COMBINED XRD AND RAMAN COMBINATORIAL SCREENING SYSTEM

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Combinatorial investigations require rapid screening techniques to test and evaluate variations of composition, structure and property within a material library. Unlike most analytical techniques, both X-ray diffraction and Raman spectroscopy are non destructive methods that require virtually no sample preparation, thus, allowing samples to be analyzed simply and quickly in their natural form. These two techniques are also complementary to one another in that X-ray diffraction provides abundant information on the atomic arrangement of the sample revealed through the diffraction pattern and Raman spectroscopy can measure the characteristic vibration frequencies determined by the chemical composition and chemical bond. In addition the optical video image can provide the surface condition, color and shape of the sample. All three measurements (Raman spectrum, X-ray diffraction and optical imaging) can be done in a relative short time. Therefore, a combination of the three functions into one instrument is beneficial to high-throughput combinatorial screening analysis. Combinatorial screening based on spectroscopic and diffraction techniques is of high importance e. g. for drug substances and formulations since the polymorphism of active ingredients has to be controlled to achieve a reliable product quality which will satisfy the regulatory authorities. This presentation covers the development of an innovative instrument consisting of X-ray diffraction and Raman spectroscopy for combinatorial screening. The X-ray source, X-ray optics, X-ray detector, laser source, Raman probe and an auto-zoomed video microscope are all integrated into a single platform so that the X-ray diffraction pattern, Raman spectrum and optical image from the same sample or sample area can be measured simultaneously or sequentially. An X-Y-Z translation stage can bring each cell of the combinatorial library in to both measurement positions. Once the solid-state characterization data are collected and stored, samples can be generally classified into groups or quantified by a novel, statistical, pattern-matching software. Besides fast evaluation of large data bases, convenient access to each individual measurement result e.g. the Raman spectrum can be achieved to yield a deeper insight on the molecular level. The software associated with the system can treat data from the various techniques and analyze the results in correlation.

HPLC-ESI-MS/MS STUDIEN ZUM CHLORPYRIFOS METABOLISMUS

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Chlorpyrifos (CP) - O,O-Diethyl-O-(3,5,6-trichlor-2-pyridyl)phosphorthioat - ist ein in der Landwirtschaft weit verbreitetes Organophosphat-Pestizid. Wie andere Organophosphate wirkt CP durch Hemmung der Acetylcholinesterase, indem der Ser-Rest in der Bindetasche dieser Serin Hydrolase phosph(or)yliert wird. CP besitzt relativ geringe Säugetier-Toxizität, da es sehr rasch durch Metabolisierung, u.z. durch Spaltung der aromatischen Phosphoester-Bindung, inaktiviert wird. Als Hauptmetabolite können daher 3,5,6-Trichlorpyridin-2-ol (TCP) und O,O-Diethylthiophosphat (DETP), welches weiter durch oxidative Desulfurierung zum O,O-Diethylphosphat (DEP) umgewandelt wird, im Urin gefunden werden, wenn man diesem Pestizid ausgesetzt war. Aus analytischer Sicht können DETP und DEP als charakteristische gruppen-spezifische Biomarker für Organophosphate herangezogen werden, während TCP als substanz-spezifischer Biomarker für CP (und CP-Methyl) angesehen werden kann.

Wir konnten im Urin einer mit CP akut vergifteten Person mittels HPLC-ESI-MS/MS [1] noch weitere, zuvor völlig unbekannte Metabolismus-Wege nachweisen [2]. Neben oben beschriebener Aryl-Ester Spaltung konnten wir auch Alkyl-Ester Spaltung nachweisen sowie eine weitere Route, nämlich eine Glutathion-abhängige nukleophile Substitution am aktivierten Pyridin-System, bei der S-Cysteinkonjugate als charakteristische Folgeprodukte resultieren.

Neben der Identifizierung von 12 neuen Metaboliten wurden für die Hauptmetabolite auch die Eliminationsprofile über einen Zeitraum von 14 Tagen bestimmt [1,2]. Dabei wurde sowohl für die Summe der (Thio)Phosphate (Summe DETP und DEP) als auch für das Gesamt-TCP, welches zu einem Grossteil als Glukuronid vorlag, eine biphasische Elimination nach erster Ordnung gefunden. Als Halbwertszeiten wurden für DETP+DEP 22 h (anfängliche rasche Phase) und 120 h (langsame Phase) sowie für das Gesamt-TCP 41 h und 151 h bestimmt.

[1] Bicker W, Lämmerhofer M, Lindner W, J. Chromatogr. B 2005; 822: 160-69.

[2] Bicker W, Lämmerhofer M, Genser D, Kiss H, Lindner W, Toxicol. Lett. 2005; 159: 235-51.

SULFATION OF RESVERATROL, A POLYPHENOLIC COMPOUND FOUND IN GRAPES AND WINE, IN HUMAN LIVER

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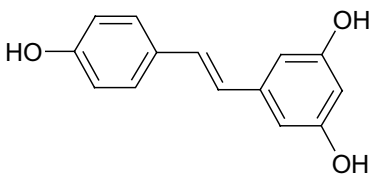
Sulfation of resveratrol, a polyphenolic compound present in grapes and wine with anticancer and cardioprotective activities, was studied in human liver cytosol. In the presence of 3'-phosphoadenosin-5'-phosphosulfate, three metabolites (M1-M3) were found, whose structures were identified by mass spectrometry and NMR as *trans*-resveratrol-3-O-sulfate, *trans*-resveratrol-4'-O-sulfate and *trans*-resveratrol-3-O-4'-O-disulfate, respectively.



