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PP01

SIBUTRAMINE IN SLIMMING DIETARY SUPPLEMENTS

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Introduction: Overweight and obesity might be classified as civilization diseases. Many people try to solve their problem with easy solutions like using herbal products. Sliming products are some of the most sold over the counter (OTC) and the food supplements products in EU. The most dangerous are the food supplements to which medicines, like sibutramine, are added as adulterants, since the patient believes he is taking a supplement, mostly herbal based and thus safe. In 2010, sibutramine was removed from the European market because of the risk of non-fatal cardiovascular events (stroke, heart attack). Since the number of slimming products grows, it is important for the regulatory instances to check these products for their quality and composition in order to evaluate the health risk for population.

Material and Methods: Our study included health quality control on sibutramine in slimming dietary supplements (tee, coffee, chocolate, capsules and all kind of syrups) purchased from Croatian market using high-pressure liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS-MS). Also, the method was developed and validated.

Results: For sibutramine and N,N-didesmethylsibutramine MS detection was performed in negative mode using Multiple Reaction Monitoring (MRM). The drugs were isolated from sample remedies using simple methanol extraction. The target drug was separated using reversed-phase liquid chromatography on chromatographic column Zorbax SB C_{18} (150 mm × 2.1 mm, 3.5 µm) with a gradient elution using acetonitrile – 0.1% formic acid mobile phase at a flow rate of 0.2 mL/min. Results showed that more than quarter of these „healthy and harmless" products are contaminated or replaced with active substance sibutramine in five years period.

Conclusions: Regular market control of slimming food supplements on sibutramine, by the method for identification and quantification described in our study, could prevent the health risk for population.

Literature Reference:

Stypułkowskaa K et al. J Pharm Biomed Anal 2011; 15:969–75.; Dunn JD et al. J Pharmaceut Biomed 2011; 54:469–74.

PP02

POPULATION PHARMACOKINETIC STUDY OF MYCOPHENOLIC ACID IN PATIENTS WITH LUPUS ERYTHEMATOSUS NEPHRITIS

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Objectives: To develop a population pharmacokinetic (PK) model Mycophenolic acid (MPA) after administration of Mycophenolate Mofetil (MMF) in patients with nephritis caused by lupus erythematosus (LE).

Methods: The study consisted of n=18 patients and all patients were receiving daily MMF doses prior to study participation. MMF doses were720 mg, 1 kg and 1.50 kg. Blood samples for MPA determination were collected at 0h (pre dose) 0.5h, 2h and 4h after commencement and were assayed by an HPLC method. After the development of the basic model covariates were screened. The final PK model was validated using nonparametric bootstrapping and a visual predictive check (VPC). Plasma concentration-time data of MPA were analyzed using NONMEM version 7.3.

Results: The final model was a one compartment model with first order absorption and additive error, with diagonal interindividual variability on all three parameters. No significant covariates were detected Final model parameter values were CL=14.6 L/h, Vd=35.2 L and ka=4.14 h^{-1} with IIV 48.9%, 83.2% and 185%, respectively. Goodness of fit assessment using diagnostic plots such as DV vs PRED were considered reasonable. Internal validation by VPC resulted that the model describes well the data including the observed variability.

Conclusions: A preliminary PopPK model for MPA after administration of MMF was developed, that is intended to serve as a prior information for the Bayesian Individualization of MPA levels in Greek hospitals. The model will be enriched with more patients including also patients receiving MPA after renal transplantation.

PP03

AN ACCOUNT OF MODIFIED RELEASE OF MELATONIN FROM SOLID PHARMACEUTICAL FORMULATIONS

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Introduction: During the last decades, a huge number of modified release systems have been developed as they display several advantages over conventional systems used in therapeutics. The aim of this work was to extend our previous studies on melatonin controlled release profiles, by comparing its release from uncoated, compression-coated and bilayer tablets.

Material and Methods: Each tablet was comprised of melatonin and combinations of the following: Ethylcellulose, polyvinylpyrrolidone (MW:10.000), dextran, low viscosity sodium alginate, Avicel PH 102, lactose monohydrate, iron oxide pigment red 30 and magnesium stearate. The coated tablets consist of the same drug and inner excipients core, but different outer coating shells. As for the bilayer tablets, they incorporate an immediate release layer and a sustained release layer. The dissolution experiments were carried out in a USP XXIII dissolution apparatus I at a rotation speed of 50 rpm, in aqueous medium, at 37 ± 0.5 ^{o}C. For simulating the conditions along the gastrointestinal tract, the release of melatonin was studied for ten hours, the first three hours at pH 1.2 and the rest seven at pH 7.4. Samples (5 ml) were withdrawn at predetermined time intervals, filtered and analyzed at λ_{max}=278 nm using a Perkin–Elmer UV spectrophotometer.

Results: The results obtained reveal that the initial release of melatonin is slower from coated tablets than from the respective uncoated. The use of lactose in the outer coating shell, instead of PVP, results in a lower release in acidic medium for the first 180 min. In the pH 7.4 medium, lactose leads to a 100% release at 420 min, whilst PVP sixty minutes later. The increase of dextran concentration in the outer coating shell delays the drug release at both pHs (coated tablets). In uncoated tablets, the formulations with ethylcellulose, dextran and lactose monohydrate show similar release profiles, although the presence of PVP seems to delay the drug release. The release of melatonin from bilayer tablets, with their immediate release layer consisting of lactose monohydrate, sodium alginate and Avicel PH 102, is initially faster than from both coated and uncoated tablets.

Conclusions: This study demonstrates that the assembly of tablets with and without coatings and one or two layers, has a profound effect on the controlled release profile of melatonin.

Literature Reference: M. Vlachou, A.Tsiakoulia, A. Eikosipentaki, Controlled release of the pineal hormone melatonin from hydroxypropylmethylcellulose/sodium alginate matrices in aqueous media containing dioctyl sulfosuccinate. *Curr. Drug Discov. Technol.,* 4, 31-38, (2007).

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PP04

ANALYSIS OF PATIENTS' BELIEFS, OPINIONS AND ATTITUDES TOWARDS GENERIC DRUGS AND GENERIC SUBSTITUTION IN REPUBLIC OF CROATIA

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Abstract:

Introduction: The ever increasing drug use and consequent rise in healthcare cost led to the search for opportunities for cost reduction and saving. One of the cost-saving measures that can be taken without reducing the quality of healthcare is to increase the share of generic drugs. The launch of generic drugs on the market brings along, apart savings, a better availability of drugs to a wider range of patients in comparison with the original drugs.

The extent to which the policy of generic substitutions is effective depends on the actions taken not only by prescribers and pharmacists, but also by patients. The aim of the study was to map and analyze patients' opinions and attitudes towards generic drugs after two years from its legislative embodiment in Republic of Croatia.

Material and method: The questionnaire consisted of questions concerning the issue of generic drugs from patients' perspective. All collected data were analyzed using descriptive statistics and correlations were tested by selected parametric tests.

Results: A significantly high proportion of patient was generally confused (85%) about introductions of generic substitution. Major issues related with generic medicines seem to be represented by diffuse skepticism about safety and therapeutically effects of generic drugs (73%). However, the frequency of patients opposing substitution was low (23%).

