

**Retinoic Acid Regulates the Expression of the Anti-Apoptotic Protein PKC $\delta$ VIII.**

PATEL NA<sup>1,2</sup>, COOPER DR<sup>1,2</sup>

James A. Haley Veterans Hospital<sup>1</sup> and College of Medicine, Department of Molecular Medicine<sup>2</sup>, University of South Florida, Tampa, FL

**Background:** The human teratocarcinoma cell line, NT2 cells differentiate into hNT neurons upon treatment with retinoic acid (RA). This is a widely accepted model to investigate the expression of genes involved in neurogenesis, neuronal differentiation and early development of nervous system. Protein kinase C (PKC)  $\delta$  plays an important role in the regulation of cell apoptosis. We have recently described a novel PKC $\delta$  isozyme- PKC $\delta$ VIII that is expressed in human NT2 cells upon RA treatment. PKC $\delta$ I and PKC $\delta$ VIII are the alternatively spliced variants expressed in human cells. Expression of PKC $\delta$ VIII peaks at day 1 of RA treatment and then declines by 7 days. RT-PCR analysis and sequencing data revealed that this isozyme is generated via utilization of a downstream alternate 5' splice site of exon 10 which results in an insertion of 93 bp in the caspase-3 recognition sequence within the V3 domain. We have shown that PKC $\delta$ VIII is resistant to caspase-3 cleavage and that PKC $\delta$ VIII regulates *anti-apoptotic* effects in these cells.

**Methods:** RNA isolations of NT2 cells treated with retinoic acid were performed using RNA Bee (Tel Test, Inc.) RT-PCR was performed using primers that detect both PKC $\delta$  isoforms as well as primers specific for PKC $\delta$ VIII.

**Results:** We have identified the nuclear serine-arginine rich splicing factor SC35 (i.e. SRp30b) which promotes the expression of PKC $\delta$ VIII mRNA via utilization of the alternative 5' splice site II on PKC $\delta$  exon 10. Western blot analysis demonstrates that the expression of SC35 increased with RA treatment concurrent with the increase in PKC $\delta$ VIII expression. Overexpression of SC35 in NT2 cells promotes the expression of PKC $\delta$ VIII. To further decipher the mechanism of alternative splice site selection we have designed and cloned a minigene which includes PKC $\delta$  exon 10 and its flanking introns in the pSP3 splicing vector. We show that this minigene is responsive to retinoic acid. Further, co-transfection of SC35 with PKC $\delta$  minigene promotes selection of 5' splice site II. Transfection of cells with SC35 siRNA along with PKC $\delta$  minigene results in a decline of PKC $\delta$ VIII expression.

**Conclusions:** (1) PKC $\delta$ VIII plays a role in development of nervous system (2) RA regulates expression of PKC $\delta$ VIII via SC35.

**(-)- $\alpha$ -Bisabolol – a Specific Ergosterol Biosynthesis Inhibitor ?**

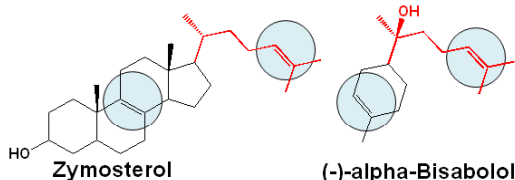
PAULI A<sup>1</sup>, SCHILCHER H<sup>2</sup>

<sup>1</sup>ReviewScience, Zirndorf, Germany; <sup>2</sup>Immenstadt, Germany

**Background:** At the level of zymosterol in the ergosterol biosynthesis all subsequent intermediates are solely produced by fungi. The recognition of a specifically acting inhibitor within this part of ergosterol biosynthesis would lead to a new class of antifungal drugs. To obtain information on the existence of compounds having both, structural similarities to fungi specific ergosterol precursors plus antifungal properties, previous findings in literature were analyzed in view of this aspect.

**Methods:** A chemical (sub)structure and MIC searchable computer database on antimicrobials (Amicbase) was used. In the data query antifungal compounds were searched, which include one of four different side chains types as they occur in the section from zymosterol to ergosterol.