trans-Resveratrol

The kinetics of M1 formation in human liver cytosol exhibited a pattern of substrate inhibition with a Ki of 21.32 ± 8.73 µM and a Vmax/Km of 1.63 ± 0.41 µl/min/mg of protein. Formation of M2 and M3 showed sigmoidal kinetics with about 56-fold higher Vmax/Km values for M3 than for M2 (2.23 ± 0.14 and 0.04 ± 0.01 µl/min/mg). Incubation in the presence of human recombinant sulfotransferases (SULTs) demonstrated that M1 is almost exclusively catalyzed by SULT1A1 and only to a minor extent by SULTs 1A2, 1A3, and 1E1, whereas M2 is selectively formed by SULT1A2. M3 is mainly catalyzed by SULTs 1A2 and 1A3. In conclusion, our results elucidate the enzymatic pathways of resveratrol in human liver, which must be considered in humans following oral uptake of dietary resveratrol.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF MORPHINAN-6-CARBONITRILES

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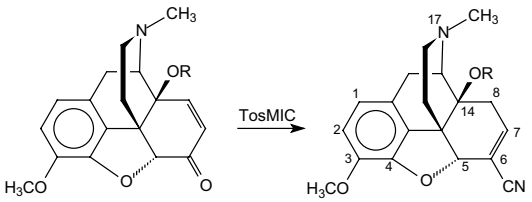
Recently, the reductive cyanation of ketones with tosylmethyl isocyanide (TosMIC) was applied to 6-ketomorphinans for the first time [1]. The proposed mechanism includes opening of the 4,5-ether bridge when 7,8-saturated morphinans are used. In contrast, 7,8-didehydromorphinans lead to 6,7-didehydro-6-carbonitriles with retainment of the 4,5-ether bridge [2] (see scheme). Opioid receptor binding studies show that the novel compounds have high affinity and selectivity for the μ-opioid receptor. In *in vivo* studies the compounds exhibit high analgesic potency.



The chemically highly versatile acrylonitrile substructure allows for easy conversion into more polar derivatives and bears the potential to open up a new field in morphinan chemistry and opioid pharmacology. It is expected that hydrolysis of the nitrile group will lead to hydrophilic compounds from which it is known to have decreased ability to cross the blood brain barrier [3].

[1] Greiner E, Schottenberger H, Wurst K, Schmidhammer H. Novel Class of Morphinans with Acrylonitrile Incorporated Substructures as Key Intermediates for Non-Oxygen-Bridged Opioid Ligands. J. Am. Chem. Soc. 2001; 123:3840-1.

[2] Schütz J, Windisch P, Kristeva E, Wurst K, Ongania K-H, Horvath U E I, Schottenberger H, Laus G, Schmidhammer H. Mechanistic Diversity of the van Leusen Reaction Applied to 6-Ketomorphinans and Synthetic Potential of the Resulting Acrylonitrile Substructures. J. Org. Chem. 2005; 70:5323-6.

[3] Fürst S, Riba P, Friedmann T, Timar J, Al-Khrasani M, Obara I, Makuch W, Spetea M, Schütz J, Przewlocki R, Przewlocka B, Schmidhammer, H. Peripheral versus Central Antinociceptive Actions of 6-Amino Acid-Substituted Derivatives of 14-O-Methyloxymorphone in Acute and Inflammatory Pain in the Rat. J. Pharmacol. Exp. Ther. 2005; 312:609-18.

ESTABLISHMENT OF A HPLC-MS/MS PLATFORM FOR THE THERAPEUTIC DRUG MONITORING OF IMMUNOSUPPRESANTS STRATEGIES, REALIZATION AND RESULTS

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Within the last years the hyphenation of HPLC (high performance liquid chromatography) with tandem mass spectrometry (MS/MS) has proved to be an alternative to antibody-based immunoassays commonly used for therapeutic drug monitoring (TDM) of immunosuppressants. These immunoassays show methodological drawbacks of antibody cross reactivity with active and inactive drug metabolites. In contrast, LC-MS/MS platforms measure circulating drug levels with lower associated costs and higher specificity, accuracy and precision. Our LC-MS/MS assay established for sirolimus, everolimus, cyclosporin A, and tacrolimus (SECT) requires a sample volume of 100 µl whole blood. Ion suppression is minimized by a sample preparation protocol combining cell lysis, online SPE for further matrix removal, and reverse phase chromatography. The validated assay shows sufficient intraday and interday repeatability (accuracy and precision) and a LLOQ of <1 ng/ml for all analytes. Participation in proficiency testing gave results within the required thresholds. Quantitative comparison with the antibody-based immunoassays showed linear relationships between the data series with bias values in agreement with the literature. The established LC-MS/MS platform will allow replacement of the current antibody-based assays. The major advantage of this technique is the ability to simultaneously acquire absolute quantitative concentrations of each of the therapeutic drugs administered from one sample. Therefore, the requirement for different sample preparation schemes or parallel measurements on different analytical instruments are no longer needed. The high sample throughput assures timely TDM data reporting to the ward. A significant reduction of costs is expected due to the lower consumable expenses in this LC-MS/MS assay.

RESVERATROLANALOGA - SYNTHESE UND DEREN BIOLOGISCHE AKTIVITÄT

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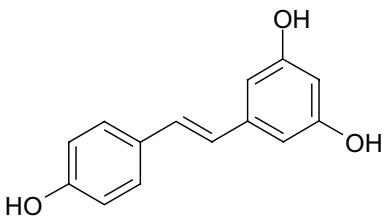
Ausgehend von Resveratrol, das einige sehr interessante pharmakologische Wirkungen besitzt (z. B. COX-Hemmung, Vasodilatation, antioxidative und tumorhemmende Wirkung [1, 2, 3])



konnten durch gezielte synthetische Modifikationen der Grundstruktur neue Substanzen mit

- verbesserten **antiinflammatorischen** Eigenschaften
- verbesserten **vasodilatierenden** Eigenschaften
- verbesserter **Antitumor**wirkung

dargestellt werden. Bei der Präsentation wird sowohl auf die Synthesen als auch auf die bemerkenswerten biologischen Aktivitäten der Verbindungen näher eingegangen.

[1] Murias M, Handler N, Erker T, Pleban K, Ecker G, Saiko P, Szekeres T, Jäger W. Resveratrol analogues as selective cyclooxygenase-2 inhibitors: synthesis and structure-activity relationship. Bioorg. Med. Chem. 2004; 12:5571-8.

