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THE ROLE OF N-SULFATION IN THE PROMOTION PHASE OF CARCINOGENESIS BY N-HYDROXY-2-ACETYLAMINOFLUORENE IN MALE RAT LIVER.

E.D.Kroese, M.L.M van de Poll, G.J.Mulder and J.H.N.Meerman

The liver is one of the organs in the male rat that is highly susceptible to the carcinogenic action of N-hydroxy-2-acetylaminofluorene (N-OH-AAF). A major route for the formation of reactive intermediates and macromolecular adducts from this carcinogen is N-sulfation through sulfotransferases. The role of this N-sulfation in the promotion phase of carcinogenesis by N-OH-AAF was the objective of this study. We used an initiation-promotion (selection) model for tumor-induction as originally developed by Roberts and colleagues (1). This model consists of treatment with diethylnitrosamine (single dose; initiation) followed by N-OH-AAF (several doses) coupled with partial hepatectomy (promotion/selection). The focal liver cell populations (foci), which are the first aberrant cells that appear with this treatment are considered to be preneoplastic lesions and can be detected by  $\gamma$ -glutamyl-transpeptidase staining (GGT<sup>+</sup>). The effects of inhibition of sulfotransferase activity towards N-OH-AAF with pentachlorophenol (PCP (2)), during N-OH-AAF treatment on the number and volume of GGT<sup>+</sup> foci was investigated. PCP treatment during promotion with N-OH-AAF reduced the volume occupied by GGT<sup>+</sup>-cells by 65%, without significantly affecting the number of GGT<sup>+</sup> foci found per cubical cm. It is therefore concluded, that promotion (selection) by N-OH-AAF of initiated cells depends for a large part on the sulfotransferase pathway.

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2. J.H.N.Meerman and G.J.Mulder, Life Sciences 28 (1981) 2361.

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CADMIUM INHIBITION OF CALCIUM TRANSPORT IN FISH GILLS

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Freshwater fish take up most of the Ca necessary for growth and Ca-homeostasis from the water via their gills.

Ca<sup>2+</sup>-inflow is a transcellular process involving an ATP-dependent Ca<sup>2+</sup>-transport mediated by a "high-affinity" Ca<sup>2+</sup>-ATPase at the basolateral membrane system (1).

Exposure of rainbow trout (*Salmo gairdneri*) to cadmium in the water rapidly leads to hypocalcemia. Experimental evidence is supplied that such disturbance is the result of decreased branchial Ca<sup>2+</sup>-transport due to inhibited Ca<sup>2+</sup>-ATPase activity.

We have tested the effects of Cd<sup>2+</sup> in the water on net branchial Ca<sup>2+</sup>-flow (inflow minus efflow) in perfused trout gills (2) and on the Ca<sup>2+</sup>-ATPase activity in the isolated gill plasma membranes. Characteristics of the Ca<sup>2+</sup>-ATPase activity are: an affinity for Ca<sup>2+</sup> in the  $\mu$ M range, ATP preference, and calmodulin dependency. The desired Ca<sup>2+</sup>-concentration ( $10^{-6}$  M) and the free Cd<sup>2+</sup>-concentrations ( $10^{-9}$  -  $10^{-6}$  M) in the Ca<sup>2+</sup>-buffer were calculated on the basis of established (Ca)(3) and newly determined (Cd) binding constants of the ligands.

Exposure of trout to  $10^{-7}$  M Cd<sup>2+</sup> reduced the Ca<sup>2+</sup>-inflow by 79%, while the Ca<sup>2+</sup>-efflow remained unaffected. Cd<sup>2+</sup> also proved to specifically inhibit (*in vitro*) the "high-affinity" Ca<sup>2+</sup>-ATPase activity (IC<sub>50</sub>:  $4 \cdot 10^{-7}$  M Cd<sup>2+</sup>). This inhibition is apparently not caused by Cd<sup>2+</sup> binding to calmodulin but rather by a direct competition with Ca<sup>2+</sup> for the Ca<sup>2+</sup>-transport site of the Ca<sup>2+</sup>-ATPase.

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2. P.M. Verboost, G. Flik, R.A.C. Lock and S.E. Wendelaar Bonga, Am. J. Physiol., 253 (1987) 216
3. M.P.E. van Heeswijk, J.A.M. Geertsens and C.H. van Os, J. Membrane Biol., 79 (1984) 19

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DNA ALKYLATION AND CROSSLINKING BY REDUCTIVELY ACTIVATED 2,5-BIS(1-AZIRIDINYL)-1,4-BENZOQUINONE ANTITUMOUR COMPOUNDS

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Bisaziridinyl benzoquinones are potential antitumour compounds, that are assumed to be bioreductively activated. We investigated a series of bisaziridinyl-benzoquinones synthesized by the Organic Chemistry Department of the Technical University of Twente. Previously, these compounds were shown to kill DNA-repair deficient E.coli and to inactivate bacteriophage-M13 DNA.

Alkylation of DNA by the unsubstituted title compound (TW13) was studied by means of UV-absorbance after removal of unbound quinone. Crosslinking of DNA was measured with an ethidium bromide fluorescence assay.

DNA alkylation as well as crosslinking appeared to increase strongly with decreasing pH, indicating the role of protonation of the aziridine rings in the alkylation process. The increase of alkylation and crosslink formation occurs at higher pH when the quinones are reduced.

This is expected because reduction facilitates protonation of the aziridine groups. At pH 7, DNA alkylation increased linearly with the amount of reduction. The similarity in pH dependence of M13-DNA inactivation and alkylation indicates that DNA inactivation is mainly caused by alkylation. The relationship between the extent of crosslinking and inactivation of M13-DNA was only weak.

Methylation of the aziridine group was shown to decrease strongly M13 inactivation and DNA crosslinking.

Generally, our results are in agreement with the concept that reductive activation is the major mechanism of action by producing modifications at the DNA level.

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CYTOTOXICITY AND BIOTRANSFORMATION STUDIES WITH BROMOBENZENE IN RAT HEPATOCYTE CULTURES

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Bromobenzene (BrB) is toxic to hepatocytes *in vivo* as well as *in vitro*. This toxicity is related to biotransformation and GSH depletion. BrB hepatotoxicity is elicited by metabolites that are generated by phenobarbital (PB) inducible forms of cytochrome P-450, i.e. isoenzymes belonging to family II. In hepatocyte primary cultures a loss in cytochrome P-450 level is observed. In rat hepatocytes this loss is greater than in hepatocytes derived from other mammalian species (1). Little is known about the behaviour of the different cytochrome P-450 isoenzymes and their residual activities in primary hepatocyte cultures.

In order to validate the use of hepatocyte cultures as *in vitro* model system for studying biotransformation, we investigated BrB cytotoxicity and biotransformation in rat hepatocytes immediately after cell isolation and after 24 h in primary culture. Toxicity (at conc up to 2 mM) was only observed in the freshly isolated cells. In these cells the levels of GSH were considerably lower than in cells after 24 h in culture. A BrB-dependent decrease in GSH was found in cells after exposure for 24 h. GSH/GSSG ratios changed from about 3 in control cells to about 1.5 in cells exposed to 2 mM BrB. BrB was metabolised to 2-, 3-, and 4-bromophenol, which were conjugated with glucuronic acid and sulphate. No changes in the ratio of 4-bromophenol/2-bromophenol were observed.

These results indicate that cytochrome P-450 isoenzymes involved in BrB metabolism belonging to family I and II are approximately equally stable in rat hepatocyte primary culture. Similar studies with liver cells derived from other mammalian species are in progress.

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