressed efflux pump systems of *Escherichia coli*, *Salmonella* Enteritidis, *Enterobacter aerogenes*, *Enterococcus faecalis* and *Enterococcus faecium*.

The clinical importance of P-glycoprotein related resistance in cancer has led to the investigation of the inhibiting properties of several compounds of natural and synthetic sources. The real-time fluorometry has been employed to detect and demonstrate the activity of the eukaryotic ABC-transporter P-glycoprotein (Pgp or ABCB1), in the presence of potential P-gp modulators.
The aim of the real-time fluorometric method is to easily and accurately detect and quantify the transport of the Pgp substrate EB through the cell membrane, at working concentrations that will not affect cell viability nor perturb cellular function, in order to readily assess efflux activity in L5178 mouse T-cell lymphoma cells transfected with the human *ABCB1* gene.

The fluorometric assay is a new application of the Rotor-GeneTM 3000 (Corbett Research, Sydney, Australia) real-time thermocycler, provides information about transport kinetics and thereby offers a rapid, high-throughput, reproducible, accurate and inexpensive screening of efflux pump inhibitors.

INFLUENCE OF CALCIUM AND PH IN THE ACCUMULATION AND EFFLUX OF EB

Ana Martins[1,2], Gabriella Spengler[1,2], Susana Costa[1,?], Miguel Viveiros[1] and Leonard Amaral[1,2]

[1]Unit of Mycobacteriology, [2]UPMM, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL), Rua da Junqueira, 96 1349-008 Lisbon, Portugal

Efflux of antibiotics by *E. coli* AG100 is performed by a variety of transporters, efflux pumps (EP), that ensures the survival of the bacterium in widely diverse media. Because calcium ions (Ca) are extremely important for cell signalling, membrane transport channels and activity of ATPases that provide energy functions, the role of Ca in the extrusion of an efflux pump substrate under conditions that challenge the bacterium was investigated.

Ethidium bromide (EB) is a substrate of bacterial EPs. Accumulation and efflux of EB inside *E. coli* AG100 was followed at 37ºC, under different pH of medium, with and without metabolic energy (glucose), and in the presence and absence of Ca and/or EDTA. The signal of EB inside the cell was automatically detected by a real time method that distinguishes accumulation from efflux.

At pH 8 the accumulation of EB is glucose (GLU) dependent and greater than at pH 5. At pH 8, chlorpromazine (CPZ) augments the retention of EB, especially with the omission of GLU. This retention, at pH 8, can be nullified by the addition of Ca. The role of Ca is further illustrated with the addition of the divalent chelator EDTA. The addition of Ca to an EDTA containing medium nullifies the accumulation promoted by EDTA. The simultaneous presence of CPZ and EDTA synergistically increases accumulation. At pH 5 the effects of CPZ, EDTA and Ca are minimal.

Ca is needed for a variety of metabolic and energy deriving pathways within the cell like ATPases that hands protons for activation of ABC type transporters. Our results suggest that there are 2 general types of transporter systems in *E. coli*: one that is dependent upon metabolic energy and is evident at pH 8, and another, general, that is demonstrable at pH 5 and which consists of 8 or more EPs that include the main EP of this organism, the AcrAB-TolC pump. These latter EPs are dependent upon protons present in the periplasm for their activation. The concentration of periplasmic protons is controlled by the concentration of protons at the surface of the cell that is lower pH, due to their attraction to the lipopolysaccharide layer. Whereas at pH 5 the concentration of surface protons is readily maintained, at pH 8 the dissociation of protons from the surface into the bulk medium is great, reducing the availability of protons to the periplasm. Hence, when *E. coli* is challenged by a noxious agent, the extrusion of this agent is made possible, at pH greater than 7, by an ABC type transporter.