[2] Jang M, Pezzuto JM. Cancer chemopreventive activity of resveratrol. Drugs Exp. Clin. Res. 1999; 25:65-77.

[3] Murias M, Jäger W, Handler N, Erker T, Horvath Z, Szekeres T, Nohl H, Gille L. Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure-activity relationship. Biochem. Pharmacol. 2005; 69:903-12.

COMMON CHEMICAL FEATURES OF PDE5 INHIBITORS

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Phosphodiesterases (PDEs), of which currently 11 subfamilies are known, are a superfamily of enzymes that are ubiquitously distributed in mammalian tissues. They play a major role in cell signaling by hydrolyzing cAMP and cGMP [1]. In recent years, especially the phosphodiesterase 5 (PDE5) subtype has received considerable attention as the binding site for compounds that have been marketed for the treatment of erectile dysfunction, such as Sildenafil ("Viagra"), Vardenafil and Tadalafil [2].

In this study, we present pharmacophore models generated with the software packages Catalyst [3] and LigandScout [4]. Ligand-based models using structural information from known PDE5 ligands were compared to structure-based models derived from recently published X-ray structures of protein-ligand complexes. The resulting pharmacophore models were evaluated for their ability to retrieve known ligands and whether they are able to discriminate against compounds that are selective for other PDE subtypes. Furthermore, possible binding modes of ligands are discussed for which no X-ray structures of protein-ligand complexes are available. Our results show that both ligand-based and structure-based models are able to increase the hit ratio of screening libraries, and that the combination of both methods may be useful to gain knowledge about essential interactions, allowing further improvement of the models.

[1] Lugnier C. Cyclic nucleotide phosphodiesterase (PDE) superfamily: A new target for the development of specific therapeutic agents. Pharmacol. Ther. 2006; 109:366-98.

[2] Carson C C, Lue T F. Phosphodiesterase type 5 inhibitors for erectile dysfunction. BJU International 2005; 96:257-80.

[3] Catalyst, Version 4.11; Accelrys, Scranton Road, San Diego, CA.

[4] Wolber G, Langer T. LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters J. Chem. Inf. Model; 2005; 45:160-169. Version 1.0 beta 4 available from Inte:Ligand GmbH, Clemens Maria Hofbauer-G. 6, A-2344 Maria Enzersdorf, Austria.

COBALT ALKYNE COMPLEXES AS CYTOSTATICS

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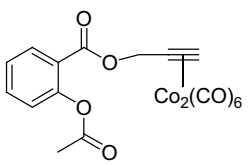
Cobalt-alkyne complexes are drugs with remarkable cytotoxicity. From the complexes tested up to now we selected the aspirin derivative [2-acetoxy-(2-propynyl)benzoate]hexacarbonyldicobalt (Co-ASS, see figure) as lead compound. Effects of Co-ASS on human breast cancer cell lines were superior to those of already established cytostatic agents. First structure activity studies showed that the activity depended strongly on the nature of the intact cobalt complex. In order to get more insight into the mode of action, we systematically modified the alkyne ligand and determined the cytotoxic properties of the resulting cobalt complexes. Further investigations were performed on the drug lipophilicity, the cellular uptake into MCF-7 and MDA-MB 231 breast cancer cells, the DNA-binding efficacy and the nuclear drug content. The ability to inhibit cyclooxygenase (COX) enzymes was examined for selected compounds. The missing correlation of the high intracellular drug levels (e.g. approximately 150-fold accumulation grade for Co-ASS into MCF-7 cells), the low drug content of the cell nuclei and the diverging results from the DNA binding experiments made a “Cisplatin-like”-mode of action unlikely. Interestingly, the most antitumor active compounds were potent COX-inhibitors (COX-1 and COX-2). They were by far more active than acetylsalicylic acid (ASS, Aspirin). The presented results indicate that cobalt-alkyne complexes of the Co-ASS type, represent a new class of organometallic cytostatics with a mode of drug action in which COX-inhibition probably plays a major role. [1]



Co-ASS

[1] Ott I, Schmidt K, Kircher B, Schumacher P, Wiglenda T, Gust R Antitumor-active cobalt-alkyne complexes derived from acetylsalicylic acid: studies on the mode of drug action. J. Med. Chem. 2005; 48:622-9.

^{18}F-MARKIERTE TRYPTOPHANDERIVATE FÜR DIE EPILEPSIE-DIAGNOSTIK MITTELS POSITRONENEMMISIONSTOMOGRAPHIE (PET)

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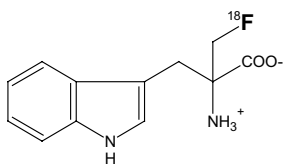
20-30% der Patienten, die an Epilepsie leiden, bleiben trotz medikamentöser Behandlung nicht anfallsfrei. In manchen Fällen bleibt nur noch die Möglichkeit eines chirurgischen Eingriffes, jedoch ist genaue Lokalisation des epileptischen Focus ist schwierig. Eine vielversprechende Methode ist die Detektion mittels PET unter Verwendung eines Tryptophan Derivates. [^{11}C]-alpha-Methyltryptophan (AMT) konnte bei vielen therapierefraktären Patienten mit unklarer Lokalisation durch konventionelle Methoden (MRI, EEG) eine genaue Lokalisation ermöglichen.



Wir berichten über die Synthese von [^{18}F]-alpha-Fluormethyl-tryptophan (FMT) zur Epilepsiediagnostik mittels PET. Aus Literaturdaten [1, 2] ist zu erwarten, daß FMT - ähnlich wie AMT - primär über den Kynurenin-Stoffwechsel metaboliert wird.

Ausgehend von Nalpha-Methoxycarbonyltryptophanmethylester gelang es uns, das gewünschte nicht markierte Produkt [3] über eine alternative Synthesestrategie zugänglich zu machen. Durch Umsetzung eines Zwischenproduktes mit [^{18}F]-CH_2BrF konnte die ^{18}F-Markierung in hohen Ausbeuten realisiert werden. Versuche zur direkten nukleophilen Umsetzung mit ^{18}F-Fluorid sind derzeit in Durchführung. Biologische Evaluierung an aktivierten PBM-Zellen und im Ratten-Epilepsie-Modell sind geplant. Durch eine potentiell höhere Selektivität von FMT für den Kynurenin-Stoffwechsel wird eine erhöhte Spezifität und Sensitivität für PET in der Epilepsiediagnostik erwartet.

