Uptake of bile acids (BAs) into hepatocytes and excretion into the bile canaliculi are achieved by a panel of transporters. Cholestasis is a particular type of hepatotoxicity, consisting in the failure of bile to reach the duodenum due to alterations of hepatobiliary transport. In this context, there is a need for reliable in vitro models to predict the cholestatic potential of new drug candidates. We have synthesized a series of cholic acid (ChA) derivatives containing an appropriate fluorophore (F), specifically the 4-nitrobenzo-2-oxa-1,3-diazole (NBD)- or dansyl (Dns)- amino conjugates 3a-, 3b-, 7a- and 7b-F-ChA. Flow cytometry assays using these derivatives have allowed us to study ChA transport in freshly isolated rat hepatocytes and its modulation by drugs.¹²

Binding of bile acids to serum albumins has also attracted considerable attention over the last decades, as it determines the BA levels in plasma, which can be used as a test for liver function. However, very little is known about the role that BA@HSA complexes play in
hepatic uptake. We have found that the singlet excited state properties of the synthetic NBD and Dns derivatives of ChA are useful to clarify key aspects of BA-HSA interactions.\(^3\) Emission enhancement upon addition of HSA has been used for determination of the binding constants, which are in the order of \(10^4\) M\(^{-1}\) for C7-NBD, C7-Dns and C3-NBD derivatives; in the case of the C3-Dns analogs two binding constants with values in the range of \(10^4\) and \(10^5\) M\(^{-1}\) have been calculated. Accordingly, formation of fluorescent ChA@HSA complexes with 1:1 (for C7-NBD, C7-Dns and C3-NBD) and 1:2 stoichiometry (C3-Dns) has been proven.

Energy transfer from tryptophan to F-ChAs occurs by a FRET mechanism; the donor-acceptor distances have been determined according to Förster’s theory. The estimated values (21-30 Å) are compatible with both site I and site II occupancy and do not provide sufficient information for a safe assignment. Nevertheless, fluorescence titration using warfarin (site I probe) and ibuprofen (site II probe) for displacement clearly indicates that C7-NBD, C7-Dns and C3-NBD derivatives bind to HSA at site I whereas C3-Dns-ChAs are preferably bound at site II. Moreover, C3-Dns-ChAs are displaced by litocholic acid.

Finally, 3a- and 3b-NBD-ChA have been incorporated into ChA aggregates and submitted to fluorescence quenching by the two enantiomers of several tryptophan derivatives. In all cases, a significant stereodifferentiation is observed; the most remarkable effect seems to be associated with C3 stereochemistry.\(^4\)


Universitätshauptgebäude, Hörsaal 3, Dienstag, den 5. Oktober 2010 um 17 Uhr c.t

ACHTUNG, DER VORTRAG FINDE AN EINEM DIENSTAG STATT!

gez. Prof. Dr. Thomas Koop, Prof. Dr. Jochen Mattay, Prof. Dr. Norbert Sewald