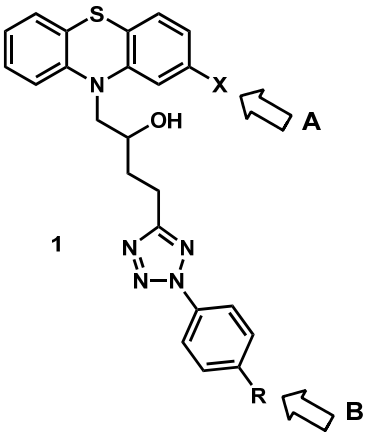
NEW HETEROCYCLIC MOLECULES IN THE SERVICE OF RESEARCH ON MDR INHIBITION

György Hajós, Zsuzsanna Riedl and Daniella Takács

Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences

In the Laboratory for Heterocyclic Chemistry of the Institute, considerable efforts have been made during the recent years in order to recognize new active MDR modulators. In the lecture, a concise survey on the most important compound-types will be provided and, furthermore, results of some recent synthetic studies will be summarized.

As a continuation of earlier investigations[1], new synthetic pathways have been elaborated to a new group of phenothiazines having unprecedented substitution pattern.



The general structural formula (1) of the new derivatives reveals that modification of the substituents on the phenothiazine ring (replacement of X, arrow A) and the benzene ring (replacement of R, arrow B) allow the synthesis of a series of new molecules of high diversity.

Methodologies of these structural changes will be summarized and results of the first measurement of MDR reversal will be evaluated.

Reference

[1] I. Nagy, Zs. Riedl, Gy. Hajós, A. Messmer, N. Gyémánt, and J. Molnár: Synthesis of New Tetrazolyldienylphenothiazines as Potential Multidrug Resistance Inhibitory Compounds. *Arkivoc,* **vii**, 177-182 (2004).

INHIBITION OF DRUG RESISTANCE OF BACTERIA AND CANCER CELLS.

Joseph Molnár[1], Ilona Mucsi[1], Yvette Mándi[1], Mihály Novák[2], Attila Zalatnai[3], Leonard Amaral[4]

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In 1891 Ehrlich supposed that the distribution of the dye stuff in the body is not uniform due to different affinity of various tissues. The observation was a basis of selective toxicity against pathogens. After the introduction of chemotherapeutics the resistance appeared. Genes responsible for emergence of multiple resistance to antibiotics in bacteria and cancer cells were suppressed by down regulation of the gene expression and the inhibition of drug-transporters of bacteria and tumour cells. Antibacterial and antiplasmid effects of phenothiazines and structurally related compounds were shown on Gram positive and negative bacteria: *B. anthracis, C. hoffmanni, S.pneumoniae S.aureus, S.pyogenes, S.viridans and E.coli, K.pneumoniae, P.vulgaris, P.aeruginosa, Mtb.* Phenothiazines formed charge transfer complexes with bacterial endotoxin, complex was not able to induce tumor necrosis factor. Elimination of plasmids as *E. coli* R, F-prime, HlyB, *Acinetobacter* R, *Rhizobium melliloti* Nod, virulence plasmid *Yersinia enterocolitica* vir*, and Agrobacterium tumefaciens* Ti, were induced.
in ecosystems. Resistance modifiers acting on ABC and proton pumps synergize the activity on various antibiotics in *E. coli, E. faecalis, H. pylori, P. aeruginosa and S. epidermidis.* Inhibition of plasmid replication was found in monocultures and in the presence of other bacterial populations.

One of the main reason for the failure of cancer chemotherapy is the ABC transporter-mediated multidrug-resistance which can be reversed by various compounds (phenothiazines, Sila derivatives etc), without stereo selectivity in vitro by and ex vivo on different tumor cells by measuring R123 uptake in flow-cytometry. The biological effects were dependent on chemical structures of resistance modifiers of cancer cells. The binding of the resistance reversing compounds to their targets occurs via charge transfer complex formation. The kinetic resistance is the consequence of discontinuous growth of solid tumors. The differences in entropy production of normal and cancer tissues will be compared and discussed based upon some current research. The reversal of entropy flow may modify the development of solid tumors due to the changes in signal transmission in the tumor host entity.

