Poster

High Correspondence of *Botulinum* Neurotoxin C1 (BoNtC1) 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 BoNtC1 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 predominatly caused by type C1 toxin (BoNtC1). 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 of viable BoNtC1 toxigenic clostridia was developed and evaluated.

The potential of BoNtC1 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 BoNtC1 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 BoNtC1 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 BoNtC1 toxigenic Clostridia in environmental and veterinary samples.

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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 both human and animal foodstuffs. All 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 (GI_{50}) in LLCPK-1 and NRK-53E cells lines was 21µM, with PKCs displaying a GI_{50} 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 indicated dysregulated proliferative control which could be a factor in disease development.

Keywords : Cell cycle, Renal carcinogenesis

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

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Zum Zwecke der Reduzierung von Tierversuchen zur Bestimmung der akuten oralen Toxizität entwickelten wir auf praktische Belange zugeschnittene Verfahren, um mit Hilfe von *in vitro* Zytotoxizitätsdaten die Startdosis eines Stoffes für die nachfolgenden *in vivo* Tests vorherzusagen. Dazu dienen die Daten im Register der Zytotoxizität (RC), in dem von 347 Stoffen neben der mittleren IC_{50} (IC_{50x}) auch die akuten oralen LD_{50}-Werte (LD_{50} p.o.) für Ratte/Maus verzeichnet sind (Halle, 1998). Mit dem einfachen linearen Regressionsmodell wurde für die log-transformierten 347 Wertepaare IC_{50x}-LD_{50} p.o. eine Standardregressionsgerade mit den Parametern Intercept a=0,625 und Regressionskoeffizient b=0,435 erstellt. Von den 347 Stoffen liegen 252 Stoffe (72,6%) in einem Dosisbereich um die Regressionsgerade, der durch den empirischen Faktor $F_G \leq \log 5$ mit der oberen und unteren Prädiktionsgrenze definiert ist.

Im nächsten Schritt wurden die Daten im RC zusammen mit dem Regressionsmodell IC_{50x} auf LD_{50} p.o. zur Einstufung der 347 Stoffe des RC in die vier EU-Toxizitätsklassen für die akute orale Toxizität und für vergleichende Untersuchungen von neun Stoffen aus einer Arbeit von Lipnick et al. (1995) verwendet, die im RC registriert sind und von denen die Autoren im Tierversuch mit der „*Up and Down-Procedure*" (UDP) die orale LD_{50} in mg/kg ermittelten. Mit den Daten des RC konnte für sieben der neun Stoffe der gleiche LD_{50}-Dosisbereich vorhergesagt werden, der auch für die LD_{50}-Werte gilt (Spielmann et al., 1999). Nur zwei der neun Stoffe weichen mehr als eine Größenordnung einer Dosiseinheit von den *in vivo* Werten ab. Diese zwei Stoffe sind demnach außerhalb der durch den Faktor F_G definierten Prädiktionsgrenzen lokalisiert.

Die Ergebnisse mit den neun Stoffen lassen eine weitere Möglichkeit erkennen, mit den RC-Daten die LD_{50} vorherzusagen. Für diesen Zweck wird ein schrittweises *in vitro/ in vivo* Verfahren vorgeschlagen. Im ersten Schritt wird eine laboreigene Zelllinie (z.B. 3T3) an die Bedingungen des Regressionsmodells im RC angepasst. Anschließend kann mit dieser Zelllinie von neuen Stoffen, für die Tierversuche vorgeschrieben sind (z.B. Industriechemikalien), die Stärke der Zytotoxizität (IC_{50}) bestimmt werden. Aus den IC_{50}-Werten lässt sich dann die LD_{50} in mg/kg vorhersagen. Das hier vorgestellte Verfahren kann prinzipiell auch zur Prädiktion der intravenösen LD_{50} (LD_{50} i.v.) bei der Neuentwicklung von Arzneistoffen übernommen werden (Halle, 1998).

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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 Ethylnitrosourea (ENU), Methylnitrosourea (MNU), Methylmethansulfonate (MMS), Hydroxyurea (HU) and Mitomycin C (MMC). After calibrating the *in vitro* EG 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.