Unit Toxicology and Chemotherapy (D0301)

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Aims and scope of the Unit
The Unit of Toxicology and Chemotherapy originated in 1993 from the former Division of Carcinogenesis and Chemotherapy. The objective of this unit is to better understand mechanisms of toxicity and carcinogenesis of drugs and chemicals and to develop improved methods for diagnosis and treatment of cancer.

In this context the mechanism of action of the carcinogenic cytostatic agent 5-aza-2'-deoxycytidine was investigated. In addition, the spectrum of anticancer and anti-protozoan activities of alklyphosphocholines was studied and, moreover, colorectal micro-metastases as well as isolated tumor cells derived from K-ras mutated colon carcinoma were identified by using a sensitive assay specific for K-ras mutations.

Sensitive detection and quantitation of colon carcinoma cells
M.R. Berger, C.C. Schimanski, M. Conzelmann, C. Dieterle, A. Wittmer, T. Stein

Cooperation with: Dr. Ulrich Linnemann, Klinikum Nürnberg Nord, Nürnberg; Dr. Matthias Seelig, Klinikum Ludwigshafen, Ludwigshafen

A PCR-RFLP assay was established for detecting mutations of codons 12 and 13 of the K-ras gene in tissue samples from patients with colorectal carcinoma. The assay is sensitive enough to detect one mutant in 10^6 wild type cells. Based on this assay, a K-ras mutation rate of 46% was found in 146 colorectal cancer patients. The most prevalent mutation at codon 12 was a G→C transition, followed by a G→A transition which resulted in amino acids alanine and asparagine, respectively, instead of glycine. At codon 13 G→A transitions were found only, resulting in amino acid asparagin instead of glycine. Concomitantly investigated tissues with potential metastatic spread (lymph nodes, liver) were found positive for K-ras mutants even if histologic examinations failed to detect any metastases. Thus, it was demonstrated that K-ras mutated isolated tumor cells can be detected earlier than with conventional diagnostic means by using the PCR-RFLP assay for tissue samples from critical organs such as the liver [1,2].

In a separate approach on quantitating colon cancer cells, human [3] and rat [4] colon cancer cells were grown in nude and normal rats, respectively. Human colon cancer cells were quantitated by using a nested PCR assay and an external standard, the number of rat colon cancer cells was determined by assaying the activity of β-galactosidase after transfecting the cell line with this marker gene. The latter model will be used for therapy experiments.

Chemical carcinogenesis
M.R. Berger

5-Aza-2'-deoxycytidine (5-AdC) is a cytostatic agent which after incorporation into DNA prevents methylation of cytosine residues. This property has been used to reverse the malignant growth of cancer cells which contain tumour suppressor genes silenced by hypermethylation. However, epigenetic changes of DNA might well harbour a carcinogenic risk due to the activation of silenced oncogenes. To assess the long term toxicological risk of 5-AdC the cytostatic was administered to Sprague Dawley rats in a carcinogenicity study. Administration of 5-AdC was well tolerated but caused significant reductions in mean survival time due to the induction of malignant tumours. The percentage of rats with malignant tumours was doubled in treated animals. Histological examination revealed the hematopoietic system, skeleton, nervous tissue, skin and mammary gland (females only) to be target organs of 5-AdC's long-term toxic action. From these results it is concluded that 5-AdC is a multipotent carcinogen in rats.
To investigate a possible relationship between imprinting or loss of imprinting due to demethylation caused by 5-Ac and its target organs of carcinogenesis, polymorphisms of implanted rat genes (IGF2 and H19) were identified. Ongoing studies focus on differences in the imprinting status between control and treated animals for normal and tumour tissues.

**Pharmacological characterisation of antineoplastic alkylphosphocholines**

M.R. Berger, S.M. Konstantinov, M. Georgieva

Cooperation with: Prof. Dr. Hansjörg Eibl, Max Planck Institut für Biophysikalische Chemie, Göttingen; Prof. Dr. Clemens Unger, Klinik für Tumorbiologie, Freiburg; Dr. Uwe Zillmann, Zentrales Tierlabor des DKFZ

High anti cancer activity in methylnitrosourea-induced primary rat mammary carcinoma, as first described by our group (Berger et al., Cancer Treat. Rev., 1987, Muschiol et al., Lipids, 1987) and then by others (Hilgard et al. Eur. J. Cancer, 1988), prompted the clinical use of hexadecylphosphocholine (HPC; INN: miltefosine) in the topical treatment of skin metastases of locally recurring breast cancer. Corresponding to our pre-clinical results, anti-cancer activity was observed in patients, leading to remissions or stable disease in 50% of treated women. Systemic administration of HPC, however, failed to result in tumour responses since gastrointestinal toxicity prevented the administration of sufficient dose levels. Nevertheless, the low dose levels administered caused significant increases in platelet and leukocyte counts. Alkylphosphocholines have been a main topic of interest since then and were followed to establish dose/response and structure/activity relationships as well as new pharmacological effects and the mechanisms of action.

a) **Structure activity relationships of alkylphosphocholines in mammary carcinoma**

Studies on the structure/activity relationships of alkylphosphocholines concentrated on the length of the alkyl chain, the introduction of double bonds into the alkyl chain and on modifications of the polar phosphocholine head. We demonstrated that an elongation of the alkyl chain was associated with increased toxicity, but introduction of a double bond reduced toxicity. A completely different physical property was obtained with an alkyl chain length of 22 carbon atoms since this structure no longer forms micelles in solution and can be administered i.v. due to the absence of haemolytic properties. Compounds of this chain length, like erucylphosphocholine, have a superior therapeutic ratio and are considered to be second generation alkylphosphocholines [5].

b) **Structure activity relationships of alkylphosphocholines in hematopoietic cells**

In spite of its antineoplastic and anti viral effects, HPC was shown by us to have no toxic influence on mouse bone marrow cells. In contrast to this effect on normal hematopoietic cells, alkylphosphocholines inhibited effectively the growth of a panel of leukemic cell lines (HL60, U937, Reh, Molt4, Jurkat, Ramos, Raji, except K562) and their cyto-

toxic activity depended on the length of the respective alkyl chain. In addition, they induced apoptosis in HL60 and U937 cells, but not in the other cell lines. The expression of BCR-ABL in chronic myeloid leukemia cell lines influences the activity of alkylphosphocholines negatively, with the least antineoplastic activity observed in the cell line expressing the highest BCR-ABL level [6].

c) Activity of alkylphosphocholines in urinary bladder cancer cells

Alkylphosphocholines showed high antineoplastic activity in two urinary bladder cancer cell lines. The activity was based, at least in part, on induction of apoptosis. The distinct anti-cancer activity led us to predict that the respective topical treatment in man (instillation of solutions containing alkylphosphocholines into the urinary bladder) might be of therapeutic benefit for patients suffering from urinary bladder neoplasia [7].

Publications (* = external co-author)


