Our results provide basis for further applied research, *e.g.* developing new antimicrobial peptides in therapy, pest control and food preservation.

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Opening of the blood-brain barrier for drug delivery to the brain: the effects of tesmilifene and short-chain alkylglycerols on brain endothelial cells

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The blood-brain barrier (BBB) forms a dynamic interface between the blood and the brain. It selectively regulates the transcellular and paracellular transport of molecules and passage of cells between the blood and the central nervous system. The BBB restricts drug penetration to the brain preventing effective treatment of several neurological diseases. Therefore it is an increasing need to find new ways to improve drug delivery to the brain. Brain capillary endothelial cells constitute the anatomical and functional basis of the BBB. One of the strategies to increase drug delivery to the brain is changing cerebral endothelial functions by opening the BBB through the modification of the paracellular or the transendothelial transport pathways.

In this study two agents, tesmilifene and short-chain alkylglycerols (AGs) were selected for detailed examination. Tesmilifene, a tamoxifen-related compound, has chemopotentiating properties in experimental and in clinical cancer studies. Treatment with tesmilifene caused temporary, acute CNS side-effects in patients indicating the opening of the BBB. Previous studies from our laboratory have shown that tesmilifene increases the permeability of the BBB in rats. Intraarterial injection of short-chain AGs, such as 1-*O*-pentylglycerol and 2-*O*-hexyldiglycerol, open the BBB and increase the delivery of molecules to rodent brain parenchyma *in vivo*. The mechanism underlying AG and tesmilifene-mediated modification of BBB permeability is still unknown. The aim of the present study was to test the direct effects of tesmilifene and AGs on barrier properties of cultured brain microvascular endothelial cells, a model of the BBB.

The triple co-culture BBB model was constructed on cell culture inserts using primary rat brain endothelial cells, rat cerebral glial cells and rat pericytes. Barrier integrity of the BBB endothelial monolayers was analyzed by transendothelial electrical resistance and permeability measurements. In addition to functional assays, toxicity tests, immunostainings for junctional proteins and freeze fracture electron microscopy were performed.

Short-term tesmilifene and AG treatment decreased the resistance of endothelial monolayers, and increased the permeability for fluorescein, a marker of paracellular flux. Tesmilifene also enhanced the transcellular transport of albumin. These short-term changes were accompanied by changes in cell morphology and immunostaining for junctional proteins. AG and tesmilifene-mediated increase in brain endothelial permeability was reversible. Short-term treatments did not alter the viability of brain endothelial cells. Tesmilifene did not affect the functions of P-glycoprotein, but decreased the activity of the multidrug resistance associated protein-1 and the production of nitric oxide in endothelial cells. Tesmilifene also altered the mRNA expression of several tight junction proteins measured by a custom Taqman gene array.

Our data support previous clinical observations and the results of animal experiments, and clearly indicate that AGs and tesmilifene increase the permeability of the BBB by directly acting on brain endothelial cell functions. Tesmilifene and AGs are promising adjuvants in the transient opening of the BBB for clinical use, especially for treatment of brain tumors.

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Metagenomics of biogas producing microorganisms

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The production of renewable energy carriers is currently receiving increasing attention worldwide. Biogas is a promising technology as its production may combine the treatment of various organic wastes with the generation of energy. Biogas can be converted to heat and/or electricity, and its purified derivative, biomethane, is suitable for every function for which fossil natural gas is used today. The degradation

of organic materials by a microbial community is carried out under anaerobic conditions. The composition of this microbial consortium depends on various factors. A clear understanding of the organization and behavior of this multifarious community is crucial for optimization of their performance and attainment of the stable operation of the process. Classical microbiological methods are principally based on studies of isolated pure strains of microbes, and hence are of little help when the goal is elucidation of the relationships among members in this complex microbial consortium. The development of high-throughput sequencing technologies has opened up new avenues for such investigations. The „next generation" sequencing methods employ various chemical reactions for the rapid determination of DNA sequences. Huge databases and sophisticated bioinformatics are prepared to analyze the results. This metagenomic approach allows the real-time study of live consortia in various environments through identification of the members and/or determination of the relative abundances of particular physiological functions.

The aims of the study were to determine the possibility of applying the next-generation sequencing technologies for the characterization of the microbial consortium in different biogas fermenters and to find a novel substrate to replace expensive maize silage, which is commonly used to feed biogas fermenters today.

First the microbial composition of maize silage fed fermenter with an extremely parallel SOLiD™ type short-read DNA sequencing platform was determined. The results showed the members of the Firmicutes and Bacteroides phyla played the most important role in the hydrolysis of the plant biomass and in the secondary fermentation. In particular, many *Clostridium* species were identified, which possess cellulolytic and H_2-producing activities, both properties being apparently essential for the efficient degradation of the biomass. In the Archaea domain, Methanomicrobiales is the most abundant order that uses CO_2 as a carbon source and H_2 as an electron donor for methanogenesis. The results demonstrate the importance of the metabolism of hydrogen beside acetate within the biogas producing microbial community.

Unfortunately maize silage is an expensive substrate for biogas production. Therefore biogas power plants are looking for more economical substrates. Algae are promising candidates. Algae can produce biohydrogen and the remaining alga biomass is a good substrate for biogas fermentation. Thus more renewable energy can be obtained through the combination of these two technologies. We tested an algal mixture consisting of *Scenedesmus* and *Chlamydomonas* species. These algae contain large amount of starch that is a good substrate for the microbial community inhabiting the biogas fermentor. It was found that, depending on the starch content and the algal cell wall destruction methods, a higher concentration of biomethane as produced from microalgae relative to corn silage. Microbial composition of alga-fed fermenters were examined with the Ion Torrent™ next generation DNA sequencing platform. The result showed that the composition of microbial community was significantly changed in the microalgae fed fermenters relative to maize silage fed technologies. Instead of the Firmicutes and Bacteroides the Proteobacteria phylum dominated the Bacteria and in the Archaea domain the acetotrophic Methanosarcinales represented the overwhelming majority.

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