Conclusions: For increasing generic medicine prescription rate, patients needed more information and educational interventions because substitution is a collaborative act: it does not depend solely on the will of government regulation and experience of doctors and pharmacists.

Literature reference:

1. M. Geitona, D. Zavras, M. Hatzikou, J. Kyriopoulos (2006) Generics market in Greece: The pharmaceutical industry's beliefs. *Health Policy* 79, 35 - 48.

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PP07

NOVEL $Al^{18}F$-CHELATES-CONJUGATED MANNOSYLATED DEXTRANS AS SENTINEL NODE DETECTION AGENT FOR PET IMAGING

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Introduction: Sentinel node imaging is a nuclear medicine procedure that identifies the first lymph node receiving lymphatic flow from the primary tumour site. It is performed by injecting small radiolabelled particles (20 to 500 nm) in the area where a tumour is located. Studies with ^{99m}Tc-labelled mannosylated macromolecules like albumin, polylysine and dextrans have shown that particles with smaller size (<10 nm) migrate faster from the injection site but they can be trapped, in a saturable mode, by the sentinel node. The retention is due to the recognition of the mannosylated macromolecules by the mannose receptors of the lymph node. Among them, ^{99m}Tc-labeled Lymphoseek recently, approved by the FDA, consists of a dextran backbone, 23 amino, 55 mannose and 8 DTPA moles per dextran unit.

Purpose: Aiming to synthesize two new novel mannosylated dextrans compounds carrying the $Al^{18}F$ chelator NOTA.

Materials and methods: The mannosylated DCM20, DCM30 were synthesized followed by the coupling with NCS-Bz-NO2A. Purification of the compounds has been performed by ultrafiltration and characterization has been based mainly on NMR. $Al^{18}F$ was prepared by adding 100 μL of 2 mM $AlCl_3$ solution to a 0.4 ml ^{18}F. To the prepared $Al^{18}F$ solution, the DCM20(NCS-Bz-NO2A)(**1**), DCM30(NCS-Bz-NO2A)**(2**) compounds (0.5 mL of 1 mg/mL solution) were added and the labeling mixture was heated at 110 °C for 15 min for **1** and 30 min for **2**. Quality control and stability studies have been performed by TLC and HPLC.

Results: Reaction of a 10 KD dextran with allyl bromide yielded the intermediate allyl dextran with about 40% coupling. Addition of cysteine to allyl dextran resulted in the dextran-S-cysteine derivatives DC15 and DC25. This compounds were mannosylated (approx. 65%) by coupling to the *in situ* activated cyanomethyltetraacetyl-1-thio-D-mannopyranoside. Subsequently, reaction of the NCS-Bz-NO2A with free amine groups resulted in the formation of the final compounds. $Al^{18}F$-DCM20(NCS-Bz-NO2A) and $Al^{18}F$-DCM30(NCS-Bz-NO2A) with high radiochemical purity were successfully prepared and was proven to be stable at physiological conditions.

Conclusions: The results of this study suggest that the easily labeled $Al^{18}F$-based compounds provide a highly promising approach for the development of a PET radiotracer for sentinel lymph node detection.

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PP08

ANTIBODY- DRUG CONJUGATES ARE ALREADY MARKETED: LESSONS LEARNED

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Purpose: The present study deals with the examination of clinical use and pharmacological properties, as well as the safety of antibody-drug conjugates that are marketed. Ado-trastuzumab emtasine and brenduximab vedotin were examined regarding their mechanism of action, pharmacology, clinical use and safety.

Materials and Methods: Systemic search and review of papers regarding approved of antibody-drug conjugated took place via MedLine and abstract presentations of international conferences.

Results: *Antibody-drug conjugates represent an innovative therapeutic application that combines the unique properties of monoclonal antibodies with the potent cell killing activity of cytotoxic bioactive compounds.* Ado-trastuzumab emtasine (T-DM1) is a an antibody-drug conjugate consisting of the HER2 monoclonal antibody trastuzumab conjugated to the maytansinoid DM1 via a nonreducible thioether linkage with potential antineoplastic activity. It was approved for use in advance breast cancer. It was approved for use in advance breast cancer in February 2013 and in November 2013 by FDA and EMA respectively under the brand name Kadcyla® [1]. On the other hand, Brenduximab vedotin is an antibody drug conjugate consisting of cAC10 anti-CD30-specific chimeric IgG1 antibody, monomethylauristatin E (MMAE) a microtubule-disrupting agent and a protease cleavable dipeptide linker. It was approved for use in refractory Hodgkin and systemic anaplastic large cell lymphoma in August 2011 and in October 2012 by FDA and EMA respectively under the brand name Adcetris® [2].

Conclusions: In conclusion, the unique properties of the above antibody-drug conjugates are that these so-called *armed antibodies* selectively dispatch highly potent cytotoxic anticancer chemotherapies directly to tumor tissues while, at the same time, leaving healthy cells unaffected.

References: [1] Krop IE, LoRusso P, Miller KD, et al. 2012. A Phase II Study of Trastuzumab Emtansine in Patients With Human Epidermal Growth Factor Receptor 2-Positive Metastatic Breast Cancer Who Were Previously Treated With Trastuzumab, Lapatinib, an Anthracycline, aTaxane, and Capecitabine. J Clin Oncol 30(26):3234-41. **[2]** Younes A, Bartlett NL, Leonard JP, et al, 2010. Brentuximab Vedotin (SGN-35) for Relapsed CD30-Positive Lymphomas. N Engl J Med, 363(19):1812-21.

PP09

TEMPERATURE-DEPENDENT DRUG RELEASE FROM INNOVATIVE POLYMER GRAFTED LIPOSOMES.

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Purpose: Novel polymer-modified thermoresponsive liposomes were developed for the delivery of indomethacin in order to control its release profile.

Materials and Methods: When attached to 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes, the end functionalized $C_{12}H_{25}$-poly(N-isopropylacrylamide)-COOH (C12-PNIPAM-COOH) polymer was membrane-disruptive in a temperature-dependent manner. The interest for this polymer is driven by the famous lower critical solution temperature (LCST) behavior, where heating an aqueous dispersion of PNIPAM above 32°C induces nanophase separation.

Results: The physicochemical/structural behavior of these polymer-modified thermoresponsive liposomes was found to depend on the PNIPAM: lipid molar ratio and the composition of the polymeric guest. The incorporation of polymer has caused alterations in the thermotropic behavior of DPPC liposomes, as the Differential Scanning Calorimetry (DSC) curves revealed. The drug loading and the release were found to be strongly dependent on the thermotropic characteristics of the polymer grafted DPPC liposomes. Namely, the *in vitro* release is immediate at 37°C (>LCST) ("burst" effect), while the prepared mixed nanocarriers did not released the encapsulated bioactive substance at 32°C (<LCST). The modulation of the thermosensitivity of polymer grafted liposomes was achieved by varying the ratio of the nanocarriers' components. Temperature-dependent release of indomethacin was observed from chimeric liposomes, due to the well known thermotropic conformational transition of the grafted C12-PNIPAM-COOH chains. The inhomogeneous drug distribution inside the polymer liposomes could be a possible explanation for the "burst" release of the incorporated indomethacin at 37°C.