COMPARISON OF THE ANTITUMOUR ACTIVITIES OF SELECTED STEROIDAL COMPOUNDS ON MDR AND A2780CIS CELL LINES

Julianna Serly[a], Iren Vincze[b], Laszlo Hodoniczki[c], Csaba Somlai[d] and Jozsef Molnar[a]

[a]Department of Medical Microbiology and Immunobiology, [b]Department of Organic Chemistry, [c]Department of Oncotherapy, Division "B", [d]Department of Medical Chemistry, University of Szeged, Szeged, Hungary

Cancer treatment by chemotherapy is often inefficient due to the appearance of multidrug resistance as the main reason for treatment failure. A number of different mechanisms can mediate the development of multidrug resistance including the increased drug efflux by ABC-transporters that due to the over-expression of certain P-glycoprotein.

Various compounds of natural or synthetic origin have been studied previously to reverse multidrug resistance in cancer cells, but androstane and estrane-type compounds have never been investigated. The aim of the presentation is to determine the effect of substituted steroids on the modulation of multidrug resistance on human *mdr1*-gene transfected mouse T-lymphoma (MDR) and cisplatin resistance on human ovary carcinoma (A2780cis) cell lines.

In our experiments, 35 chemically synthesized androstane and estrone-type compounds were studied on both cell lines. The antiproliferative activity and resistance reversal effects were measured. In the next step, the resistance modifiers with chemotherapeutic agents doxorubicin or cisplatin were evaluated. The reversal of multidrug resistance was investigated by flow cytometry using rhodamine 123. In the antiproliferative and checkerboard assays, MTT was used to determine the cell proliferation, doxorubicin (on MDR cells) or cisplatin (on A2780cis cells) was applied for combination assays.

Some particular substitution with groups containing N on steroidal skeleton resulted in effective resistance modifiers (20 compounds) on MDR cell line. A few substituted steroids (7 compounds) reduced the cisplatin resistance of human ovary cancer cells, all of them were effective resistance modifiers on MDR cell line as well. Correlation was found between cytotoxicity and the presence of O-acetyl or N-acetyl groups on the D-ring. Furthermore, the O and N atom of the functional group in 1,3-position resulted in the most active compounds in both cell types.

On the basis of the structure-activity relationships of the steroid samples, certain tendencies seem to be outlined. Both androstane and estrane derivatives can be found among the active and inactive groups, consequently the activity does not depend on the structure of the skeleton. Thus, it is rather the nature and arrangement of the functional groups that determine the activity of the compounds.

The effects of selected resistance modifier steroidal compounds in combination with anticancer agents were evaluated. One of them (11) was found to act in synergistic manner with doxorubicin on MDR cells. The modification of resistance in ovary carinoma cell line was less effective than in the transfected MDR cells since the difference between the resistance of the two cell lines is based upon various mechanisms. Therefore, different resistance modifiers have to be prepared for the two different systems according to the rational drug design.

QUORUM SENSING INHIBITION BY PHENOTHIAZINES AND ESSENTIAL OILS

Zoltán Gábor Varga[1], Mira Ágnes Szabó[1], Zsuzsanna Schelz[1], Ernő Szegedi [3], Robert Berkecz[2], Leonard Amaral[4], and József Molnár[1]

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A quorum is a the smallest number of people able to organize the decisions concerning functional activity. Similarly microbes use chemical signal molecules to make population size-dependent ”decisions” by changing their gene regulations. With a quorum sensing (QS) system, microbes can accommodate readily to changing habitats, can be responsible for antibiotic resistance, organize biofilms, produce virulence factors and antibiotics.
The inhibition of QS by phenothiazines and structurally related molecules, e.g. amitriptyline, promethazine, acridine orange, imipramine, promazine, diethazine, desipramine, desertomycin and essential oils and 5-Fluorouracil as positive control was studied with *Chromobacterium violaceum* 026 as a sensor strain, which detects short carbon chain acyl homoserine lactones (AHLs) by the development of a purple pigment.

