High Correspondence of Botulinum Neurotoxin C1 (BoNT/C1) Detection by Mouse Bioassay and Corresponding Gene Detection by PCR after Enrichment with Cooked Meat Medium: An Alternative for the Mouse Bioassay Investigating the Presence of Viable BoNT/C1 Toxigenic Clostridia

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Avian botulism, a paralytic and most often fatal disease, is considered an intoxication of birds caused by the active ingestion of pre-formed botulinum neurotoxin from the surrounding environment and can be a critical factor in the protection of species especially for endangered ones. The toxin is produced by BoNT-toxigenic clostridia cells/spores under suitable anaerobic conditions. Among the seven well known BoNT types designated from A to F, avian botulism is predominantly caused by type C1 toxin (BoNT/C1). Toxin detection is performed by means of the only available and appropriate mouse bioassay, an expensive, laborious and increasingly ethically questionable technique. In this work, an alternative based on PCR investigating the presence of toxigenic viable BoNT/C1 toxigenic clostridia was developed and evaluated.

The potential of BoNT/C1 production of environmental and avian samples after enrichment with cooked meat media (CMM) was detected by the conventionally used mouse bioassay. In order to evaluate alternatives, a nested PCR targeting the corresponding BoNT/C1 gene was adapted for gene detection before and after the CMM enrichment step. A (highly) significant correlation between the mouse bioassay and the PCR after CMM enrichment could be observed. In contrast, PCR BoNT/C1 of unamended samples (no CMM enrichment) showed low correlation with mouse bioassay.

We suggest the PCR technique, using CMM enriched samples, as an alternative for the currently used mouse bioassay for testing the presence of viable BoNT/C1 toxigenic Clostridia in environmental and veterinary samples.

Antiproliferative and Cell Cycle Specific Effects of Ochratoxin A in LLCPK-1, NRK-52E and Porcine Primary Proximal Kidney Cells

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Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite by certain Aspergillus and Penicillium species. It is commonly found as a contaminant of human blood samples tested to date have proved positive for OTA. Average daily intake in humans is estimated to be approximately 5-10ng/kg. OTA has been demonstrated to induce nephropathy as well as to be immunotoxic in pigs (antiproliferative effect in T-cells), teratogenic in several species and to cause renal tumours in rodents following chronic dietary intake. A disruption in the normal cellular proliferation control could contribute to the observed induction of nephropathy, immunotoxicity and teratogenicity of OTA as well as to its carcinogenic effects. Therefore, we investigated the effect of OTA on proliferation rates of LLCPK-1, NRK-52E and porcine primary proximal kidney cells (PKC) following acute exposure. Cells were exposed to increasing concentrations of OTA over 24, 48 and 72hrs. Antiproliferative effects were determined using a Coulter counter. The concentration producing a 50% proliferative arrest (G1) in LLCPK-1 and NRK-53E cells lines was 21μM, with PKCs displaying a G1 of 15μM. Standard flow cytometric analysis of propidium iodide stained DNA was used to investigate the nature of this growth inhibitory effect. OTA was found to increase the percentage of cells residing in the G2/M phases of the cell cycle. More detailed analysis, (fluorescence-microscopic mitotic index of Hoechst-stained DNA) demonstrated no increased numbers of cells in M-phase thus suggesting an OTA mediated specific blockade of the cell cycle located to the G2 phase in all three cell types. As the G2 phase of the cell cycle is where proof-reading of replicated DNA and intracellular protein expression (cyclins, cdks) occur before entry into mitosis, an increased residency of cells in this cell cycle phase could indicate dysregulated proliferative control which could be a factor in disease development.

Keywords: Cell cycle, Renal carcinogenesis
Poster

Ermittlung der Startdosis aus Zytotoxizitätsdaten vor
der Bestimmung der akuten oralen Toxizität

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In the next step, the data were included in the RC with the regression model IC₅₀ to LD₅₀. In the first step, a standard regression model was used for the log-transformed 347 data pairs IC₅₀, LD₅₀ (p.o.) for rat/mouse weight. With the help of the IC₅₀, LD₅₀ were also included in the RC. In this way, the IC₅₀, LD₅₀ values can be set for acute oral toxicity (LD₅₀) for the prediction of acute oral toxicity (LD₅₀) and for the prediction of acute oral toxicity (LD₅₀) for the prediction of acute oral toxicity (LD₅₀).

Literatur


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Poster

Permanent Female and Male EG-Cell-Lines of Balb/CJ Mice – an Alternative Concept for Reproductive Toxicity Testing?

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While several in vitro tests are available for replacing animal experiments in base-level screening of new substances, few have been defined adequately for quantitative studies in reproductive toxicology. In order to offer a sensitive and predictive in vitro method to assess the genotoxic potential of chemical agents on male and female reproduction, we established primordial germ (PG) cell-derived permanent female and male embryonic germ (EG) and embryonic stem (ES) cell lines of the mouse (strain Balb/cJ). All EG and ES cell lines were characterised (by PCR and karyotype) and periodically checked for quality criteria like alkaline phosphatase activity and mean generation time (MGT) to ensure clone stability.

The differences in developmental sensitivity of EG cells, ES cells and differentiated fibroblast cells of the mouse cell line 3T3 regarding genotoxicants were comparatively tested under identical test conditions. Cytotoxicity assay was based upon determination of growth inhibition (MTT-test) and genotoxic effects were determined by sister chromatid exchanges (SCE) induced by standard reference mutagens like Ethylisourea (ENU), Methylisourea (MNU), Methylmethylsulfonate (MMS), Hydroxyurea (HU) and Mitomycin C (MMC). After calibrating the in vitro ES cell assay by testing positive and negative control substances, we are now starting to clarify if our in vitro test system can reliably and reproducibly discriminate between known genotoxic and non toxic agents. Furthermore, a distinction between different degrees of genotoxic effects is mandatory for our assay. To classify the genotoxic potential of all tested chemicals we will develop a biostatistical prediction model on the basis of concentration-response curves and exclusion of a major impact of cytotoxicity. Finally, we would like to establish an additional in vitro test focusing on EG cell progression through meiotic prophase as predictive endpoint for sex-specific genotoxicity testing.