Conclusions: In conclusion, we modulated the thermoresponsivity and the drug loading/release properties of the prepared formulations by varying the ratio of components, as well as the molecular characteristics of the polymeric guest.

PP10

HERBAL DRUGS WITH ANTIOXIDANT ACTIVITY IN CROATIAN ETHNOMEDICINE

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The traditions of folk medicine in Croatia have been preserved for centuries (from the 14th to 20th centuries) in the manuscript collections of recipes for preparing remedies called folk medicine books (*Ljekaruše*). *Ljekaruše* are usually written by priests who often took on the role of doctors in the Middle Ages. They were written under the influences of Slavic, Greek, Roman, Arabic and medieval Salerno medicine. Folk medicine books in Croatia were written in Latin alphabet, old Slavic script called *glagoljica*, and Croatian Cyrillic alphabet. These manuscripts are important for ethnographic and philological studies but they also have a historical medical and pharmaceutical importance because they reflect the traditions of folk medicine. They represent an interesting source of data about treatments with plants that are neglected in official medicine regardless of possessing healing effects.

Herbal drugs are a natural source of antioxidants, which are widely used for the treatment of many diseases caused by oxidative stress. Oxidative stress occurs due to a disturbance in the balance between the production of reactive oxygen species and cellular defense by cellular antioxidants.

Here, the medicinal herbs with antioxidant activity that are the subjects of scientific research were compared with those used in Croatian ethnomedicine (*Artemisia absinthium* L., *Achillea millefolium* L., *Betula pendula* Roth., *Centaurium erythraea* Rafn., *Morus alba* L., *Sambucus nigra* L., *Salvia officinalis* L.) which can be found in folk medicine books.

PP11

OPTIMIZATION OF CYCLODEXTRIN-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM MEDICAGO SATIVA L.

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Purpose:
In this study, the response surface methodology (RSM) was applied in order to optimize the cyclodextrin-assisted extraction of phenolic compounds from *Medicago sativa* L., as well as to optimize the antioxidant activity of the extracts prepared.
Material and Methods:
Box Behnken design was used to investigate the effects of three variables: concentration of (2-hydroxypropyl)-β-cyclodextrin (HPβCD) (mM), time of extraction (min) and ultrasonication strength (W) on the extraction of polyphenolic compounds and the antioxidative potential of the obtained extracts. Selected variables were coded at three levels and their actual values were selected on the basis of preliminary experimental results. Phenolic content of the extracts was determined using Folin Ciocalteu reagent. The radical scavenging and chelating properties of the extracts were determined through reactivity with the stable 2,2-diphenyl-1-picrylhydrazyl radical and ferrozine, respectivelly. Results were expressed as EC_{50} in mg of herbal material equivalent (HME) per mL. Data was analysed using Design Expert software.
Results:
The obtained results indicated that the optimal conditions for aqueous extraction of phenolic compounds were 25 mM HPβCD, 45 min, and 456 W. Extraction using 25 mM CD, 42 min and 760W gained extracts with simultaneously maximised antiradical and chelating activity. Extraction was repeated under optimum conditions to verify the validity of the model. The experimental value obtained for total polyphenols was 2.33 mg/mL, which was closely matched with the predicted value of 2.46 mg/mL. In addition, EC_{50} values for antiradical and chelating activity were 76.3 and 296.1 mg HME/mL, while predicted values were 80.6 and 294 mg HME/mLrespectively.
Conclusion:
Experimental values were in close agreement with those predicted, thus indicating suitability of RSM in optimizing the cyclodextrin-assisted extraction antioxidative compounds from *M. sativa*.

PP12

NOVEL INHALED MEDICINES FABRICATED USING NATURAL CELLULOSE NANOCARRIERS

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Purpose: The natural polymer cellulose is a renewable, biocompatible organic compound suitable for the ecologically friendly production of pharmaceutical dosage forms. The aim of the project was to determine if cellulose derived from the "recalcitrant" cell walls of the microalgae *Nanochloropsis gaditana* could be used for the fabrication of nanoparticles which provide on demand drug delivery.

Materials and Methods: Cellulose derived from the *N. gaditana* (algae cellulose) was characterized using attenuated total reflectance Fourier transform infra-red spectroscopy (ATR-FTIR), thermogravimental analysis (TGA) and diffractive scanning calorimetry (DSC). Nanoparticles were prepared using a nanoprecipitation technique. They were characterized using photon correlation spectroscopy (PCS) and atomic force microscopy (AFM). Pluronic L62D, a non-ionic surfactant, was used to trigger the opening of the nanoparticles. The response to the trigger was determined using PCS through changes in the size distribution and attenuated light scattering at t = 0, 3 and 20 hours.

Results: The ATR-FTIR, TGA and DSC data indicated that algae cellulose displayed different characteristics compared to the commercial cellulose commonly used in pharmaceutical science. AFM measurements showed that the algae cellulose formed spherical nanoparticles with sizes of 164 ± 38 × 171 ± 21 nm (height × horizontal measurement). When Pluronic L62D was added into the nanosuspension, the profile of the particle size distribution changed from a unimodal to a bimodal distribution and the derived count rate dropped significantly ($P<0.01$, t-test) from 167380 ± 33847 kcps in the control sample (without the trigger) to 37063 ± 11750 kcps in the test sample (with the trigger) 5 min after the addition of Pluronic L62D. Loaded nanoparticles with the anti-tuberculosis drug rifampicin showed an immediate drug release after the application of the trigger which suggested successful "on-demand" delivery.

Conclusions: Algae cellulose formed nanoparticles which have the potential to be used in medicines. Nanoparticles responded immediately to surfactant trigger, therefore they have the potential to be used for controlled delivery.

PP13

PHARMACOKINETIC STUDIES: IN VITRO AND IN SILICO APPROACHES

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The prediction of the pharmacokinetic phenomena involved during the drug release from a pharmaceutical form after an oral administration is a key topic.

Purpose. This work aims to study these phenomena and the drug fate along the human body using both in vitro and in silico models.

Materials and Methods. To reproduce the stomach fluid-dynamics and the absorption phenomena taking place through the intestinal wall, two in vitro models are proposed. The first mimics the peristaltic waves and the mixing behavior in the stomach and was tested using commercial tablet to evaluate the mechanics influence on drug release. The second model was realized using an hollow fibers filter, which mimics the intestinal wall and the mass transport between the intestine and the circulatory system. Finally, a physiologically based pharmacokinetic model is proposed to evaluate the drug concentration along the human body. The in silico model is composed by 7 compartments, each with a specific function and it is able to simulate the plasma concentration starting from the in vitro drug release profiles.

Results. The release behavior of a commercial tablet tested in the in vitro stomach was found very different to the one obtained using a conventional dissolution method (i.e. USP Apparatus II). The drug concentrations at the top and at the bottom of the in vitro model are different, reflecting the real physiological behavior, in which the stomach content is not perfectly mixed. Using the mass transport model, the drug decrease in the intestine due to the exchange phenomena can be evaluated. Finally, the model simulations were compared with the experimental data founding a good agreement between the two tools.

