Mechanism of TCDD-induced oxidative stress, role of estrogen receptor, and modulation by estradiol in liver cells

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defensins *in vivo* was verified by cytotoxicity assays, including the determination of the transepithelial resistance of CaCo-2 monolayers and analysis of the glucosylation status of Rac1 in toxin-treated cells. *In vitro*-glucosylation was utilized to determine the corresponding IC_{50}-values. HNP-1, HNP-3 and HD-5, but not β-defensin-1 or LL-37, inhibited toxin B-catalyzed *in vitro*-glucosylation of Rho GTPases in a time- and concentration dependent manner. The IC_{50} values range from 0.6 – 1.5 µM, depending on the defensin, the GTPase and the utilization of either holotoxin B or the N-terminal catalytic glucosyltransferase domain. Precipitation and turbidity assays demonstrated a concentration-dependant complex formation, comparable to *Bacillus anthracis* protective antigen (PA) and lethal factor (LF). The formation of aggregates was found not to be responsible for the inhibitory potential. Our data indicate that toxin B but not toxin A interacts with high affinity with defensins HNP-1, HNP-3 and HD-5 and suggest that defensins may provide a defense mechanism against some types of clostridial glucosylating cytotoxins.

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Cytoxic action of the ADP-ribosyltransferase SpvB from *Salmonella enterica*

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Salmonella enterica, a Gram-negative food-born pathogen, causes human diseases ranging from mild gastroenteritis to severe systemic infections. The virulence of *Salmonella* is closely associated with its intracellular replication in macrophages and the bacterial ADP-ribosyltransferase SpvB is absolutely crucial for the intracellular growth. We cloned and expressed the catalytic domain of SpvB (C/SpvB) and demonstrated that SpvB mono-ADP-ribosylates G-actin at position Arg-177. In infected cells, SpvB is directly secreted from intracellular growing *Salmonella* into the host cell cytosol, most likely by type-III-secretion through a bacterial protein needle. Thus SpvB is not taken up into cells when applied to the medium and therefore, we chose two different approaches to investigate the effect of SpvB on intact mammalian cells. At first, we took advance of a recombinant fusion toxin (C2IN-C/SpvB) to deliver C/SpvB into the cytosol via the binary *Clostridium botulinum* C2 toxin. Treatment of various mammalian cell lines with C2IN-C/SpvB resulted in the depolymerization of actin filaments and cell rounding. However, the cytopathic effect of C2IN-C/SpvB was transient due to degradation of the toxin in the cytosol. Intoxicated cells regained a flat morphology but failed to divide, resulting in enlarged binuclear cells. Currently, we investigate whether the observed effects reflect the situation in the infection model. To this end, we infect cultured J774.A1 macrophages with a genetically modified *S. enterica* strain, over-expressing SpvB. Following infection, the SpvB protein is detected in the cytosol of J774.A1 cells by a specific antibody raised against SpvB and the degradation of SpvB is monitored.

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Organic anion transporter 1 (OAT1) as mediator of nephrotoxicity of 5-sulphooxymethylfurfural (SMF)

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5-Hydroxymethylfurfural (HMF) is formed when sugars are acidified or heated. It is present at high levels in numerous foods. HMF itself was inactive in various genotoxicity tests. However, it has been shown that HMF can be metabolised in animal models *in vivo* as well as by human enzymes *in vitro* into a chemically reactive metabolite, 5-sulphooxymethylfurfural (SMF), which is mutagenic and carcinogenic. In a recent *in vivo* study conducted in our department SMF was highly toxic in mice: most animals died on the 5-11th day after the treatment with a single dose of 250 mg/kg body mass. The most severe effects were seen in the kidneys with abundant acute necrosis and proteinaceous casts in the proximal tubules. Since proximal tubule cells of the kidney are the main site of the active organic anion secretion we hypothesised that the transporter-mediated uptake of the SMF in to the cells could be the reason for the selective organotoxicity. To test this hypothesis, we studied whether renal basolateral transporter OAT1, primary responsible for the basolateral concentrative uptake of organic anions into the proximal tubule cells, could use SMF as substrate. Human OAT1 was stably expressed in the Chinese hamster V79 cell line, a suitable model for genotoxicity testing. SMF inhibited the uptake of the model substrate *p*-aminohippurate in OAT1-expressing V79 cells, suggesting that it is a competing substrate. The K_i was calculated to be around 300 µM. Moreover, the expression of OAT1 significantly enhanced SMF-induced mutagenicity in the *hprt* gene mutation assay. Addition of probenecid, a known inhibitor of OAT, to the incubation medium reduced the SMF-induced mutation level to that observed in control cells. Taken together, these results indicate that OAT1 mediates the transport of SMF into the proximal tubule cells of the kidney and thus could play role in SMF-induced nephrotoxicity.