[1] Chugani D C, Muzik O J. Cereb Blood Flow Metab 2000; 20:2-9.

[2] Feldblum,S, Rougier A, Loiseau H, Loiseau P, Cohadon F, Morselli P L, Lloyd K G. Epilepsia 1988; 29:523-9.

[3] Zembower D E, Gilbert J A, Ames M M. J Med Chem 1993; 36:305-13

4-AMINOBICYCLO[2.2.2]OCTANDERIVATE MIT VERBESSERTER WIRKUNG GEGEN PROTOZOEN

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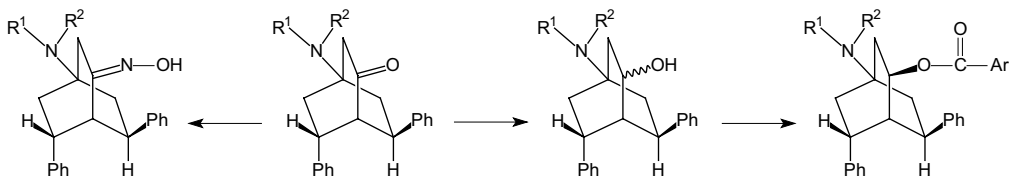
Malaria ist die wichtigste, durch Plasmodien ausgelöste Protozoenerkrankung und zugleich eine der bedeutsamsten Infektionskrankheiten überhaupt. Es gibt weltweit etwa 350-500 Millionen Neuerkrankungen und 2-3 Millionen Todesfälle pro Jahr [1].

Schlafkrankheit wird durch Trypanosomenbefall hervorgerufen und verursacht jährlich 300.000 Neuerkrankungen bei 50.000 Todesfällen [2].

Die Wirkung von 4-Aminobicyclo[2.2.2]octanon- **1** und die gesteigerte Aktivität von 4-Aminobicyclo[2.2.2]octanol-Derivaten **2** gegen chloroquinresistente Plasmodien sowie Trypanosomen wurde bereits nachgewiesen [3]. Durch Derivatisierung der funktionellen Gruppe in Position 2 sollte die Wirkung gegen die oben genannten Protozoen weiter gesteigert, die Zytotoxizität jedoch in einem akzeptablen Bereich gehalten werden. Durch die Darstellung von Oximen **3** und unterschiedliche Arten von Estern **4** gelang es diese Vorgabe weitestgehend einzuhalten. Verbindung **3c** hatte im Zellversuch sogar eine stärkere Wirkung als Chloroquin.



3a-c **1a-c** **2a-c** **4a-c**

a: $R^1 = R^2 = CH_3$
b: $R^1 + R^2 = -(CH_2)_4-$
c: $R^1 + R^2 = -(CH_2)_5-$
Ar = Aryl, Heteroaryl, Alkyl

[1] http://www.who.int/malaria/docs/press/ga_monitoring.htm

[2] WHO, *World Health Report* 2002, p. 192.

[3] Weis R., Brun R., Saf R., Seebacher W. 2003 *Monatsh. Chem.* 134: 1019-1026.

HIV PROTEASE INHIBITOREN: PHARMAKOPHORMODELL-GENERIERUNG, VALIDIERUNG UND DATENBANK SCREENING

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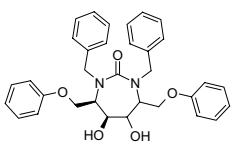
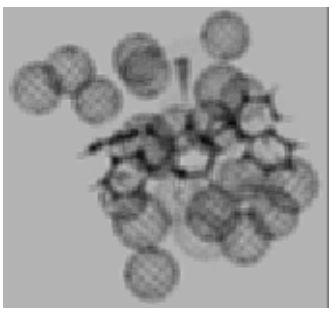
Computer unterstützte Methoden haben einen festen Platz in der rationalen Entwicklung von Arzneistoffen eingenommen. Pharmakophormodellgenerierung und Durchsuchen von virtuellen Datenbanken ermöglichen ein rasches Filtern großer Datenmengen nach Molekülen mit den geforderten Eigenschaften und bieten somit eine wichtige Quelle für neue Leitstrukturfindung.

In dieser Studie wurden am Beispiel der HIV Protease systematisch 50 Enzym-Inhibitor Komplexe aus der Brookhaven Protein Datenbank [1] untersucht. Die Software Pakete LigandScout [2] und Catalyst [3] dienten zur anschließenden Struktur-basierten Pharmakophormodellgenerierung bzw. zum Datenbank Screening. Eine sorgfältige Validierung nach verschiedenen Kriterien ermöglichte die Identifizierung des besten Modells. Dieses kann für das Filtern weiterer Datenbanken zur Auswahl von Verbindungen für die biologische Testung verwendet werden und so die Suche nach möglichen neuen Leitstrukturen für dieses Zielprotein erleichtern.





HIV Protease Inhibitor Aha001 aus Protein-Ligand Komplex 1AJX [4] (links) und daraus resultierendes Pharmakophormodell (rechts):

hydrophobe Bereiche: hellblau
Wasserstoffbrückenakzeptor: grün
Wasserstoffbrückendonoren: violett exkludierte Volumina: schwarz

[1] Brookhaven National Laboratory: Protein Data Bank, http://www.rcsb.org/pdb/.

[2] LigandScout beta 3, Inte:Ligand GmbH, http://www.inteligand.com/.

[3] Catalyst version 4.11, Accelrys Inc., http://www.accelrys.com/.

