The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/17471

Please be advised that this information was generated on 2019-01-29 and may be subject to change.
THE ROLE OF N-SULFATION IN THE PROMOTION PHASE OF CARCINOGENESIS BY N-HYDROXY-2-ACETYLAMINOFLUORENE IN MALE RAT LIVER.

E.D. Kroese, M.H. 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 the carcinogen is N-oxidation through sulfotransferases. The role of this N-oxidation 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 coworkers (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 p-glutamyl-transpeptidase staining (GPT). 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 GPT -foci was investigated. PCP treatment during promotion with N-OH-AAF reduced the volume occupied by GPT -cells by 65%, without significantly affecting the number of GPT foci found per cubical cm. In the theoretical model (3), that promotion (selection) by N-OH-AAF of initiated cells depends for a large part on the sulfotransferase pathway.


Division of Toxicology, Center for Bio-Pharmaceutical Sciences, University of Leiden, Wassenaarweg 72, 2333 AL Leiden, The Netherlands.

CACODYLUM INHIBITION OF CALCIUM TRANSPORT IN FISH GILLS

R.A.C. Lock, P.M. Verbrust, G. Flik and S.E. Wendellaar Bonga

Freshwater fish take up most of the Ca necessary for growth and Ca-homeostasis from the water via their gills. Ca2+ inflow is a transcellular process involving an ATP-dependent Ca2+ transport mediated by a "high-affinity" Ca2+-ATPase at the basolateral membrane system. 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 Ca2+-transport due to inhibited Ca2+-ATPase activity. We have tested the effects of Cd2+ in the water on net branchial Ca2+-flow (inflow minus outflow) in perfused trout gills (2) and on the Ca2+-ATPase activity in the isolated gill plasma membranes. Characteristics of the Ca2+-ATPase activity are: an affinity for Ca2+ in the uM range, ATP preference, and calcium dependency. The desired Ca2+ concentration (10^-6 M) and the free Ca2+-concentrations (10^-9 to 10^-6 M) in the Ca2+-buffer were calculated on the basis of established (Ca2+/GTP) and newly determined (Ca2+) binding constants of the ligands. Exposure of trout to 10^-7 M Cd2+ reduced the Ca2+-inflow by 75%, while the Ca2+-outflow remained unaffected. Ca2+ also proved to specifically inhibit (in vitro) the "high-affinity" Ca2+-ATPase activity (Fig: 4. 10^-7 M Ca2+).

This inhibition is apparently not caused by Cd2+ binding to calmodulin but rather by a direct competition with Ca2+ for the Ca2+-transport sites of the Ca2+-ATPase.


Dept. of Zoology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.

DNA ALKYLATION AND CROSSLINKING BY REDUCTIVELY ACTIVATED 2,5-BIS[1-AMIDRINYL]-1,4-BENZOQUINONE ANTITUMOUR COMPOUNDS

K.J. Lusthof, N.J. de Mol, L.H.M. Janssen

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 (Twind) 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. A 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 alkylation was only weakly related. 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.

Dept. Pharmaceutical Chemistry/Chemical Pharmacy, Faculty of Pharmacy, University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht, The Netherlands.