Conclusions. In this work a combined in vitro/in silico approach to mimic the pharmacokinetic phenomena involved during the drug release closer to the real physiology is successfully used

PP14

HYDROGEL-BASED CRSs ANALYSES: TESTING AND MODELING

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Hydrogel-based Controlled Release Systems (CRSs) are widely used in pharmaceutical preparations. The hydrogel swollen layers play a key role to control the release pattern, therefore the understanding of the hydrogel mechanical response, beside the drug release kinetic, is needed.

Purpose. This work aims to characterize the behavior of an hydrogel-based system using both an experimental and a mathematical modeling approaches.

Materials and Methods. HydroxyPropyl-MethylCellulose (HPMC, Methocel K15) and theophylline were used to obtain pure HPMC gels and matrices for oral controlled release. The pure gels were subjected to mechanical characterization via indentation and confined compression tests with a texture analyzer. Drug loaded matrices were subjected to dissolution tests to evaluate both the release kinetic and the concentrations (drug, water, and polymer) inside the swollen matrices. The drug release was quantified via spectrophotometric analyses, water uptake and polymer release were quantified via gravimetric analyses. Using indentation tests the water distribution inside the swollen system was obtained [1]. The matrices behavior was virtualized in COMSOL using the mass transport equations coupled with a moving mesh approach to describe the system swelling [2].

Results. The mechanical analyses performed on pure gels showed the viscoelastic nature of the HPMC, leading to a characterization of its mechanical behavior after several dissolution times. From the dissolution of the matrices the evolution of the global masses of water, drug and polymer in the system was obtained for several times. The model curves were compared with the experimental results, showing that the key phenomena taking place during the dissolution were correctly identified. The water distribution inside the swollen systems was measured, at different dissolution time, finding a good agreement with the model prediction.

Conclusions. In this work the behavior of HPMC-based systems for controlled release was deeply investigated, both under an experimental and a modeling point of view.

Literature Reference

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2. Caccavo D, Cascone S, Lamberti G, Barba AA (2015)

PP15

PHARMACOKINETICS OF REMIFENTANIL: METABOLISM AND MODELING

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Remifentanil is an opioid derivative, characterized by high potency and reduced cardiovascular toxicity. It is an ultra-short acting drug and it is subjected to metabolism by esterases in blood and other tissues.

Purpose. The aim of this work is to study the Remifentanil pharmacokinetics using both an in vitro approach to study its metabolism and modeling its concentration evolution along the human body to individuate the key parameters which can lead to an individualized therapy.

Materials and Methods. To evaluate the in vitro degradation kinetic, the Remifentanil concentration evolution in a solution (initial concentration of 20000 ng/mL) kept at 37°C and Ph 7.4 (mimicking the blood value) has been evaluated during the time. Samples of the medium have been withdrawn and analyzed by HPLC. With this method, the degradation due to the temperature effect can be evaluated. Following the same procedure, the degradation effect due to the metabolism caused by the addition of esterase has been evaluated. Finally, the Remifentanil pharmacokinetics has been modeled using a physiologically based pharmacokinetic model, in order to predict the drug concentration in the blood.

Results. The in vitro results have shown that the drug is subjected to a degradation if exposed for prolonged time at physiological temperature. Moreover, the addition of esterases in the Remifentanil solution causes a further decrease in the concentration values, demonstrating a metabolic effect due to the enzymes presence in solution. The model simulations have been compared with in vivo plasma concentrations of the drug finding a good agreement between the model curves and the experimental data, both in the case of intravenous constant-rate infusion and of bolus injection.

Conclusions. The metabolism of Remifentanil has been studied in vitro to understand the key factors which influence its degradation and it has been modeled to describe, and eventually predict, its pharmacokinetics.

PP16

ENTERIC DOSAGE SYSTEMS BY ULTRASONIC ATOMIZATION OF NATURAL BIOPOLYMERS COUPLED TO POLYELECTROLYTE COMPLEXATION

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Purpose: Aim of this work was to produce enteric shell-core microparticles, based on the polyelectrolyte complexation between a cationic natural biopolymer and the anionic (meth)acrylate copolymers Eudragit®, to encapsulate gastrolesive drugs.

Material and Methods: Shell-core microparticles were produced by the home-made apparatus described in [1], using the ultrasonic atomization for the microencapsulation process [2]. Core and shell solutions, made of the cationic chitosan, a viscous agent and the anionic indomethacin (a nonsteroidal anti-inflammatory drug), and only chitosan, respectively, were nebulized by the coaxial ultrasonic atomizer and placed in contact with the complexing solution of the anionic Eudragit S100 (with a dissolution pH above 7). Then they were washed, centrifuged and freeze dried.

Results: The produced micro-particles showed high encapsulation efficiency and good gastroresistance properties. Thus they are suitable for the production of smart enteric tablets. In effect, in an environment at a nearly neutral pH (for example, in the empty stomach), common tablets can undergo a surface damage that causes leakages of the drug in a site different from the target, on the contrary in smart tablets made of micro-particles, only the micro-particles located on the tablet surface are damaged, keeping intact the more internal ones. Thus, only a little percentage of active principle is lost in the stomach, assuring that the smart tablet arrives almost unaltered to the intestine, where the drug is absorbed.

Conclusions: The polyelectrolyte complexation together with the ultrasonic atomization, used to produce shell-core fine droplets of a natural biopolymer, can be performed using mild conditions, aqueous solutions, in absence of organic solvents and chemical cross-linkers, proving to be more effective than traditional methods to obtain enteric delivery systems.

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PP17

dsDNA ENCAPSULATING IN NANOLIPOSOMAL STRUCTURES TOWARDS GENE THERAPIES

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Purpose: Aim of this work is to prepare stable and highly loaded cationic liposomal small unilamellar vesicles (SUVs) encapsulating double-stranded DNA (dsDNA simulating siRNA molecule) for the delivery in gene therapy. The liposomal vectors are produced by thin film hydration method and reduced to nanosize using a dedicated ultrasonic irradiation protocol.

Material and Methods: Liposomal vesicles were prepared by Bangham method [1] using two different formulations: PC, CHO and DOTAP at 1:0.125:1 (mol:mol) ratio and at 1:0.1:0.5 (mol:mol) ratio. Liposomes were then reduced in size in order to achieve SUVs, using a protocol based on duty cycle sonication [2]. Briefly, lipids were dissolved in organic solvent removed by evaporation, the dried lipid film was then hydrated with Tris-buffered saline containing dsDNA simulating siRNA. The preparations containing MLVs were then subjected to a first cycle of sonication. The samples were stored at 4°C for one night and then a second cycle of sonication was used to obtain SUVs. Liposomes size and zeta potential (□) analysis were performed using the Zetasizer Nano ZS (Malvern, UK). dsDNA load in SUVs was determined by lysing with ethanol followed by UV spectrophotometric assay (λ=260 nm, PBS as blank). dsDNA release studies were performed in PBS solution (pH 7.4) at 37°C. Release profile has been evaluated during 10 days.