[4] Backbro K, Lowgren S, Osterlund K, Atepo J, Unge T., Hulten J, Bonham, N M, Schaal W, Karlen A, Hallberg A. Unexpected binding mode of a cyclic sulfamide HIV-1 protease inhibitor. J. Med. Chem. 1997; 40:898-902

DESIGN AND EVALUATION OF A RISK ASSESSMENT TOOL FOR ACTIVE PHARMACEUTICAL INGREDIENT (API) PROCESS VALIDATION

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In 2005 the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has released a guideline for risk management, which sets the framework for its application to the pharmaceutical industry [1]. Process validation is the documented evidence that a process results reproducibly in a product, which meets its predetermined specifications. This is one of the areas that is most stringently influenced by the ICH guideline on risk management. As a result of our work, a risk-assessment model has been established that is in compliance with current regulatory requirements for manufacturing active pharmaceutical ingredients, but can also be applied to the finished pharmaceuticals industry. In addition, the risk assessment model covers the risk management elements of risk control and risk communication. The tool designed incorporates the elements of the widely used techniques of Failure Mode and Effects Analysis (FMEA) [2] and Hazard Analysis and Critical Control Points (HACCP) [3].

The applicability has been evaluated by model applications and by interviewing representatives of the industry and regulatory authorities of an EU-member state.

[1] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Q9: Quality Risk Management, 2005.

[2] International Organisation of Standardisation. ISO 14971:2000 - Application of Risk Management to Medical Devices, 2000.

[3] World Health Organisation. Technical Report Series No 908 - Annex 7 Application of Hazard Analysis and Critical Control Point (HACCP) Methodology to Pharmaceuticals, 2003.

COMPARATIVE IN VIVO MUCOADHESION STUDIES OF THE THIOMER PCP-CYS USING MRI AND FLUORESCENCE DETECTION

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The aim of this study was to investigate the impact of application forms on mucoadhesive properties of the thiomer polycarbophil-cysteine (PCP-Cys) in vivo. Therefore PCP-Cys was coazervated with the fluorescence marker fluorescein-diacetate (FDA) [1], lyophilised and either pressed into tablets or filled in gelatine capsules. Both application forms were enteric coated and subsequently fed to rats which have been fasted for 24 hours. In order to examine the point when the thiomer is released from the application form in the intestine, PCP-Cys was additionally enriched with the gadolinium complex Gd-DTPA. Because of the positive magnetic resonance signal that occurs when Gd-DTPA gets in contact with water i.e. when the enteric coating has been dissolved, t = 0 could be ascertained. 3 hours from t = 0, the rats were sacrificed, the intestine was excised and cut into 5 equal pieces. The amount of remaining fluorescence marker was determined by incubating the intestine with 5 M NaOH, in order to quantitatively hydrolyse FDA to the actual fluorescent marker sodium fluorescein [1]. Subsequently the fluorescence of each sample was measured [1].

Results show that a tablet is more suitable for gaining higher mucoadhesive properties of PCP-Cys in vivo, because more marker adhered to earlier parts of the intestine compared to administration of the thiomer in a capsule.

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DEVELOPMENT OF A NOVEL METHOD FOR THE PREPARATION OF THIOLATED POLYACRYLIC ACID NANOPARTICLES

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The aim of this study was to develop a novel method for the preparation of thiolated polyacrylic acid nanoparticles. In a first step nanoparticles were generated by ionotropic gelation of polyacrylic acid (PAA) of three different molecular weights (100 kDa, 240 kDa and 450 kDa) and various cations including Ca^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+} and Fe^{3+}. Via in vitro characterization of the particles (particle size, size distribution and zeta potential) optimized preparation conditions were identified. Taking into consideration, that thiolated polyacrylic acid (PAA-Cys) displays higher mucoadhesive and permeation enhancing properties than unmodified PAA, PAA-Cys nanoparticles were produced in the same manner with Ca^{2+}, as the most promising results concerning particle size and stability of particles could be achieved with this ionic crosslinker. The nanoparticles were stabilized via the formation of inter- and intrachain disulfide bonds within these particles due to oxidation with H_2O_2. Ca^{2+} was removed proximately by the addition of EDTA and exhaustive dialysis.

Results showed that by using the preparation method described above PAA-Cys nanoparticles of a mean diameter of about 220 nm (PAA_{100}-Cys), 250 nm (PAA_{240}-Cys) and 295 nm (PAA_{450}-Cys) can be generated. In comparison to PAA nanoparticles ionically crosslinked with Ca^{2+}, the removal of the crosslinker Ca^{2+} from PAA-Cys particles led to a nearly 3-fold increase in the zeta potential, from about -7 mV up to -20 mV. Consequently, covalently crosslinked particles should thereby provide higher mucoadhesive and permeation enhancing properties. Apart from this advantage, covalently crosslinked PAA-Cys nanoparticles were more firm as they remained stable when incubated in hydrochloride solution, whereas ionically crosslinked particles dissolved at pH lower than 5.

APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR OPTIMISING GALENICAL FORMULATIONS

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Response surface methodology (RSM) is the suitable method for indicating the relative significance of a number of independent variables and their various interactions in the production of an obtained result. Such factorial designs offer the possibility of evaluating the influence of individual parameters and their interaction at the same time with a minimum of experiments. They are favoured for characterization and optimising the galenical processes, pharmacodynamics, etc.

Inversion of sucrose is a stability problem of candies with acidic taste that contain sucrose and small amounts of organic acids, since the free D-fructose produced by hydrolysis is hygroscopic. The following possibilities were investigated for preventing the hydrolysis of sucrose in tablets containing sucrose and citric acid: Adding various amounts of tri-sodium citrate to the formulation in order to neutralize the citric acid, (Hot) melt coating of citric acid and tri-sodium citrate with a vegetable fat at different coating ratios, variation of the ratio of coated citric acid and tri-sodium citrate in formulations, and compressing the formulations with different compressing forces. After tablet processing and storage of tablets, the concentration of D-fructose was determined based on enzymatic reactions. A response surface central composite design was used. The abovementioned variations were chosen as independent variables and the amount of D-fructose was chosen as response variable. The lowest rates of inversion could be achieved by increasing the content of tri-sodium citrate and the ratio of coating material and decreasing the ratio of coated citric acid and tri-sodium citrate in the tablet formulations. The compression force had no significant effect on the inversion of sucrose.

