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Research and identification of polymorphic variants of the gene of human carboxylesterase 2, an enzyme involved in the activation of irinotecan into SN-38

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Irinotecan is widely used in the treatment of metastatic colorectal carcinoma. It is a prodrug requiring hepatic activation to the topoisomerase I - interactive drug, SN-38. We had shown earlier a wide individual variation of irinotecan activation by human liver microsomes. Plasma levels of SN-38 also vary in large proportions in patients treated with irinotecan. Carboxylesterase 2 (hCE2) is the enzyme which is mostly responsible for irinotecan activation. There is a possibility that a genetic polymorphism of this enzyme explains the individual variability of SN-38 production in humans and, therefore, some of the variability of the therapeutic and toxic effects of irinotecan in patients. The carboxylesterase 2 gene (16q22.1) contains 12 exons. Human genome sequence in public databases has listed 6 single nucleotide polymorphisms (SNP) in the gene, but no data is yet available about their frequency in the population and their possible role on the expression and activity of the enzyme. None of these variations is able to alter protein sequence, but one of them is located in the promoter of the gene, at position -332 before the transcription initiation codon. In a first attempt, we have explored in a population of 11 individual human DNA samples the existence of allelic variants (i) at the level of each exon and at the intron-exon junctions; (ii) at the level of the 6 SNPs already mentioned in the databases (promoter, intron 2, intron 10, intron 12). This has been done using denaturing high performance liquid chromatography (dHPLC) of 14 different fragments amplified by PCR and sequencing of the amplified fragments which had a modified profile. We have identified a total of 6 allelic variations which constitute three different haplotypes in addition to the wild-type sequence. These variations occurred at the level of the promoter, intron 10 and intron 12 (12a and 12b). We have then extended this study to 53 human DNA samples using dHPLC of the 4 fragments where an alteration had been detected. We have quantified the frequency of the 3 haplotypes in this population: haplotype *1 (promoter, intron 10, intron 12b): 9.4%; haplotype *2 (intron 10): 5.7%; haplotype *3 (intron 10, intron 12a, intron 12b): 11.3%. Another SNP was identified in exon 12 at position 1703, associated with the SNP in intron 10, constituting a 4th haplotype with a frequency of 1.9% and no alteration of protein sequence. A study of the whole gene is ongoing on the 53 DNA samples.

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New structural analogues of lycobetaine with high anticancer activity *in vitro*

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The phenanthridine alkaloid lycobetaine, a minor constituent of Amaryllidaceae, showed very good anti-tumor activity *in vitro* and was found to act as a specific topoisomerase IIβ poison (BJC, 2001, 85(10):1585-91). Here we report on the antitumor activity of 25 lycobetaine analogues, as well as 11 synthesis intermediates and closely related compounds. In a first step, inhibition of tumor growth was studied in a cellular proliferation assay in a panel of 12 human tumor cell lines comprising colon, central nervous system, gastric, non-small cell lung, mammary, melanoma, renal and uterus cancer cell lines. Three compounds (E758, E759, and E761) showed a better antitumor activity than the lead compound lycobetaine (IC_{50} = 6.9 μM). The mean IC_{50} values of the three lycobetaine derivatives were 0.46 μM (E761), 1.2 μM (E759), and 1.4 μM (E758). Compared to the activity of established anticancer agents acting via topoisomerase inhibition, the novel derivatives were similarly potent to etoposide (IC_{50} = 1.0 μM). Lycobetaine, E758, E759 and E761, as well as etoposide were further studied in the clonogenic assay, using 28 human tumor xenografts growing subcutaneously in nude mice. Again, the three derivatives of lycobetaine were more active than the lead compound lycobetaine. The mean IC_{50} values of the three derivatives were 2.0 μM (E761), 3.3 μM (E758), and 4.4 μM (E759), compared to a mean IC_{50} of 11.3 μM of lycobetaine. Regarding the fingerprint profiles of the compounds over 28 human tumor xenografts in the clonogenic assay, the most sensitive tumors were the melanoma MEXF 989, the uterus carcinoma UXF 1138, and the renal cancers RXF 944 and RXF 631 followed by the lung model LXFA 526 and the mammary model MAXF 401. In conclusion, 3/36 analogues of lycobetaine showed a higher antitumor activity than the lead compound lycobetaine. Further *in vivo* studies in nude mice in human tumor xenografts found as sensitive in the clonogenic assay are warranted.