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Establishment of an insect cell expression system for rat Abcb6, an ATP-binding cassette transporter involved in copper tolerance

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Several transporters of the ATP-binding cassette (ABC) superfamiliy are involved in cellular protection by extruding potentially toxic substances through biological membranes. Rat Abcb6 (rAbcb6) is a half-transporter that is expected to achieve functionality as part of a dimer. We have previously shown rAbcb6 expression to confer tolerance to copper. Fluorescence microscopy revealed that rAbcb6 was distributed intracellularly, displaying co-localization with lyosomal/endosomal markers. Although rAbcb6 is related to the multidrug/xenobiotic resistance transporter MDR1, its substrate spectrum remains to be defined. To establish procedures for systematic screening of substrate preferences, systems enabling high levels of transporter expression are required. Thus, baculovirus-dependent expression of rAbcb6 in the SF9 (*Spodoptera frugiperda*) insect cell line was attempted. The cDNA sequence for rAbcb6, extended by a C-terminal foreign V5-epitope, was integrated into the baculovirus (BaculoDirect™) genome. SF9 cells were transfected with the recombinant viral DNA to produce infectious virus particles that were released into the culture medium. To scale up the viral stock, several rounds of infection with culture supernatants were performed. Viral titers were evaluated by detecting virus producing foci in infected cultures with a baculovirus antibody. Finally, high baculovirus expression vector titers were used for SF9 infection, and viral concentration- and time-dependent rAbcb6 expression within cells was examined by immunoblot analyses of cell lysates. Rat Abcb6-V5 was probed for with a primary antibody against its V5-tag. Substantial rAbcb6-V5 expression was observed within two days of infection. In mammalian expression systems, rAbcb6 molecules were shown by immunoprecipitation to interact with each other, supporting the conclusion that homodimers are formed and that co-expression in SF9 cells of a different half-transporter is not required to achieve a functional status. In summary, the presented results demonstrate that the baculovirus/SF9 system is suitable to obtain high rAbcb6 expression, which provides an important basis for systematic functional analyses.

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Effect of liposomes on the uptake of catechols into V79 cells

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Compounds containing a catechol structure, i.e. an ortho-diphenolic group, are often encountered among plant constituents or are formed as metabolites of aromatic substances. In general, catechols are chemically and metabolically unstable, and numerous reactions of catechols are known leading to toxic effects, e.g. oxidation to semiquinone/quinone intermediates which generate reactive oxygen species through redox-cycling, or complex formation with metal ions. Due to their instability and also to their rather low lipophilicity, which hampers diffusion through cell membranes, the concentration of catechols in cells after *in vitro* exposure is usually low. The aim of the present study was to increase the uptake of quercetin, a model catechol, into cultured male Chinese hamster V79 lung fibroblasts by packing it into liposomes. The use of this vehicle was expected to increase the concentration and also the induction of DNA strand breaks by quercetin in these cells. When the stability of free quercetin was studied in cell culture medium, a fast decline was observed, probably due to chemical degradation; this degradation was much slower when quercetin was packed in liposomes. Incubation of V79 cells with liposomes containing quercetin also exhibited a slower degradation of quercetin in the incubation medium, and an increased amount of quercetin was determined in these cells as compared to cells incubated with free quercetin. Surprisingly however, no elevated level of DNA strand breaks was observed in the liposome-exposed cells. These results show that packing a catechol into liposomes leads to a higher stability and increased cellular uptake of the catechol. The failure of the elevated amount of catechol to increase DNA strand breaks suggests that the catechol is not or only partly released from the liposomes.

Supported by the Deutsche Forschungsgemeinschaft (Grant ME 574/26-1).

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Mechanism of TCDD-induced oxidative stress, role of estrogen receptor, and modulation by estradiol in liver cells

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic member of a large family of halogenated aromatic hydrocarbons present as contaminants in food, mother's milk and environmental samples. TCDD was classified as a group I human carcinogen by the International Agency for Research on Cancer (IARC). Its effects are mediated by the arylhydrocarbon receptor (AhR) that regulates transcription of target genes including cytochromes P450 (CYP) 1A1, 1A2, and 1B1. TCDD acts as a liver carcinogen in female but, to a much lower extent, in male rats. There is good evidence for a role of estrogens in the mechanism of TCDD-induced hepatocarcinogenesis in the rat. Estrogens may act as genotoxic procarcinogens. Particularly, the 17β-estradiol (E2) catechol metabolite 4-hydroxyestradiol formed mainly by CYP1B1 can undergo redox cycling and thus generate DNA damaging reactive oxygen species (ROS). As previously seen, induction of CYPs by TCDD led to increased formation of ROS and increased DNA levels of 8-oxo-2'-deoxyguanosine (8-oxo-dG) in rat H4IIE hepatoma cells and in rat primary hepatocytes but not in human HepG2 hepatoma cells. E2 alone increased ROS formation only in hepatocytes while the combination with TCDD had no additive effect. Real-time RT PCR analysis revealed that TCDD induced CYP1B1 mRNA level to a slightly higher extent in rat hepatocytes than in hepatoma cells, while E2 had no clear effect. Since estrogen receptor alpha (ER)α mRNA was present in hepatocytes but not in hepatoma cells, it can be speculated that the presence of ERα may be responsible for the elevated ROS formation after E2 treatment.

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Protection by indolo[3,2-*b*]carbazole against DNA-damage by benzo[*a*]pyrene and hydrogen peroxide in Caco2-cells

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Epidemiological studies suggest that high dietary intake of fruit and vegetables protects against tumour development in many organs including colon. Especially compounds derived from the Brassica genus, e.g. broccoli, cauliflower, brussels spout or chinese cabbage, appear to be protective against colorectal cancer. Brassica vegetables are rich