The most effective tricyclic compounds as inhibitors of QS were amitriptyline, imipramine, promethazine, acridine orange and desertomycin. Imipramine and diethazine were moderately active, while chlorprothixene was ineffective relative to 5-Fluorouracil as positive control. Natural compounds such as essential oils were also studied in the system. The most potent oils were: rose-, geranium- and rosemary oils. The quorum sensing was moderately inhibited by lavender, eucalyptus and cirus oils, while the chamomile-, orange and juniper oils were ineffective for QS inhibition. In view of the demonstration that certain QS inhibitor compounds can block the QS signal response of diverse bacterial species, it is conceivable, that in the future these compounds may be used to modify biological signal transmission at a population level. The modification of antibiotic resistance in biofilms and the production of virulence factors may have practical importace. The authors suppose that the inhibition of QS in combination with conventional therapy can reduce the growth rate and virulence of certain types of pathogens.

LASER AND OPTICAL TECHNIQUES USED TO MODIFY MOLECULAR STRUCTURES OF MEDICINES

Mihail–Lucian Pascu

National Institute for Laser, Plasma and Radiation Physics, Bucharest

Recent clinical experience shows the development of the acquired resistance at drug treatment (MDR), signalled for antibiotics, cytostatics and other classes of drugs. Maladies like tuberculosis, malaria, viroses, hepatitis which once were considered as eradicated reappeared, exhibit now a new stage of development and require new treatment procedures. In line with these, the malignant tumours develop also resistance to treatment with cytostatics, particularly with the most often used in cancer treatment, such as MTX (methotrexate), 5-FU (5–fluorouracil) and others.

In general, as far as the medicines proper are concerned, there are currently two ways to fight maladies by using drugs:

- production of new drugs, which requires extensive research and development, is costly, time consuming and needs special quality assurance procedures.
- modify existing drugs by using different procedures in order to make the modified drug forms more efficient in fighting the maladies; this is recommended mainly for medicines used in cancer treatment and action against bacteria.

In order to improve the medicines action one may also develop new methods and means to transport them to targets, such as micro- and nano-droplets with controlled content.

The cytostatics chosen for the applications are given in the table and were developed for the project needs at the University of Marseille, Faculty of Pharmacy.

Code number	Series	Bp (°C)	Chemical formula
BG 186	Acridine	64	*(4-methoxy,9-thio [2-N-N-diethyl diazantracene)*
BG204	Acridine	128	*(3-amino,9-thio [2-N-N-diethyl-1,9-diazantracen-10-one]*
BG 558	Naphthyridine	130	*1,9-diazantracen-10-one] amino-ethyl] acridine)*
BG1120	*Pyridoquinoline*	*115 – 117*	*(1,8 bis [thio(2-N-N amino-ethyl acridine) diethylaminoethyl)], 9methyl 4,5-diazantracene)*

The cytostatics were prepared in water solutions at different pH values (between 4.0 and 8.4) controlled by addition of NaOH; they were exposed to optical radiation emitted by a cw Xe lamp; the irradiation / exposure time varied, typically, between 4min and 180min.

The measurements of the absorption and fluorescence spectra were performed for all the above mentioned compounds after exposure to uncoherent optical radiation. Important modifications of the spectra were measured for BG 204 and BG1120 showing that the molecular structures are strongly modified under the influence of the optical radiation. According to the spectral data, tautomeric forms of both molecules are obtained.

The same kind of data were obtained for cytostatics which have, at the same time, antibiotic action, such as doxorubicin and daunorubicin.

The medicines solutions were exposed to laser radiation as well; new data about this type of experiments will be reported.

In order to measure the cytotoxic effects of the BG compounds, particularly of the BG1120 the action of solutions of these compounds was measured on different types of cell lines.

Further measurements of the effects of irradiated cytostatics in the BG class were performed on pseudotumour tissues produced on cornea of rabbit eyes; the more efficient action of the BG compounds modified after exposure to optical radiation was evidenced by anatomopathological studies performed on the pseudotumour tissues.

The laser beam interaction with micro-droplets produced under controlled conditions, becomes interesting due to the fact that by choosing the right characteristics of the laser radiation and the suitable dimensions and compositions of the droplets one may generate droplets with pre-designed characteristics.