Results: The choice of both the phospholipids formulation along with the number of duty cycle sonication used have allow to achieve dsDNA loaded liposomes of nanometric size, useable for intravenous administration. Furthermore, SUVs bilayer, being characterized by cationic phospholipids, improves the cellular uptake and provides lipids-dsDNA charge interaction. The first formulation used have leaded to achieve stable nano-liposomal vesicles of 66.3 ± 2.3 mV in □ and 0.075 ± 0.02 μm in size, but with a poor content of dsDNA, the entrapment efficiency (e.e.) achieved was 37%. The second formulation was prepared by doubling the total quantity of phospholipids, mantaining costant that of dsDNA. By this way the encapsulation efficiency was improved (55% e.e.) leading to the formation of SUVs with 52.8 ± 1.58 mV in □ and 0.056 ± 0.004 μm in size. SUVs were also found to be stable at 37°C in PBS media release for a ten days period, load and structure are remained unaltered.

Conclusions: This study demonstrated the successful preparation of a stable, highly loaded SUVs formulation containing dsDNA, to yield the basis for an improved nucleic acid based drugs shelf life for gene therapies.

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PP18

BIOACTIVE SMALL MOLECULES OF *PISTACIA LENTISCUS L. (MASTIC TREE)*: LEAF STRUCTURE, HISTOCHEMISTRY AND PHYTOCHEMICAL INVESTIGATION

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Introduction: The mastic tree is a dioecious evergreen sclerophyllous shrub that secretes, when injured, a highly appreciated ivory-colored resin. Plant parts are extensively used in folk medicine for their antimicrobial, anticancer, antiulcer, antioxidant and anti-inflammatory properties. The resin obtained from the plant is known as gum mastic or masticha. It is used in cosmetics, perfumery and food industry. There are data shown that it can cure peptic ulcers by eliminating the populations of *Helicobacter pylori.* Smashed leaves release a pleasant, resinous essence which is far more attractive and delicious in taste when it comes from the mastic trees cultivated on Chios Island.

Materials and methods: Leaves, stem and root were detached, fixed, sectioned and investigated using light, transmission and scanning electron microscopy. Histochemical tests were performed on fresh and fixed tissue employing common histochemical reagents. Extracts of the leaves and of the resin were investigated phytochemically and analyzed by GC-MS, LC-HRMS and NMR.

Results: Leaves are composite. Palisade and spongy parenchyma are rather compact. Stomata appear on the lower surface and most of them are actinocytic. The major nerves of the leaf contain a resin duct. From the stem to the leaf, the resin ducts find their way through the petiole and the central nerve. Five ducts of different magnitude are located within the phloem. The phytochemical investigation of the resin revealed terpenoids (mono, di-, and triterpenoids), while the leaves where mainly characterized by phenolic derivatives and flavonoids.

Conclusions: Stem, root and leaf anatomy of *P. lentiscus* revealed important details that can be correlated to the phytochemical profile and the bioactive small molecules produced on each part of the plant. Those data can partially explain and justify the traditional applications of the plant in the Mediterranean region.

This work was supported by IKY - State Scholarship Foundation, Athens, Greece.

PP19

INVESTIGATIONS ON THE MOST POISONUS PLANT OF THE WORLD: LEAF STRUCTURE, HISTOCHEMISTRY AND BIOACTIVE INGREDIENTS OF *RICINUS COMMUNIS* L. (EUPHORBIACEAE)

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Introduction: *R. communis* (or castor plant) holds the title of the most poisonous plant of the world according to the *Guinness World Records*. It is a perennial shrub or occasionally a soft wooded small tree with high traditional and medicinal value. The pharmacological activities reported are: antibacterial, antifungal, antivirus, anti-oxidant, antitumor, antihistamic, antidiabetic, hepatoprotective, laxative, purgative lipolytic and wound healing beyond its extremely high toxicity. Its therapeutic value is attributed to several phytochemical constituents.

Purpose: Leaf was observed to disclose its anatomical features and secretive structures. The nature of the secondary metabolites produced was also approached.

Materials and methods: Leaves were detached, fixed, sectioned and investigated using light, transmission and scanning electron microscopy. Histochemical tests were performed at fresh and fixed tissue. Extracts of leaves have been investigated phytochemically and analyzed by GC-MS and LC-HRMS.

Results: The leaf is compact, the epidermal cells posses a thick external wall, accumulate secondary metabolites and host hairs. Mesophyll cells are characterized by the accumulation of osmiophillic metabolites within their vacuole. Leaf nerves are small and compact. SEM micrographs reveal interesting features trichomnes. TEM micrographs confirm the accumulation of secondary metabolites. In the preliminary phytochemical study steroids, alkaloids (eg ricinine), flavonoids, and triglycerides were detected.

Conclusions: *R. communis* is a very important indigenous medicinal plant of the Mediterranean basin. It has various pharmacological actions that range from highly toxic to highly therapeutic depending on the plant part and the concentration of its bioactive ingredients.

This work was supported by IKY - State Scholarship Foundation, Athens, Greece.

PP20

LEAF STRUCTURE, HISTOCHEMISTRY AND SCREENING FOR BIOACTIVE METABOLITES OF *GLOBULARIA ALYPUM* L. (GLOBULARIACEAE)

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Introduction: *Globularia alypum* is a phryganic species in a genus composed of various herbs, chamaephytes or shrubs growing in Europe and the Mediterranean region in particular. The plant is known for its bioactive metabolites and thus it has been used in folk remedies for the treatment of cardiovascular, renal diseases and also as a laxative, purgative, anti-inflammatory, hypoglycemic and antibacterial agent. **Purpose:** The aim of this work was to investigate leaf structure and antibacterial activity of bioactive metabolites in leaves extracts from *G. alypum* growing in Greece.

Materials and methods: Summer and winter leaves were detached, fixed, sectioned and investigated using light, transmission (TEM) and scanning electron microscopy (SEM) along with histochemical tests. The antimicrobial activity was determined in three different chemical composition of leaf extract by using agar disc diffusion method. The antibacterial activity of winter leaves was tested against one Gram-positive (*Staphylococcus aureus* ATCC29213) and one Gram-negative (*Escherichia coli* ATCC25922).

Results: The compact, isolateral, amphistomatic summer leaf appears rather xeromorphic. The epidermal cells posses a thick external wall, accumulate secondary metabolites and host numerous small, capitate secretive hairs among them. Mesophyll cells are characterized by the accumulation of osmiophillic metabolites within their vacuole. SEM micrographs reveal interesting features of the secreting trichomnes. TEM investigations confirm the accumulation of secondary metabolites within the vacuole of the mesophyll cells. Histochemical treatments identified these metabolites as various phenolic compounds. Winter leaves did not have antibacterial activity to the tested strains.

Conclusions: *G. alypum* seems to respond to the stressful environmental conditions of the Mediterranean climate employing pathways of the secondary metabolism. It is very important to mention the season of plant collection because it is widely accepted that under stressful conditions (eg summer drought), plants accumulate many different metabolites. Those metabolites are used in various fields (medicine, pharmacology, food industry). Various researches suggest that *G. alypum* may be a promising alternative source.

