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Project

Assessment of active accumulation of xenobiotics into milk with help of MDCKII-bABCG2 cells: A novel in vitro model of the lactating bovine mammary gland

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06/2014 – 11/2015

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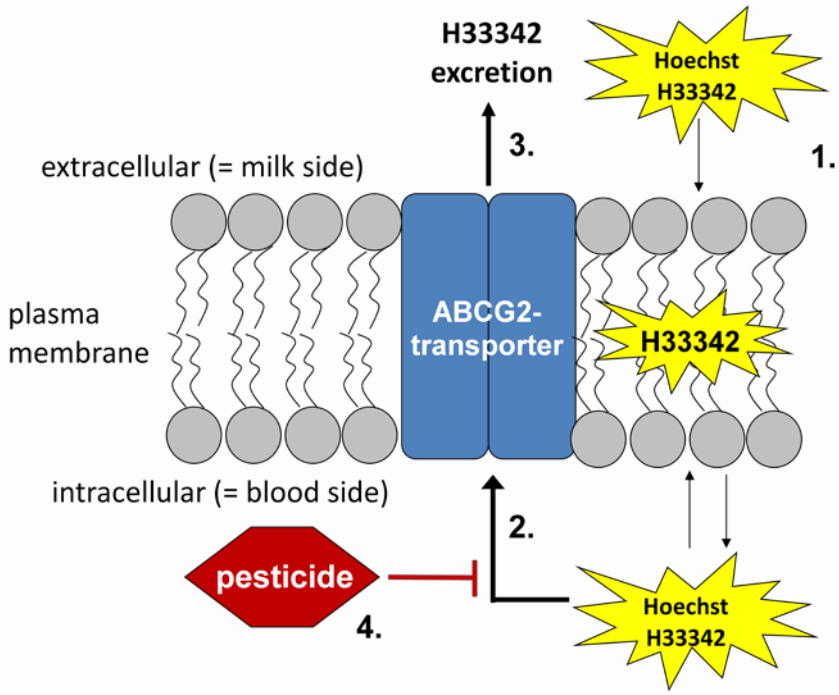
Assessment of active accumulation of xenobiotics into milk with help of MDCKII-bABCG2 cells: A novel in vitro model of the lactating bovine mammary gland

Pesticides are extensively used in agriculture leading to an exposure of the general population and accumulation of these potential harmful substances throughout the food chain including milk. Hence, pesticides are of central public concern due to the risk of serious adverse health effects. Assessment of potential milk residues as part of drug or pesticide approval studies is often performed in dairy goats as a model for dairy ruminants (OECD guideline 503). If a chemical is secreted into the milk, its legal maximum residue level (MRL) has to be further determined (Regulation (EC) No 396/2005). However, these long-term feeding studies induce extraordinary stress in the animals and are very high in price.

In the human, murine and ruminant mammary gland the cell membrane-bound ABCG2 transport protein was identified as the central transfer mechanism for xenobiotics into milk. As so far there was no adequate in vitro model of the ruminant lactating bovine mammary gland, MDCKII cells stably expressing bovine mammary ABCG2 (bABCG2) were generated in our institute. In this project, this cell culture model should be validated as an efficient screening method to assess the active ABCG2-mediated accumulation of chemicals in milk. Frequently used pesticides were therefore representatively applied. Potential ABCG2 pesticide substrates should be identified by the established Hoechst H33342 accumulation test through their ability to competitively inhibit ABCG2-mediated secretion of the tested pesticide in MDCKII-bABCG2 cells. Additionally, dioxin-like pesticides that potentially stimulate ABCG2 secretory activity should be identified by the ethoxyresorufin-O-deethylase (EROD)-enzyme assay. In cooperation with Prof. Dacasto et al. the regulatory mechanism of pesticide-depended bABCG2 regulation will further be explored by molecular biological methods like quantitative RT-PCR. In particular, the effect of multiple pesticide residues on ABCG2 activity in the low MRL concentration range should be investigated. Thus, it could be possible that an ABCG2-stimulating pesticide induces the secretion of a coexisting ABCG2 pesticide substrate into milk. Consequently, this might cause an accumulation of pesticides above the legal MRL in milk. Additionally, an interaction between two ABCG2 pesticide substrates may result in a reduction of their milk secretion and in increased pesticide residues in edible bovine tissues. Altogether, this novel in vitro method may complete the existing in vivo metabolism studies in ruminants and reduce the number of necessary animal experiments. Therefore, this study may overall contribute to improving risk assessment of pesticide residues in milk and thereby enhance protection of consumers of dairy products.



Principle of the Hoechst H33342 accumulation test: The MDCKII-bABCG2 cells are cultivated in 96-well cell culture plates for 3 days. At the third day the cells are pre-treated with the selected pesticide for 4 hours. In order to adequately reflect the in vivo situation, elected concentrations corresponded to the one- or ten-fold MRL value of edible ruminant tissues. Then, cells are incubated for 15 min with H33342 (20 µM) in the presence or absence of the investigated pesticide. In general, the fluorescent H33342 dye diffuses passively into cells and accumulates in the cytoplasm (1.). Subsequently, H33342 is excreted actively by the efflux transporter ABCG2 (2. + 3.). If the pesticides are substrates, they will cause a competitive inhibition of ABCG2-mediated cellular H33342 excretion (4.) resulting in an increase of intracellular H33342 levels. H33342 accumulation is detected by fluorescence spectrophotometry (360 nm/465 nm). Then, cellular H33342 accumulation is calculated as Relative Fluorescence Units (RFU) per mg protein.

Results (Jan 2016)

MDCKII-bABCG2 cells: A novel in vitro model of the lactating bovine mammary gland to assess the active accumulation of xenobiotics into the milk Assessment of potential milk residues as part of chemical approval studies is often performed in dairy ruminants. In the bovine mammary gland, the cell membrane-bound ABCG2 transport protein (bABCG2) was identified as the central transfer mechanism for xenobiotics into milk. Hence, in this 3R research project we aimed to establish the MDCKII-bABCG2 cell line as an efficient in vitro screening method to assess the active ABCG2-mediated 2 accumulation of chemicals in milk.

In screening studies using efficient transport or enzyme assays we examined selected pesticides in concentrations reflecting the legal maximum residue level (MRL) in edible ruminant tissues. In this project we identified several pesticides like the insecticide chlorpyrifos or the growth inhibitor glyphosate as potential substrate or modulator of the bABCG2 transport protein. Moreover, we detected additive effects of pesticides including the insecticides chlorpyrifos-methyl and methiocarb at the bABCG2- transporter. The pesticide-bABCG2 interaction may result in altered milk pesticide secretion and thereby in increased pesticides residues in edible bovine tissues or milk. Consequently, this might cause an accumulation of pesticides above the legal MRL in milk.

Altogether, this novel MDCKII-bABCG2 cell model may complete the existing in vivo metabolism studies in ruminants (OECD Test No. 503: Metabolism in Livestock) and reduce the number of necessary animal experiments. Therefore, this study may overall contribute to improving risk assessment of pesticides residues in milk and thereby enhance protection of consumers of dairy products.

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Project leader

Dr. Sandra Halwachs

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Duration

06/2014 – 11/2015