The resonant interaction of the micro-droplets that contain solutions of BG1120 and Doxorubicin in ultra-pure water, with laser radiation was, for the first time, studied. In both cases the molecules exhibited significant modifications after exposure to laser radiation (the pH remained all the time neutral), which were evidenced by measuring the fluorescence spectra. These modifications are obtained in shorter time in micro-droplets than in bulk irradiation of solutions.

Acknowledgements: The results were obtained within the CNMPS project 41-018/2007 and the COST networks BM 0701 – ATENS and P21-Physics of Droplets.

CULTURE OF PRIMARY HEPATOCYTES: A USEFUL MODEL FOR STUDYING ABC TRANSPORTER ACTIVITIES

László Vereczkey

Department of Pharmacobiochemistry, Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences

Biliary elimination of xenobiotics is often a very important phase of drug disposition. The determination of activity of hepatocytes in this process is perequisite in the investigation of toxic effects of a drug, in its pharmacokinetic properties and in drug development, in general.
Two kinds of hepatocyte cultures are used: Rigid and „Sandwich" cultures. This latter allows the quantative determination of the biliary and sinusoidal elimination of xenobiotics.
Modulation of activities of mrp-2, bsep, bcrp and mdr-1 has been studied by using different – mainly endogeneous - substrates and different known modulators in rat and human hepatocytes.
Mrp-2 activity was determined by using bilirubin as substrate. Indomethacin, benzbromarone, rifampicine and probeniicid decerased the activity of mrp-2, which was partially compensated for by increased activity of sinusoidal transporters (possibly by mrp-3 and mrp-4).
Bsep function was studied by using taurocholate as substrate. Cyclosporine A, glibenclamide and troglitazone inhibited the activity in a concentration dependent manner.
Bcrp activity was determined by using estrone-3-sulfate as substrate. Ko134, mitoxantrone and novobiocin inhibited its activity.
Mdr-1 activity was determined by using rhodamine-123 as substrate in the rigid culture of rat hepatocytes. A series of new compounds were investigated for inhibitory activity and a structure-activity relationship has been found.

IN VIVO EFFECTS OF MDR-REVERTANT ORGANOSILICON COMPOUNDS (SILA-409 AND SILA-421) ON HUMAN PANCREATIC CANCER XENOGRAFTS

Zalatnai,A., Füredi,A., Krizsán Á., Molnár, J.

The third-generation MDR-revertant organosilicon compounds (SILA-409, SILA-421) have been designed for enhance the effect of chemotherapeutics by inhibiting the efflux of drugs. This feature was evidenced by numerous *in vitro* studies, but *in vivo* experiments have also revealed some unexpected antitumor effects in immunosuppressed mice bearing human pancreatic cancer xenografts. Continuous (3-4 weeks) sc. or ip. administration of these compounds at a larger doses have resulted in a delay of tumor growth, an increased apoptotic, and a decreased proliferative activities. The immunohistochemical expression of membrane p-glycoprotein has also diminished in the tumor cells. Combination treatments with cytostatic drugs (vincristine, irinotecan, paclitaxel or even gemcitabine) have yielded a slight, but recognizable enhanced tumor-inhibiting effect. The compounds have altered the expression of pro-, and antiapoptotic molecules (bax, blc-2), but SILA-409 and SILA-421 behaved differently. In collaboration studies by using a mini-PET it was found that the extent of necrotic areas in the tumors was increased. The direct peritumoral administration of SILAs has shown no benefit over the ordinary sc. injection.

The *in vivo* experimental data indicate that in addition to the *in vitro* MDR-revertant properties, these organosilicon compounds do exert an antitumor effect on human pancreatic cancer xenografts without detectable side effects. Although the tumor-inhibiting effect is mild, the drugs are worth further investigating also in this field, optimizing the dosage and mode of administration.