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PP21

INVESTIGATION OF THE INTERACTIONS OF SUMATRIPTAN WITH CYCLODEXTRINS THROUGH PHYSIOCOCHEMICAL TECHNIQUES AND COMPUTATIONAL METHODS

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Purpose: The aim of this work was to study the interactions of sumatriptan with selected cyclodextrins (α-, 2-HP-α-, β-, 2-HP-β-, γ-, 2-HP-γ-CD) and to elucidate the role of gastrointestinal tract conditions and changes of molecular parameters in modification of solubility, stability and dissolution of sumatriptan-cyclodextrin complexes.

Material and Methods: The complexation products of sumatriptan with CDs were characterized by differential scanning calorimetry (DSC), infrared (FT-IR) and Raman (RS) spectroscopy. Possible mechanisms of formation of sumatriptan inclusion complexes were proposed as a result of theoretical calculations based on molecular modeling. The initial structure of sumatriptan and CDs were set equal to crystallography structures, if available. Otherwise, molecular dynamics was employed in order to obtain optimal conformations. The DFT method was used to minimize the structures of separated sumatriptan and cyclodextrins. Finally, optimized structures of separated molecules underwent docking with AutoDock Vina with preservation of atomic charges calculated *ab initio*. Binding modes and the affinities between sumatriptan and selected CDs were predicted by higher level calculations on docked structures. The aqueous solubility and dissolution characteristics of complexes at pH conditions (pH=2; 4.5; 6.8) simulating those of upper gastrointestinal tract were applied. The stability of sumatriptan after complexation was studied in aqueous solutions as well as in the solid state. The protective action of CD on sumatriptan was studied in condition of acidic-basic hydrolysis, oxidation, photolysis and thermolysis in dry air (RH=0%, T>353 K) and at an increased relative humidity (RH>76%). Changes of sumatriptan concentrations during stability, solubility and dissolution studies were determined by using UPLC-DAD method.

Results: The first part of the work was devoted to preparation of complexes of sumatriptan with CDs. We proved that the formation of sumatriptan complexes can be characterized by changes in DSC thermograms as well as in FT-IR and Raman spectra. Theoretical results were in accordance with those obtained in experiments. The second part of work aimed at studies of modification of properties of sumatriptan after introduction of it into cavity of CDs. The studies confirmed that interactions between sumatriptan and CDs resulted in modification of its chemical stability, solubility, dissolution.

Conclusions: The presented studies clearly demonstrate that the solubility, chemical stability, dissolution of sumatriptan can be modified as the effect of formation of its complex with CDs. The introduction of sumatriptan into CD cavities can be particularly valuable in formation of its drug delivery systems for pharmaceutical application as it can increase low bioavailability (~25%) of free sumatriptan.

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PP22

EFFECTS OF SILVER NANOPARTICLES ON PLEURAL SODIUM TRANSPORT AND ON MIGRATORY AND ADHESIVE PROPERTIES OF PLEURAL MESOTHELIAL CELLS

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Purpose: The extensive use of engineered nanoparticles (NPs) has been associated with lung and pleura pathology such as inflammation, fibrosis and cancer. Silver (Ag) NPs are used in a large number of consumer products and medical devices increasing therefore the possibility of user exposure. The aim of this study was to investigate the effects of engineered AgNPs on the sodium transport of sheep pleura and on the migration and adhesion of pleural mesothelial cells.

Material and Methods: The short circuit current (I_{SC}) and the transmesothelial resistance (R_{TM}) of sheep parietal pleura was monitored in Ussing chambers after the preincubation of the tissue with spherical AgNPs of 20nm and 60nm in size for 30 min. The effect of AgNPs on the function of the epithelial sodium channel (ENaC) was investigated by the addition of the inhibitor amiloride (10^{-5}M) apically. Moreover, the expression of αENaC subunit was analyzed by Western Blotting. The effects of AgNPs on cell migration were studied by the wound healing assay and on cell adhesion by the crystal violet assay in Met-5A cell line.

Results: AgNPs (20 nm; 2μg/ml) increased the amiloride sensitive I_{SC} indicating that ENaC function is enhanced by this treatment. The R_{TM} of the sheep parietal pleura was not altered, suggestive of no effects on the tight junctions. The levels of αENaC expression were similar in all groups. AgNPs of 60nm had no significant effects in any of the parameters. Regarding the cell functions of Met-5A cells, AgNPs of 20nm partially inhibited migration while AgNPs of 60nm caused no significant change in cell migration index. Finally, the adhesion of Met-5A cells was enhanced by AgNPs of both sizes.

Conclusions: These data suggest that AgNPs increase the ENaC activity of sheep parietal pleura and differentially affect the migratory and adhesive properties of pleural mesothelial cells.

PP23

INFLUENCE OF HYDROLYZED COLLAGEN IN COMBINATION WITH OTHER ACTIVE INGREDIENTS AND BETA-ALANINE ON SKIN ELASTICITY AND BODY MASS INDICES

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Introduction: The aim of this study was to investigate the effects of a collagen supplement (also containing grape extract, pomegranate extract, vitamins A & C) on skin elasticity after 30 days of daily administration. The impact of beta-alanine on lean body mass and skeletal muscle was also explored.

Materials and Methods: Twenty-two healthy, adult women (aged 22-50 years), who exercised twice or three times per week and did not take any other protein supplements were included in the study. All subjects ingested 5g of collagen supplement and 3.2g of a beta-alanine supplement daily for a period of one month. The elastic properties of the skin were measured before and after treatment using the 'Cutometer MPA 580' elasticity measuring probe on different skin sites (right and left arm, right and left thighs). Lean body mass and skeletal muscles were measured using the 'In Body S10' (multiple frequency bedside-BIA Instrument). Statistical analysis was applied to all indices.

Results: Twelve subjects successfully completed the study and were included in the statistical analysis. Paresthesia, due to beta-alanine co-administration, was the sole reason for the withdrawal of subjects from the protocol. The statistical analysis showed that daily ingestion of the hydrolyzed collagen supplement for 30 days produced a statistically significant increase in two skin elasticity parameters ($p < 0.000$). Beta-alanine did not produce a statistically significant increase in either lean body mass or skeletal muscle.

Conclusions: Despite the small sample-size, the daily administration of 5g of the collagen supplement improved skin elasticity after only one month of treatment, while collagen ingestion alone was reported to have no effect on elasticity[1]. Beta- alanine did not produce any increase in lean body mass.

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PP24

THE DEVELOPMENT OF A SOFTWARE SYSTEM FOR THE MANAGEMENT OF CHEMOTHERAPY FOR COLORECTAL CANCER: A PILOT STUDY

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Introduction: Time-consuming methods, characterised by a substantial risk of error occurrence, are still implemented today in the planning and preparation of chemotherapy treatments. The aim of the present study was to develop a software system capable of supporting and improving the management of chemotherapy protocols for colorectal cancer patients.

Methods: The software database created was based on globally recognised and used chemotherapy protocols from the international bibliography and updated in accordance with current treatment concepts. Overall 35 metastatic colorectal cancer and 14 adjuvant therapy protocols were included in the software. Information regarding drug side effects was also included.