RECENT STUDIES ON BONE IMAGING RADIOPHARMACEUTICALS REVEAL NEW INSIGHTS INTO BINDING CHARACTERISTICS AND MECHANISMS

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Bone imaging using radioactive labelled pharmaceuticals is one of the major tasks in clinical nuclear medicine. Although the first polyphosphonates were introduced as bone imagers in the early 1970s, mechanisms involved in their uptake into bone tissue have remained speculative.

In an attempt to provide a model for the evaluation of parameters influencing tracer-matrix interactions, the authors introduced a feasible in-vitro method involving binding studies on collagen, hydroxyapatite and amorphous calcium phosphate [1]. Correlations of the findings with published in-vivo data brought first evidence for the suitability of the method. In a follow up study, our model was verified replacing the synthetic matrices by powdered human bone allografts [2].

Using our model, we defined isotopic effects, the preparation method, the amount and the nature of carriers, and the ligand concentration as parameters significantly influencing the uptake of bone tracers [1,2,3]. Regarding the binding mechanisms, we could demonstrate that the binding to bone tissue is an irreversible, mineral associated process with insignificant involvement of collagen fibres and osteoblasts [4].

In summary, we conclude that the presented model has the potential to be a helpful tool in the pre-vivo evaluation of novel bone imaging radiopharmaceuticals.

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PHYSICO-CHEMICAL AND THEORETICAL ASPECTS OF THE PHARMACEUTICAL APPLICATION OF CYCLODEXTRINS

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Cyclodextrins (CDs) are widely used in pharmaceutical technology as recipient, e.g. to improve the solubility and consequently the bioavailability of drugs [1]. The geometries and the stabilities of the complexes are of great importance for their applications. The determination of the thermodynamic parameters K, ΔG, ΔH and ΔS supported by molecular calculations gives some quantitative insight into the interaction between the cylcodextrins as host and the drug molecules [2].

Spironolactone is a partial synthetic steroid-analogue of aldosterone and works as competitive aldosterone-antagonist. It has been chosen for the investigations on the host-guest complexation mechanism, because of its extremely high affinity to β-CD and a consequently large solubility enhancement. Although the association constants of the compound are comparable for various β-CDs, the reaction enthalpies and entropies differ significantly, which is an indication that diverse driving forces are responsible for the inclusion reaction. Particularly, the reaction entropy is of high importance for the host-guest interaction. For some β-CDs enthalpy-entropy compensation is observed.

The geometries of the inclusion complexes were determined by ab initio and DFT calculations in order to obtain some information about the specifity of the interactions between the guest molecule and the host concerning steric and electrostatic contributions.

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PHARMAKOLOGISCHE UND PHARMAKOKINETISCHE STUDIEN MIT ECHINAFORCE® TROPFEN UND TABLETTEN

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Echinacea-Zubereitungen werden zur Behandlung grippaler Infekte, chronischer Infektionen im Bereich der Atemwege und der ableitenden Harnwege verwendet. Kürzlich konnten für die enthaltenen Alkamide neue Erkenntnisse zum molekularen Wirkmechanismus und zur Bioverfügbarkeit gewonnen werden [1, 2, 3]. Um die Bioverfügbarkeit dieser Alkamide aus unterschiedlichen Arzneiformen, wie Tropfen und Tabletten aus *E. purpurea* (Echinaforce®) zu vergleichen und *ex vivo* Effekte am Immunsystem zu beobachten, wurde nun eine randomisierte, offene Studie mit 8 gesunden Probanden durchgeführt. Sie bekamen entweder 4 ml einer standardisierten alkoholischen Echinaforce® Tinktur oder 12 Echinaforce® Tabletten, entsprechend jeweils einer Dosis von 0,07 mg Dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraensäure-isobutylamiden. Das Konzentrationsmaximum nach Applikation der Echinaforce® Tropfen wurde nach 30 min mit einem C_{max} von 0,40 ng/ml Serum erreicht. Im Vergleich dazu betrug T_{max} nach Applikation der Tabletten 45 Minuten mit einem C_{max} von 0.12 ng/ml. In der *ex vivo* Studie zeigten beide Arzneiformen denselben Effekt bezüglich Freisetzung von TNF-α, IL-8 bzw. IL-6. 23 Stunden nach der Applikation wurde eine signifikante Downregulierung LPS-stimulierter TNF-α und IL-8 Konzentrationen detektiert. Bezüglich IL-6 konnten keine signifikanten Effekte beobachtet werden.

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ANTIMYKOBAKTERIELLE WIRKSTOFFE AUS *EUCLEA RACEMOSA SSP. SCHIMPERI*

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In der traditionellen Medizin Ostafrikas besitzt *Euclea racemosa* Murr. *ssp. schimperi* (A. DC.) F. White (Ebenaceae) einen hohen Stellenwert und wird u.a. bei Indikationen wie Lepra, Ekzemen und Wunden, die eine antimikrobielle Wirkung vermuten lassen, eingesetzt [1, 2]. In dieser Studie wurden nun verschiedene Extrakte aus der Wurzelrinde dieser Arzneipflanze, für die das Vorkommen von Naphthochinonen beschrieben wurde [3], untersucht.

Aus dem Dichlormethanextrakt konnten fünf Substanzen isoliert werden, die in einem Mikrodilutionstest [4] auf Wachstumshemmung schnell wachsender Mykobakterien getestet wurden. Dabei erwies sich die im Extrakt vorliegende Hauptkomponente 7-Methyljuglon als stark wirksam (MIC = 4 µg/ml (*M. aurum*) bzw. 8 µg/ml (*M. fortuitum*)), während ein weiteres Naphthochinon, Neodiospyrin, nur eine moderate Wirkung aufwies (MIC = 128 µg/ml (*M. aurum* und *M. fortuitum*). Die minimalen Hemmkonzentrationen (MIC) der Standardantibiotika Ethambutol und Isoniazid betrugen 1 und 4 µg/ml (*M. aurum*) bzw. 8 und 1 µg/ml (*M. fortuitum*). 4(*S*)-Shinanolon, Isodiospyrin und Mamegakinon zeigten keine Wirkung bei 128 µg/ml.