**Results:** Among antifungals the side chain of zymosterol was most frequently found. Addition of oxygen and limiting to *Candida albicans* resulted in 19 different compounds, whose MIC increased with molecular volume ( $r = 0.77$ ). Like zymosterol, (-)- $\alpha$ -bisabolol contains a cyclohexene ring plus a second double bond in similar interatomic distance. This may point to an inhibition of zymosterol conversion into fecosterol by (-)- $\alpha$ -bisabolol, which would offer the opportunity to disturb specifically fungal biochemistry.



(-)- $\alpha$ -Bisabolol from chamomile has antiinflammatory, wound-healing, anticancer, antispasmodic properties and inhibits fungi (3-100  $\mu$ g/ml), gram-(+) (32-500), but less or not gram-(-) bacteria. Due to its low toxicity (monkeys tolerated oral 15 ml/kg bw) and the lack of reports on allergic reactions in its cosmetic use, the compound was taken to treat fungal and bacterial infections. Own case reports will be given.

**Conclusions:** The antifungal mechanism of (-)- $\alpha$ -bisabolol is worthwhile for further investigation due to its safety and its unique pharmacological profile.

**Differential roles of physiological and physicochemical parameters on low and variable bioavailability of Saquinavir- hurdles of effective drug treatment**

PATHAK SM, MUSMADE P, UDUPA N

Department of pharmaceutical Quality Assurance, Manipal College of pharmaceutical Sciences  
Manipal-University, Manipal-576104, INDIA

**Background:** Saquinavir (SQV), the first of the Human Immunodeficiency Virus (HIV) protease inhibitors to reach the market, remains one of the most widely prescribed agent which has markedly improved morbidity and mortality in HIV-infected patients. Inclusion of SQV in 'highly active anti-retroviral therapy' has substantially improved the clinical outcomes of AIDS patients. However, the complete therapeutic potential of this class of drugs is yet to be exploited due to number of limitations related to their poor and variable transport across important biological membranes, especially gastrointestinal tract. The pharmacokinetic profile of SQV being characterized by its low and variable bioavailability is primarily attributed to metabolism by cytochrome P-450 3A4. Moreover, there is increasing understanding that membrane transporters (P-gp, MRP-2) contribute significantly to the biopharmaceutical characteristics of SQV and this entire class of drugs. However, the relative contributions of these eliminating organs to the first-pass effect and the significance of incomplete absorption of SQV have not been explored.

**Methods:** In this study, our objective was to determine the various factors governing the bio-availability of SQV. In order to understand the contributions of i) first pass metabolism in gut and liver and ii) solubility and permeability of SQV across the lumen, towards its low bioavailability, in-vitro, in-situ and in-vivo study results in rat model were compared. Though, the first-pass intestinal and hepatic metabolism has been shown to be a major determinant in the oral clearance of SQV, we found that physicochemical characteristics may also have an important role in determining the oral absorption and disposition properties of this drug. To best of our knowledge, we are the first to report a double-peak phenomenon in plasma concentration time profiles of SQV via oral administration. Although the double peaks in the plasma concentration-time profiles after p.o. doses could be attributed to a gradient expression of transporter proteins along the gastrointestinal tract, we hypothesize that the phenomenon is due to differential solubility profile of SQV across the intestine. In-vitro solubility data clearly indicates the pH dependent solubility profile of this drug. SQV has pKa values of 1.1 and 7.1, corresponding to the quinoline and octa-hydroisoquinoline nitrogens, respectively. A base pKa of the drug just above pH 7.0, so the solubility would be expected to decrease significantly as the drug moves from duodenum (fasting pH about 6) to distal ileum (at about pH 7.8), and then increase again in caecum (at about pH 5). The time of the second peak (4-5 hours) coincides with the expected arrival time in rat caecum which was confirmed with intestinal motility test using charcoal.