The software was designed to: 1) enable the user to effortlessly enter and save patients' personal information and medical history, select an appropriate chemotherapy protocol, calculate appropriate dosage regimens for the drugs included in each protocol, schedule patients' appointments and be informed about drug side-effects and interactions, 2) provide analytic guidance for the preparation and administration of the injectable anticancer agents.

Subsequently, the software was evaluated to test its reliability. Specifically, for seven patients undergoing therapy at the IASO hospital, the dosage regimens (PO, IV or IM doses, infusion time, frequency and duration of administration) and preparation guidelines (e.g. volume of solvent) for all injectable anti-neoplastic drugs, calculated both manually by hospital clinicians and by using the new software, were compared.

Results: Our evaluation showed that the use of the software produced the most precise calculations for the preparation and administration of each chemotherapeutic agent, in the shortest possible time. Also potential errors in the administration of chemotherapy protocols were avoided and more efficient use of medical personnel was achieved, due to the fully automated process. Finally, the software database may be updated regularly in accordance with current developments and thus constitutes a useful tool.

Conclusions: Tentatively, from the results of the small pilot study, it appears that the software developed for colorectal cancer chemotherapy administration, may minimise errors, while making the provision of chemotherapy faster, easier and more precise.

PP26

PERIVASCULAR ADMINISTRATION OF ATORVASTATIN FROM A HYDROGEL/MICROPARTICLES FORMULATION DECREASES STENOSIS IN MICE CAROTID ARTERIES

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INTRODUCTION

After a bypass graft surgery, almost half of the patients are prone to develop stenosis (thickening of the grafted vessel wall) within the year following the procedure. In this work, we present a single administration drug delivery system, to be applied locally during the surgical procedure, around the
vessel, to prevent the development of the stenotic pathology.

MATERIAL AND METHODS

An o/w emulsion evaporation method was used to prepare atorvastatin (ATV) - loaded microparticles of poly lactic-*co*-glycolic acid (PLGA Resomer® 503). ATV - loaded microparticles and/or free ATV were incorporated in a cross-linked hyaluronic acid (clHA) hydrogel (Fortelis Extra®, Anteis). The release of ATV from these formulations was investigated using a Franz's cell set-up.

The formulations were deposited around the carotids of wild type mice developing stenosis, after carotid artery ligature. Sacrifice took place on day 28 following the intervention. The therapeutic effect was evaluated in terms of carotid artery wall thickening. Tissues were harvested to explore the biodistribution of ATV (HPLC/MS/MS).

RESULTS

The clHA ensured the permanence of the gel *in vivo* up to 28 days. The different combinations of free ATV and/or loaded microparticles gave different release kinetics (3 days, 45 days and burst at 3 days + sustained release for 45 days).The combination of a burst and a sustained release significantly reduced by almost two-fold intimal hyperplasia and proved to be the most effective formulation. Additionally, ATV was detected in the adjacent tissues, confirming the sustained release of ATV *in vivo*. No drug was detected in distal tissues.

CONCLUSIONS

The presence of a clHA is crucial to maintain in place the delivery system for a long term period. Perivascular administration of atorvastatin should combine a burst and a sustained release for over a month in order to decrease stenosis in mice carotid artery ligature model.

REFERENCE

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PP27

GREEK PLANTS: ANATOMY AND SUBCELLULAR LOCALIZATION OF BIOACTIVE MOLECULES

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Introduction: Millions of people in the developing world rely on medicinal plants for primary health care. Between 50.000 and 70.000 plant species are known to be used for medicine production because of the accumulation of secondary metabolites. It is well known that plants grown under Mediterranean climate conditions accumulate various bioactive molecules. Those secondary metabolites protect plants from biotic and abiotic stresss and are utilized by human beings for various purposes.

Purpose: The aim of this work was to disclose anatomical features, secretive structures and subcellular localization of bioactive molecules.

Materials and methods: Four medicinal Greek plants were examined: *Ficus carica*, *Euphorbia characias*, *Osyris alba* and *Mandragoras officinarum*. Leaves were detached, fixed, sectioned and investigated using light, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). The nature of the bioactive molecules produced was also approached by histochemical assays. Histochemical reagents were used on fresh and fixed tissues.

Results: The epidermal and mesophyll cells as well as the glandular trichomnes of all the examined plants showed accumulation of osmiophillic metabolites. Histochemical treatments identified these metabolites as phenols, tannins, alkaloids, terpenes and steroids. SEM micrographs revealed interesting features of trichomnes (glandular and non-glandular) in most of the investigated plants. Laticifers were observed at *F. carica* and *E. characias*.

Conclusions: The majority of Greek plants seem to respond to the stressful environmental conditions of the Mediterranean climate employing pathways of the secondary metabolism. This leads to the production of small, bioactive molecules. Due to those molecules, all the investigated plants in this report are used in traditional medicine for their antibacterial, antifungal, anthelmintic, anti-cancer and anti-oxidative activity. The latex of *E. characias* is well known for its synergistic interaction with antibiotic compounds.

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PP28

INFLUENCE OF MODIFIED CYCLODEXTRINS ON STABILITY AND APPARENT SOLUBILITY OF BENZOCAINE

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Purpose

The studies were aimed at evaluation the stability and apparent solubility of benzocaine in complexes with various cyclodextrins (CDs).

Material and methods

Complexes of benzocaine (BZ) with cyclodextrins (CDs) such as: α-CD, 2-hydroxypropyl-α-CD, methyl-β-CD, 2-hydroxypropyl-β-CD, γ-CD and 2-hydroxypropyl-γ-CD) were prepared by direct co-grinding. Differential Scanning Calorimetry (DSC) and FT-IR spectroscopy were employed for confirmation of the formation of the complexes. Initial complex conformations were established by docking BZ and CDs. Conformations of complexes were optimized with multistep procedure basing on molecular docking, molecular mechanics modelling MMFF94 and semiempirical PM6 method. The stability of complexes BZ-CDs (in hydrochloric acid in increased temperatures) and the dependence of their solubility on the stechiometric ratio were evaluated using UHPLC-DAD method. The apparent solubility of benzocaine after release from each complex in phosphate buffer (pH = 7.4) were also determined.

Results

Analysis of DSC curves of benzocaine in bulk substance, various CDs and the mixtures of benzocaine with each CD confirmed that in all cases the BZ-CD complexes were formed. The changes of FT-IR spectra for complexes of BZ-CDs were identified based on quantum-chemical calculations by using density functional theory (DFT) method with the B3LYP hybrid functional and 6-31G(d,p) basis set. During stability studies of BZ-CDs was proved that stability of complexes is not affected by the type of CDs, however it slightly depends on the concentration of hydrochloric acid and strongly on the temperature.. Apparent solubility of benzocaine after its complexation into CDs was significantly greater than for BZ in free form. Essential differences of apparent solubility of benzocaine in complexes with CDs in phosphate buffer were observed.

Conclusions

The preparation complexes of benzocaine with various CDs may be a crucial method of improvement its stability and apparent solubility. This beneficial effect can be applied in different dosage forms of benzocaine.

