820 PUBLICATION

In vitro efficacy of transferrin conjugates of chlorambucil

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Purpose: In order to improve the therapeutic index of the alkylating agent chlorambucil (CLB), this drug was coupled to human serum transferin which exhibits a significant uptake in tumour tissue. The link between CLB and the protein was realised through ester or carboxoyl hydrazone bonds. The activity of the conjugates was evaluated *in vitro*.

Methods: The *in vitro* efficacy of the protein conjugates and free CLB was assessed in two human tumour cell lines (MOLT 4 – leukemia and MCF 7 – mamma carcinoma) using a propidium iodide fluorescence assay (concentration range of CLB from 0.01 to 40 μM).

Results: Whereas the CLB conjugates of the ester type were not as active as free CLB, the conjugates of the acid labile carboxyl hydrazone type showed an *in vitro* efficacy which was comparable or significantly exceeded that of CLB in both cell lines.

Conclusion: Acid labile transferrin conjugates of CLB represent new active formulations suitable for further *in vitro* and *in vivo* testing.

821 PUBLICATION

170-P-Glycoproteins in multidrug resistance. Immunohistochemical assays with MoAb

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Purpose: Multidrug resistance to chemotherapeutical drugs is a complex processus; the synthesis and accumulation of P-170 by MDR-1 gene activation is one of the mechanisms involved. The P-170 expression in Ehrlich ascites carcinoma cells resistent to Epirubicin as well as modulation of it's expression by Nifedipine and Verapamil, using MoAb anti-P-170 was studied.

Methods: The experiments were carried out on groups of 20 Swiss female mice, i.p. grafted with 1.5 mil. Ehrlich carcinoma cells resistant to Epirubicin (RRE-1). Immunocytochemistry on cells smears was dynamically performed and counterstained with M.G.G. We use anti P-170 (clone JSB-1 Boehringer Mannheim).

Results: Untreated carcinoma cells (control) gave negative reaction to anti P-170, as well as Ig-POD assays. Dynamic determination (15 days) on resistent cells (RRE-1), demonstrate positive reaction to P-170, extremely intensive (100% cells) in the first templates (days +15; +30) and moderate positive in the last templates (days +60; +75). The association of an i.p. treatment with Nifedipine and Verapamil, partially reduced the P-170 expression (for Nifedipine) and it was completely reduced for Verapamil.

Conclusion: The association of calcium channels blockers can reduced totally the presence of P-glicoproteins 170, expressed by multidrug resistance cells.

Head and neck cancer I

822 ORAL

Influence of allelism at GSTM1, GSTM3 and GSTP1 gene loci and enzyme expression on site-specific susceptibility to head and neck cancer

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The glutathione S-transferase and cytochrome P450 loci are susceptibility candidate genes for cancers associated with alcohol and tobacco consumption. We describe GSTM1, GSTM3, GSTP1, GSTT1, CYP2D6, CYP1A1 and CYP2E1 genotype frequencies determined by PCR and enzyme digestion procedures from leukocyte DNA in 398 Caucasians with oral cavity, pharyngeal and laryngeal squamous cell carcinomas (SCC) and 216 controls. We found no differences in CYP or GSTT1 genotype frequencies between cases and controls. Proportions of the GSTM1 AB genotype were reduced in all cancer groups compared with controls (versus oral cavity and pharyngeal SCC: p = 0.019, OR 0.1, versus laryngeal SCC: p = 0.029, OR 0.33). Site-specific differences in genotype frequencies were shown with the GSTM3 and GSTP1 polymorphisms. The frequency of GSTM3 BB was significantly lower in the glottic SCC cases than in controls (p = 0.043, OR 0.170) By contrast, we found a significantly lower frequency of the more active, wild type GSTP1 AA genotype in the oral cavity/pharyngeal SCC cases compared with controls (p = 0.042, OR 0.47). Immunohistochemical studies complemented the epidemiological findings in demonstrating strong expression of the GSTM3 subunit in the cilia lining the larynx whereas the GSTP1 staining was predominantly found in the outer layers of the pharyngeal squamous cell epithelium. Our data suggest that allelism at GSTM1 confers risk to all subgroups of head and neck SCC, whereas GSTM3 and GSTP1 are associated with altered, site-specific susceptibility to SCC.