To relate bioavailability to molecular transport characteristics, we speculated that an efflux (counter transport) mechanism might contribute to the low and variable bioavailability of SQV. Single pass in-situ absorption method was employed for the determination of the permeability ( $P_{app}$ ) of SQV in various segments of rat intestine, viz. duodenum, jejunum ileum and colon. The data reveals that the  $P_{app}$  of SQV through rat duodenum ( $2.91 \times 10^{-5}$  cm/s) is higher than jejunum ( $1.86 \times 10^{-5}$  cm/s) or ileum ( $2.11 \times 10^{-5}$  cm/sec), which is in line with the higher extent of absorption of the drug in the duodenum. On the other hand,  $P_{app}$  values of the drug in jejunum, ileum and colon were found almost similar. The results hence clearly suggest that SQV is a low permeable drug, apart from being poorly soluble at intestinal pH.

**Conclusion:** Because P-gp is found lower down the gut than is CYP3A, the exposure of SQV to P-gp is high because of its low solubility, and this could explain the very long period over which SQV is absorbed. Absorption, rather than elimination, controls the pharmacokinetics of SQV, and its very slowness is probably responsible for the low and variable kinetic profile of this drug. In conclusion, the bioavailability of SQV is controlled by a combination of solubility in the gut lumen, p-glycoprotein mediated efflux in the gut-wall, and first-pass intestinal and hepatic metabolism by CYP3A4. Given the differential and complex roles of physiological and physicochemical characteristics in SQV oral absorption, the optimization of AIDS boosting regimens requires careful consideration in order to avoid therapy limiting drug-drug transporter and enzyme interactions.

**Discovery and Use of the Magic Bullets in Human Taeniosis (Niclosamide, Praziquantel)**

PAWLOWSKI ZS

Medical University, Poznan, Poland

**Background:** Taeniosis/cysticercosis is a serious public health problem in several countries of Latin America, Africa and Asia. Approximately 2 500 000 people carry a *Taenia solium* tapeworm. Globally, conservative estimates calculate 50 000 deaths from neurocysticercosis every year.

**Older taenicides:** Several more or less toxic natural remedies, such as male fern extract, Kosso flowers, areca nuts, pomegranates have been used for centuries. In early 20<sup>th</sup> century in Germany among 22 000 cases of taeniosis treated with male fern extract 18 patients died and 71 others have lost vision. Several synthetic drugs were tried as taenicides in 20<sup>th</sup> century e.g. thymol (1912), carbon tetrachloride (1931), hexylresorcinol (1932), mepacrine (1947), dichlorophen (1956), bithionol (1962), paromomycine (1967) and mebendazole (1975). In 1950s my patients with *T.saginata* taeniosis were treated with pumpkin seeds (cure rate 65% among 163 treated), atabrine (respectively 42%, 44), acranil (88%, 89) or metallic tin compounds (88%, 226). Many of these drugs were unsafe or poorly tolerated, although some were rather effective.

**Modern taenicides:** The first magic bullet was - niclosamide, introduced in 1959. As a barely absorbed substance it is safe and well tolerated. However, it's early original version (and still some generic products) has lost the efficacy during storage due to polymerization of its active particles. The efficacy of a single dose of niclosamide in human taeniosis is about 85%. Since 1972 niclosamide has been gradually replaced by the second magic bullet - praziquantel, being more stable taenicide, more efficacious (95%) and much cheaper (10 US cents for a dose). It has been used widely in the control of schistosomes. Due to autoinfection more than 10% of *T.solium* tapeworm carriers develop neurocysticercosis. Therefore, the use of praziquantel in control of taeniosis is questioned, as this drug may damage existing brain cysticerci and change asymptomatic neurocysticercosis into a symptomatic one. The problem is partly solved by a reduction of the dose of praziquantel in taeniosis to 5-10mg/kg b.w. in a single dose. Still some uncertainty exists whether it is worth to risk such a rare side effect at a mass-treatment in *T.solium* endemic areas.

**Conclusions:**

- 1) There is still a place for another magic bullet in taeniosis/cysticercosis.
- 2) Nitazoxanide, a broad spectrum antiparasitic drug, is waiting in a row.