THE ENIGMA OF MULTIDRUG TRANSPORTERS: DIRECT DRUG EXTRUSION FROM THE PLASMA MEMBRANE

László Homolya

Membrane Biology Research Group, Hungarian Academy of Sciences, Semmelweis University

The multidrug transporters, including MDR1/Pgp, MRP1, and ABCG2 bear particular importance in both pharmacological and clinical aspects. They play an important role in ADME-Tox of pharmacologically relevant drugs, and they significantly contribute to clinical multidrug resistance that compromises cancer chemotherapy regimens. The special role of these transporters both at the physiological barriers and in cancer cells is based on their extremely broad substrate recognition. Since hydrophobic molecules are known to partition into the lipid bilayer and accumulate in membranes, the “classical pump” model for the mechanism of multidrug transporter proteins has been challenged. In the early 1990s, special transport mechanisms, the “hydrophobic vacuum cleaner” and the “floppase” models were proposed for MDR1/Pgp to explain the peculiar transport properties of this transporter. Both models were based on substrate-transporter interaction within the membrane. Although this idea was supported by a number of drug-binding experiments, unambiguous evidence for the direct extrusion from the membrane has not provided yet.

In the present work, we employed the fully functional GFP-tagged ABCG2 in online confocal microscopy analysis to determine the kinetics of drug distribution in the plasma membrane and other cellular compartments using the fluorescent anti-cancer agent, mitoxantrone. We generated kinetic models describing the transport processes for both the classical pump model and the alternative models, and compared the characteristic features of these models with the experimental observations. Our results clearly indicate that ABCG2 extrudes mitoxantrone directly from the plasma membrane, providing experimental evidence for the suggested mechanism of multidrug transporters.

P-GLYCOPROTEIN IS THE ACHILLES' HEEL OF MULTIDRUG RESISTANT CANCER CELLS

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The central tenet of our work is that Pgp, a universally accepted biomarker of drug resistance, should be considered as a molecular target of multidrug-resistant (MDR) cancer. Our earlier work has unveiled the hidden vulnerability of MDR cells (Szakacs et al. Cancer Cell. 2004;6(2)129-37. 5). As a next step, we screened the National Cancer Institute's complete drug repository to identify a series of compounds showing increased toxicity in multidrug resistant (MDR) cells. Strikingly, several of these compounds show significant structural similarity to NSC73306, the first "MDR-selective" compound identified in the DTP dataset. Analysis of these compounds uncovered initial structure activity relationships and a possible mechanism of action linked to metal chelation. Selective toxicity of the "MDR-selective" compounds is specifically tied to the activity of Pgp, suggesting that Pgp may be considered the "Achilles' Heel" of MDR cells, representing a fatal weakness that should be exploited by a new modality for tackling MDR in cancer.

ANTIMICROBIAL ACTIVITIES OF NEUROTROPICS IN VITRO AND IN VIVO

THE NEED FOR A GENERAL THEORY OF THE HOST ORGANISM, MICROORGANISMS AND DRUGS

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The aim of these investigations has been to throw light on the question whether drugs other than antibiotics and chemotherapeutics exert an antimicrobial effect. In order to elucidate this, the antimicrobial effect of selected psychotherapeutic drugs and there stereo-isomeric analogues was studied.

The development of psychotherapeutics from the blue dyes has been reviewed against the background of its history considered as a scientific idea. It is demonstrated that psychotherapeutic drugs have different antimicrobial activities.

Stereo-isomeric analogues of known psychotherapeutic drugs also have antimicrobial effect both *in vitro* and in combination with known antibiotics *in vivo*. The selectivity of the various stereo-isomeric compounds depends on which microorganism and which specific chemical compound is investigated.

The antimicrobial activities of neurotropic compounds are independent of the antihistaminic, antihypersecretory, and of the known neuroleptic and antidepressant effects of these drugs.

The examples chosen in these investigations of the antimicrobial effect of psychotherapeutic drug and there stereo-isomeric analogues *in vitro* and *in vivo* lead to the conclusion and the perspective in the present study.

There is a need for a general theory of the interplay between host organism, microorganism and drugs. This proposition is based on a concern to argue against the view that the prokaryotic effect of eukaryotic-directed drugs is without major significance, either for scientific research or for clinical treatment. Mathematical modelling might be of help in that direction!