823 ORAL

P53, PCNA, Ki-67 in head and neck carcinoma

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Purpose: To evaluate the prognostic influence of p35, PCNA and Ki-67 in head and neck squamous cell carcinoma (HNSCC) on overall survival (OS) and relapse-free survival (RFS) after accelerated split-course radiation therapy (RT) with and without chemotherapy (ChT).

Materials & Methods: 87 pts (80 male, 7 female) with HNSCC, median age 52 years were treated by RT alone or simultaneous RT/ChT. Patients with SCC of the oral cavity (19 pts), the oropharynx (38 pts) and the hypopharynx (30 pts) of UICC (1987) stages III (7 pts, 8%) and IV (80 pts, 92%) underwent RT consisting of 3 cycles of accelerated fractionation (1.8 Gy bid, total dose 70.2 Gy/51 days) and 2 splits of 10 days each. A combination of cis-DDP, 60 mg/m^2, 5-FU, 350 mg/m^2, Leucovorin (LV) 50 mg/m^2 iv bolus on day 2 as well as 5-FU, 350 mg/m^2/24 h and LV 100 mg/m^2/24 h ci on days 3–5 served for ChT, which was repeated on days 22 and 44. Using monoclonal antibodies for detection of p53, PCNA and Ki-67 antigen, IHC-staining was applied to routinely processed paraffin embedded sections and described by Labeling Indices (LI). Median follow-up was 3.5 years (range 1.5–5 years). PCR and sequencing-analysis of p35 were performed for 10 pts.

Results: OS was 39% and RFS 44% after 3 years. p-35-LI L1 (LI 0%: 54% RFS and LI > = 35%: 69% RFS vs. LI 1–34%: 0% RFS, p = 0.0001), PCNA-LI (LI > 20%: 50% OS vs. LI < = 20%: 31% OS, p = 0.0146), Ki-67-LI (LI > 20%: 55% OS vs. LI < = 20%: 23% OS, p = 0.0344) and additional ChT (RT/ChT. 41% OS vs. RT· 27% OS, p = 0.003) did significantly impact on RFS and OS.

Conclusion: Proliferative parameters like p-35, PCNA and Ki-67 together with clinical factors, grading and staging may be of predictive value in HNSCC after accelerated RT/ChT. Molecular investigations of p53 could help to determine relevant mutations.

824 ORAL

The relationship of human papillomavirus to p53 and proliferating cell nuclear antigen expression in head and neck cancer

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Purpose: Human papillomavirus (HPV) infection and p53 mutation have been implicated in many various types of carcinoma. In this study we investigated HPV infection and its relationship to the presence of proliferating cell nuclear antigen (PCNA) and p53 mutation in seventy-one head and neck carcinoma patients.

Methods: HPV DNA was detected by polymerase chain reaction with L1 consensus primer in fresh frozen tissue and the presence of PCNA and p53 mutation was investigated immunohistochemically in the same patients.

Results: Among 71 tumors HPV DNA was found in 14 cases (19.7%), twenty-five p53 positive cases (35.2%) and PCNA expression was observed in 13 cases (18.3%). HPV DNA was detected higher rate in early and NO stage than late stage of carcinoma (p = 0.046). Contrarily p53 mutation was more frequently observed in advanced cases. There was no association the presence of HPV DNA and PCNA, p53 mutation, but p53 mutation revealed its higher rate of coocurrance with the detection of PCNA.

Conclusion: In head and neck carcinoma, HPV DNA was frequently detected in early stage of disease while p53 mutation might play a role in the late stage of carcinogenesis and p53 mutation was also observed some relationship to the detection of PCNA in this study.