Morphine and Loperamide Interact Differently with Uptake Transporters of the OATP-Family

Authors: Dr. rer. nat. Anett Engel,[1] Tabea Sieling,[1] Dipl.-Ing. Annette Wegner,[1] Julia Kolbow,[2] Dr. med. vet. Markus Keiser,[2] Prof. Dr. med. Werner Siegmund,[2] Prof. Dr. med. Konrad Meissner[1]

[1]Klinik und Poliklinik für Anästhesiologie und Intensivmedizin & [2]Institut für Pharmakologie, Universitätsmedizin Greifswald, Greifswald, Germany

Background / Introduction: Transmembrane transport processes mediated by ABC (ATP-binding cassette)-type proteins play an important role in absorption, distribution and elimination of different drugs. Several opioids like loperamide, morphine, and methadone are known substrates of the efflux transporter P-glycoprotein (ABCB1, MDR1), which limits access of its substrates to the brain at the apical side of endothelial cells of the blood-brain barrier, restricts absorption from the gut at the apical side of enterocytes and supports active secretion at the apical side of hepatocytes and proximal tubule cells in the kidney. In contrast, OATP (organic anion transporting polypeptide)-type transmembrane transport proteins facilitate the uptake of drugs into the brain at the apical side of endothelial cells (OATP1A2, OATP2B1), into the liver at the basolateral side of hepatocytes for subsequent metabolism (OATP1B1, OATP1B3, OATP2B1), and enable absorption from the gut at the apical side of enterocytes (OATP2B1). Thus, OATPs have been recognized as important determinants of pharmacokinetics similar to ABCB1. However, the interaction of opioids with such uptake transporters has not been sufficiently studied so far. Therefore, the present study investigated the interaction of morphine and loperamide with multispecific uptake transporters of the OATP family *in vitro* to elucidate possible substrate and inhibitor properties of the opioids.

Methods: HEK 293 cells were stably transfected with OATP1A2, OATP1B1, OATP1B3, OATP2B1 or the empty vector as control. Inhibitory effects of increasing opioid concentrations were studied in competition assays (n=9) using the established reference substrates estrone-3-sulfate (OATP1A2) and bromosulfophthalein (OATP1B1, OATP1B3, OATP2B1). Intracellular accumulation of radiolabeled estrone-3-sulfate and bromosulfophthalein was measured by liquid scintillation counting after cell lysis. Furthermore, cellular uptake of radiolabeled morphine and loperamide (10 nM and 10 µM) into OATP-transfected cells was assessed in a preliminary uptake assay (n=9) to screen for possible substrate properties.

Results: Loperamide was a potent inhibitor of all investigated OATPs (IC_{50}: 0.21 – 2.4 µM; maximum inhibitory effect: 78 – 95%). Morphine fully inhibited the function of OATP1A2 but at much higher concentrations (IC_{50}: 87 µM) than loperamide. While morphine exhibited a higher affinity to inhibit OATP1B3 (IC_{50}: 4.5 µM), only a maximum of 50% inhibition could be reached even at 1 mM. In contrast, morphine did not affect OATP1B1

and OATP2B1. In the preliminary uptake screening both opioids seemed to be a substrate of OATP1A2, OATP1B1 and OATP2B1 but not OATP1B3.

Conclusion: Morphine and loperamide were shown to interact with various OATPs as both inhibitor and substrate but to different extent. These findings suggest that OATPs might play a distinctive role in pharmacokinetics and thus drug effect variability of these two clinically important opioids. However, additional investigations are necessary to further characterize opioid uptake by OATPs *in vitro* and to elucidate how morphine and loperamide effects vary due to drug uptake transport *in vivo*.