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PP29

DEVELOPMENT OF A POPPK MODEL FOR CLOPIDOGREL ACID METABOLITE IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Purpose: To develop a PopPK model based on the concentration data obtained from an easy to use HPLC-PDA plasma assay method of the inactive carboxylic acid metabolite (CCA) of the antiplatelet prodrug clopidogrel (CLP) in combination with genotyping information (PCR analysis).

Material and Methods: The clopidogrel carboxylic acid metabolite concentration data obtained (HPLC-PDA analysis method) from 50 patients suffering from ACD were analyzed. The covariates including demographic characteristics, laboratory indexes, combined medication, different generic formulations administered and genetic polymorphisms of related enzymes (CYP2C19) were screened for their influence on PK parameters. Population PK data analysis was performed using NONMEM software to describe the time course of CLP inactive metabolite in plasma.

Results: A one-compartment (1-CMT) PK model with first order absorption and elimination was found to best describe the concentration vs time data of Clopidogrel carboxylic acid metabolite (inactive). The model was parameterized as Cl=1.94 L/h, V=274 L and ka=1.45 h^{-1}. The interindividual variability was 85.8% and 45.2% for Cl and V, respectively. The application of an additive residual error model led to the optimum performance. No significant covariates were identified.

Conclusions: The derived model described adequately the concentration-time data of Clopidogrel carboxylic acid metabolite (inactive). The model is intended to serve as a prior information for the individualization of CCA levels in Greek hospitals. The model will be enriched with more patients till the end of the study, further elucidating the effect of various covariates on PK parameters.

PP30

PHARMACOKINETICS OF *CROCUS SATIVUS L.* AQUEOUS EXTRACT AFTER PEROS AND INTRAVENOUS ADMINISTRATION TO C57/BL6J MICE

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Purpose: To develop a PK model to describe serum pharmacokinetics and bioavailability of saffron aqueous extract, and especially the PK properties of crocetin, after peros and intravenous administration to mice.

Materials and Methods: A lyophilized aqueous extract of Crocus sativus L. stigmata was administered to 80 C57Bl/6J male mice (dose 60 mg/kg body weight) after reconstitution with sterile water. Mice were divided into groups of five and sacrificed at selected time points for blood and tissue sampling. Serum samples were analyzed with an HPLC-PDA method developed for crocetin (the metabolite of crocin, the main antioxidant component of the aqueous extract). Crocetin serum concentration data after both iv and peros administration were analyzed in NONMEM to describe serum pharmacokinetics and bioavailability of saffron aqueous extract.

Results: Crocin from saffron extract is rapidly absorbed through the GI tract and turned into crocetin. Crocetin serum levels were determined using standardized calibration curves. Cmax was measured and was approximately found 3±0.18 μg/mL (Tmax=15 min) and 2.7±0.06 μg/mL (Tmax=30 min) for i.v and peros administration respectively. Serum i.v and peros data were described by a one-compartment model parametrized for i.v. administration as V=103 mL, k_e=1.7 h^{-1} and k_{tr}=13.7 h^{-1} representing the rate of crocin to crocetin transformation (%RE 34.5); and for peros administration V=40 mL, k_e=3.2 h^{-1} and $k_{a,tr}$=2.02 h^{-1}, representing a hybrid rate of absorption and crocin to crocetin transformation (%RE 14.7). Crocetin was found 100% bioavailable after peros administration of crocin as saffron extract.

Conclusions: Both i.v. and peros pharmacokinetics of crocetin after single dose administration to C57Bl/6J male mice as saffron extract was adequately described by a one compartment PK model. Crocin was rapidly and completely absorbed from GI tract and measured serum crocetin levels were similar after i.v. and peros administration. Tissue levels (liver, kidneys, brain, spleen, lungs, heart) are in process of measuring in order to develop a PBPK model.

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EVALUATION OF MICROPOROUS ZEOLITES BEA AND ZSM AS FORMULATION PLATFORMS FOR POORLY WATER SOLUBLE DRUGS

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The objective of the study was to exploit two zeolitic carriers as hosts of three BCS class II drugs.

The inclusion of three lipophilic drugs, namely probucol (logP: 10), indomethacin (LogP: 4.27) and danazol (logP: 3.62) in the zeolitic framework types BEA (SiO_2/Al_2O_3: 25) and ZSM (SiO_2/Al_2O_3 : 23) was assessed via the solvent impregnation method. Empty and loaded carriers were characterized by means of scanning electron microscopy (SEM), thermogravimetric analysis (TGA), zeta potential, nitrogen adsorption measurements (BET) and differential scanning calorimetry (DSC). *In vitro* release studies were conducted in simulated gastric fluid environment pH 1.2 in the presence of 2% SLS. Induction of any structural defects to zeolitic carriers upon exposure to gastric fluids was recorded by SEM analysis.

Low drug content values were reported for the probucol loaded formulations, whereas almost total drug load was reported for indomethacin and danazol. Zeta potential shifted towards less negative values upon drug inclusion. A significant decrease in drug's crystallinity was evidenced in all zeolite loaded thermograms. A major decline above 60% in both micropore area (m^2/g) and surface area (m^2/g) of loaded zeolites indicated successful residence of drug molecules within the microporous framework structure. Different zeolite framework types may be possible to 'fine tune' the loading and release of the poorly water soluble drugs. SGF medium did not induce any structural defects on the zeolitic carriers.

Our findings indicate that zeolites could act as potential drug delivery system of poorly soluble active compounds.

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_IN VITRO_BUCCAL DRUG DELIVERY OF ROPINIROLE HYDROCHLORIDE IN THE PRESENCE OF PERMEATION ENHANCERS: THE EFFECT OF CHARGE

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In the current study the *ex vivo* permeation of ropinirole hydrochloride (RH) across porcine buccal mucosa in the presence of three permeation enhancers namely; N-trimethyl chitosan (positively charged), sulfobutyl ether-β-cyclodextrin (SBE-β-CD) (negatively charged) and hydroxypropyl-β-cyclodextrin (HP-β-CD) (neutral), was investigated. Buccal permeation studies were conducted using Franz diffusion cells. 5 mg/mL of RH with 0.05 % (w/v) of the permeation enhancers were prepared in PBS pH 7.4. Cumulative amounts of RH were plotted versus time. Infrared spectroscopy (IR) was employed to investigate the interaction of permeation enhancers with the epithelial lipids of porcine buccal mucosa. Finally light microscopy was performed to assess the histological changes resulting from the presence of the permeation enhancers.

The presence of the permeation enhancers significantly increased the transport of the drug across the porcine buccal epithelium compared to its plain congener (RH solution). The rank order effect of the permeation enhancers for the transport of RH across buccal epithelium was TMC > SBE-β-CD > HP-β-CD > RH solution.

IR spectroscopy revealed interactions between the permeation enhancers and intercellular lipids corroborating the permeation results. Histological assessment after 5 h treatment revealed significant changes in the porcine epithelium with the formation of vacuoles, linear detachment of the epithelium and swelling when present the permeation enhancers. However the most pronounced changes were recorded in the presence of negatively charged SBE-β-CD.

The data suggest that all enhancers tested, and particularly TMC, seems to improve the transport of RH across buccal epithelium.