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URTICA DIOICA AGGLUTININ – ISOLIERUNG, ANALYTIK UND PHARMAKOLOGIE

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Die in der Brennnesselwurzel enthaltenen Lektine (*Urtica dioica* Agglutinin, UDA) gelten neben Polysacchariden als aktive Bestandteile der Droge, deren Zubereitungen traditionell zur Behandlung der benignen Prostatahyperplasie (BPH) Verwendung finden. UDA besteht aus einem Gemisch niedermolekularer Lektine (8300-9500 Dalton), die nur aus Aminosäuren aufgebaut sind, und sich durch eine hohe Affinität zu N-Acetylglucosamin-Oligomeren auszeichnen [1,2].

Die vorliegende Präsentation versucht diese interessanten Naturstoffe näher zu charakterisieren. Die Isolierung einzelner Isolektine gelang mittels Affinitätschromatographie und präparativer HPLC, ihre quantitative Analyse und Zuordnung im Pflanzenmaterial wurde anhand verschiedenster analytischer Verfahren (HPLC, HPLC-MS, CE, CE-MS) durchgeführt [3]. Durch eine sehr potente Stimulation der Lymphozyten-Proliferation konnte die biologische Aktivität der *Urtica*-Lektine belegt werden.

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PFLANZEN IN DER ÖSTERREICHISCHEN VOLKSMEDIZIN DIE „VOLKSMED-DATENBANK“

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In Weiterführung der Arbeiten über die volksmedizinisch verwendeten Pflanzen in Österreich [1,2] wurde die VOLKSMED Datenbank erweitert. Sie umfasst nun alle in 55 Diplomarbeiten aufgenommenen Daten über Gebiete in Österreich und Südtirol zur Verwendung von Heilpflanzen und Heilmitteln. Die nunmehr vorliegende Datenbank erlaubt ein Data Mining, wie etwa den Vergleich verschiedener Regionen, aber auch eine abschließende Zusammenfassung und Auswertung aller bisher erhobenen Angaben. Etwa 400 Pflanzenarten aus 364 Gattungen werden in der österreichischen Volksmedizin zu Heilzwecken verwendet, wobei allerdings 98 Gattungen den Hauptteil (91,5% aller Nennungen) ausmachen. Interessant an diesen 98 Gattungen ist, dass ungefähr die Hälfte der Indikationen und Anwendungen wissenschaftlichen Erkenntnissen entspricht. Es gibt aber circa 30 Gattungen, die in der Datenbank häufig erwähnt werden und die in der Volksmedizin neben der allgemein anerkannten Anwendung auch bei Indikationen Verwendung finden, die durch die bisher bekannten Inhaltsstoffe nicht immer erklärbar sind: Zu dieser Gruppe zählen *Hypericum*, welches in der VOLKSMED DB nicht nur auf seine antidepressive Wirkung reduziert wird, oder *Achillea*, die nicht nur als Magenmittel verwendet wird, sondern ein häufig angewandtes Mittel bei Frauenleiden darstellt. *Dryopteris*-Arten gelten als wichtigstes Rheumamittel, das äußerlich angewendet schmerzstillend wirken soll. Ebenfalls äußerlich angewendet werden *Lycopodium clavatum/ annotinum* bei Krämpfen der quergestreiften Muskulatur.

Aus der Fülle der jetzt zugänglichen Informationen sind dies einige Beispiele, die zeigen, dass die volksmedizinische, ausschließlich auf Erfahrung beruhende Anwendung mancher Pflanzen heute wissenschaftlich plausible Erklärungen erfahren kann. Bei vielen, offenbar erfolgreich angewendeten Pflanzen fehlen aber noch immer Kenntnisse über Wirkstoffe und Wirkmechanismen; entsprechende Untersuchungen sind hier notwendig und erscheinen absolut lohnend.

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ENTWICKLUNG VON METALL-MARKIERTEN RGD PEPTIDEN FÜR DIE MOLEKULARE BILDGEBUNG VON αvß3 INTEGRINEN

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Peptide mit der Sequenz Arg-Gly-Asp (RGD) binden mit hoher Affinität an αvβ3-Integrine, die auf Tumorzellen und auf Endothelzellen u.a. bei der tumor-induzierten Angiogenese exprimiert werden. Unser Ziel war es radioaktiv markierte Peptid-Derivate für die nuklearmedizinische Anwendung zu entwickeln.

Auf Basis eines zyklischen RGD Peptids wurden am ε-amino-Rest des Lysins Derivatisierungen für die Markierung mit metallischen Radionukliden vorgenommen. Einerseits wurde 2-Hydrazinonikotinsäure (HYNIC-c(RGDyK)) für Markierungen mit ^{99m}Tc für die Anwendung in der Single-Photonen-Emissionstomographie eingesetzt, andererseits wurde 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′,-tetraessigsäure (DOTA-c(RGDfK)) für die Markierung mit ^{68}Ga für die Positronenemissionstomographie (PET) als auch ^{90}Y/^{177}Lu für den radiotherapeutischen Ansatz verwendet. Die Peptide wurden mit ^{99m}Tc, ^{68}Ga und ^{111}In (als Surrogat für ^{90}Y,^{177}Lu) markiert und in Bezug auf Stabilität, Rezeptorbindungsverhalten und Bioverteilung (im Maus-Tumormodell) untersucht.

Markierungsausbeuten >90% konnten bei hoher spezifischer Aktivität erreicht werden, die erhaltenen Metallkomplexe zeigten hohe Stabilität in Puffer und Serum. An αvβ3 positiven M21 Melanonzellen konnte eine spezifische Bindung und Internalisierung nachgewiesen werden. Im Tiermodel zeigte sich eine rasche renale Elimination, geringe Aufnahme in Leber und Darm, sowie eine hohe Tumoraufnahme in αvβ3-positiven Tumoren (>2,5%ID/G) im Vergleich zu negativen Kontrollen (<1%ID/g) 1h p.i.. Tumor / Organ-Quotienten waren am höchsten für ^{111}In-DOTA-c(RGDfK), gefolgt von ^{68}Ga-DOTA-c(RGDfK) und ^{99m}Tc-HYNIC-c(RGDyK). Die vorliegenden Daten zeigen das Potential der untersuchten metallmarkierten Peptide zur nicht-invasiven Bildgebung αvβ3-positiver Tumoren vergleichbar mit bereits etablierten ^{18}F-markierten Derivaten [1] auf.