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Pharmacokinetic and Pharmacogenetic Analysis of Irinotecan (CPT-11) Given in Combination with R115777

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The topoisomerase I inhibitor CPT-11 is a prodrug of the pharmacologically active metabolite SN-38, and is approved for the first-line treatment of advanced colorectal cancer. Recently, we conducted a phase I study in which we combined this drug with the farnesyltransferase inhibitor R115777 (Zarnestra'). The maximum tolerated dose was established at 300 mg BID R115777 administered orally for 14 consecutive days q3weeks in combination with 350 mg/m^2 CPT-11 i.v. (90 minutes) q3weeks. The pharmacokinetics (PK) of CPT-11 and SN-38 were determined during 2 courses in a total of 27 patients. In the first course, R115777 was started 2 days after the infusion of CPT-11. In the second course, it was given together. Blood samples were analyzed by HPLC. Next, a recently developed population-PK (NONMEM) model for CPT-11 (Xie et al, J Clin Oncol, 2002) was used to calculate the kinetics of the lactone, carboxylate and total forms of CPT-11 and its metabolite. As patients were treated on different dose-levels of both CPT-11 (200-350 mg/m^2) and R115777 (200-400 mg BID), a dose-normalization was performed. As both anti-cancer agents are partly metabolized by the detoxifying enzyme UGT1A1, a pharmacogenetic analysis for polymorphic variants in UGT1A1*28 was done, using pyrosequencing. In the first course, the mean AUC for the total forms of CPT-11 and SN-38 were 17,763 $\pm$ 4,234 ng.h/ml and 476 $\pm$ 181 ng.h/ml, respectively. In the second course, the CPT-11 AUC was 12.5% higher (paired, 2-sided Student t-test: P = 0.004) with a value of 19,980 $\pm$ 5,309 ng.h/ml), whereas the SN-38 AUC increased by 18%, with a mean value of 562 $\pm$ 300 ng.h/ml (P = 0.036). Also, most other parameters (AUCs and clearances for the lactone and carboxylate forms) of both compounds differed significantly between both courses. The genetic code for UGT1A1 could be determined in 22/27 patients (12 wild-type patients, 9 heterozygous for an extra TA repeat and 1 homozygous for the same variant). No apparent relation between this polymorphism and the PK of CPT-11 could be discovered. In conclusion, a substantial increase in the systemic exposure to CPT-11 and the active metabolite SN-38 was observed following continuous administration of R115777. This may be due to inhibition of UGT1A1, which could lead to less glucuronidation of SN-38. However, the clinical relevance of these findings remains to be established, since both drugs could be safely given in combination at their full-recommended single agent dose.

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Pilot studies of antimetabolites preceeded by irinotecan in advanced solid tumors

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In a Ward rat colon color carcinoma (ca) model, 5-fluorouracil (FUra) showed 95% complete tumor regression (CTR) when irinotecan (IRN) was given 24 h before, but only 38% when it was given 24 h after FUra (50% of MTD of each agent). At these doses, FUra alone gave 25% CTR and IRN gave zero (Cao and Rustum, Cancer Res 60:3717, 2000). A fourfold reduction in the dose of IRN led to only a minor decrease in activity, indicating that IRN is acting as a modulator; preliminary data indicated that IRN caused cells to accumulate in S-phase leading to potentiation of FUra activity. This effect is being explored in two pilot studies in patients (pts) with advanced solid tumors A: IRN/FUra and B: IRN/Gemcitabine (GEM). All drugs are given intravenously. IRN is given over 1.5 h, followed 24 h later by FUra 400 mg/m^2 over 0.08 h, weekly $\times$ 4 q 6 weeks (A) or by GEM 1000