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A NOVEL METHOD FOR MEDIUM-THROUGHPUT SCREENING OF HERBAL SEDATIVES AND BENZODIAZEPINE-LIKE LIGANDS ON $GABA_A$-RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES

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We present a novel method for medium-troughput screening of herbal and synthetic sedatives on $GABA_A$- receptors expressed in *Xenopus* oocytes. Voltage-clamp experiments on *Xenopus* oocytes are performed in a 15µl-bath that is covered by a glass plate. Two angular inlet channels in the glass cover enable access of two microelectrodes to the oocyte. A funnel for drug application surrounds the access channels for the two microelectrodes. The test solution is applied to the funnel by means of a TECAN pipetting robot (Miniprep 60) that is controlled by a custom made software permitting automation of the experiments.

A mean time of solution exchange ($t_{10\text{-}90\%}$) of < than 150 ms was estimated. This approach requires only small amounts of test solution (about 100 µl) for drug testing. After an initial fast perfusion step the chamber is continuously perfused at a slower rate (usually at 1 µl/second).

Examples are given to illustrate the use of the robot system for testing the modulation of $GABA_A$-receptors of different subunit compositions by herbal extracts and benzodiazepine-like ligands. In these studies we have focused in particular on $GABA_A$ receptors composed of α_1, β_2, γ_1-subunits that are expressed in only a few areas of the brain and thus represent interesting drug targets. The pharmacological profile of this receptor subtype is largely unknown. The $\alpha_1\beta_2\gamma_1$- $GABA_A$-receptors were expressed in *Xenopus* oocytes and their modulation by 21 compounds comprising different chemical structures was investigated by the new screening technique. Triazolam, Clotiazepam, Midazolam and CGS 20625 were identified as potent and efficient modulators for this receptor subtype.

SIGNALLING PATHWAYS OF THE ADENOSINE A_3 RECEPTORS ON RAT CORTICAL NEURONS

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The purine adenosine is involved in a variety of regulatory processes including its involvement in the modulation of synaptic transmission as an extracellular signal molecule in the central nervous system. Previous pharmacological studies demonstrated the presence of A_3 receptors in pyramidal cells of the rat cingulate cortex [1,2]. The activation of these receptors induced inhibition of synaptic potentials (PSPs). The aim of the present study was to investigate the signalling pathways by which A_3 receptors mediate the inhibition of the PSPs. Intracellular recordings were made in rat brain slices.

The A_3 agonist IB-MECA induced an inhibition of the PSPs. The effect was completely antagonized by the A_3 antagonist MRS 1523. Inactivation of G proteins by GDP-β-S or N-ethylmaleimide abolished the inhibitory effect of IB-MECA. The phospholipase C inhibitor U73122 prevented the IB-MECA-induced inhibition. Blockade of the protein kinase C by chelerytine (6 μM) abolished the IB-MECA-induced inhibition. The effect of IB-MECA was partially affected by the IP_3-antagonist heparin and completely blocked by BAPTA-AM, a chelator of intracellular calcium. The calmodulin antagonist W7 (30 μM) abolished the effect of IB-MECA on the PSPs. KN-93, an inhibitor of the Ca/calmodulin-dependent protein kinase II prevented the IB-MECA-induced inhibition. KN-92 that was used as negative control did not show any effect. Experiments with the adenylyl cyclase inhibitor SQ22,536 and the inhibitor of the protein kinase A HA-1004 revealed that neither adenylyl cyclase nor protein kinase A seem to participate in A_3-receptor signalling in cortical neurons.

We conclude that activation of phospholipase C followed by activation of protein kinase C and calcium-dependent processes is involved in the signal transduction of the neuronal A_3 receptor.

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FHM-1 AND EA-2 MISSENSE MUTATIONS AFFECT SURFACE EXPRESSION LEVELS OF $CA_V2.1$ CALCIUM CHANNELS

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Familial Hemiplegic Migraine Type 1 (FHM-1) and Episodic Ataxia Type 2 (EA-2) are inherited autosomal dominant disorders related to neuronal dysfunction. FHM-1 is exclusively associated with missense mutations in the pore-forming α_1 subunit of $Ca_v2.1$ calcium channels, whereas EA-2 is caused by missense as well as truncating mutations. In order to investigate the surface expression of mutant channels, we generated eight extracellularly hemagglutinine epitope (HA)-tagged $Ca_v2.1$ α_1 subunits. Thusly tagged subunits allow the detection of the channel complex on the surface of intact cells, and also constitute useful tools to study the trafficking of these channels in different cell systems. $Ca_v2.1$-HA-1722 presents the HA-tag at position 1722 in the proposed extracellular portion of the pore loop of domain IV, and shows total protein expression levels comparable to wild type after heterologous expression in tsA-201 and Jurkat cells. Furthermore, electrophysiological measurements in tsA-201 cells and Xenopus oocytes revealed current densities and kinetics indistinguishable from wild type. Immunocytochemical experiments in Jurkat cells showed specific HA surface staining in non-permeabilised cells and additional cytoplasmic staining in permeabilised cells. To quantify changes in surface expression, we incorporated selected FHM-1 and EA-2 missense mutations into $Ca_v2.1$-HA-1722, and performed a surface labelling assay based on chemiluminescence signal detection. We could show that several of the mutants indeed affect the surface expression of $Ca_v2.1$ channels, as revealed by an either enhanced or reduced HA-derived chemiluminescence signal on the cell surface. Our results will add useful information to the controversial discussion about the molecular pathology of FHM-1 and EA-2 mutations.