discordant results can be explained by insufficient increase in the marker levels or by a substantial lead-time.

Conclusion: Increasing marker levels in NSCLC stage IIIb/IV patients contribute to the clinical decision making at least in a way that these patients may no longer be treated by ineffective and toxic drugs.

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Comparison of the in vitro cytotoxicity of the antitumour antibiotics bleomycin and mitomycin on human colorectal cancer cells and endothelial cells

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Purpose: To investigate the absolute and relative cytotoxic effects of two antitumour antibiotics on human endothelial cells. Information of cytotoxicity on both proliferating and non-proliferating EC might be important in their toxic and antitumour effects. For both BLM and MMC cytotoxicity against EC is considered to be involved in pulmonary toxic reations of these compounds.

Methods: Cell cultures of the human colorectal cancer line DLD-1, the immortalised endothelial cell line HMEC and fresh harvested umbilical vein EC's were exposed to different concentrations (0.01 to 120 μg/ml) of bleomycin and mitomycin. Cytotoxicity was analysed with Alamar blue technique. Cytotoxic studies were performed against confluent and against proliferating cells. Different durations of incubation 2 h vs 18 hs were studies.

Results: Both BLM and MMC had a cytoxic effect against proliferating EC. BLM exhibited no such effect against confluent EC in the concentration range studied. The cytotoxic effect increased with increased duration of exposure, both for BLM and MMC.

Conclusion: Both MMC and BLM exert a cytotoxic effect against proliferating EC. This effect is dependent on both drug concentration and exposure duration. These results show that at concentrations obtained in patients endothelial cell toxicity might contribute to both the antitumour effect and the different toxic events.

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The influence of theobromine on angiogenic activity of human ovarian cancer cells

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Introduction: Angiogenesis plays the crucial role in growth of solid tumor and metastasis formation. The aim of present study was to evaluate if theobromine – adenosine receptor antagonist and phosphodiesterase inhibitor – shows antiangiogenic activity in human ovarian cancer cells.

Methods: Ascitic fluid was obtained from 30 patients with ovarian cancer (FIGO 3 and 4). Full suspensions of cancer cells were grafted intradermally into Balb/c mice and subsequently treated with theobromine in 0, 24, and 72 hours thereafter. Then mice were sacrificed and newly formed blood vessels were counted (TIA – tumor induced angiogenesis test). In further studies cells (full suspensions, isolated cancer cells and TILs) were preincubated with theobromine and then used to TIA test, and in vivo culture in Balb/c mice peritoneal cavity. The concentrations of angiogenic cytokines (IL8, bFGF and VEGF) in 48 hours in vivo cultures were estimated in ELISA test and shown in pg per mg of total protein. RTPCR method was used to determine the influence of theobromine on urokinase and tissue plasminogen activators (uPA and tPA).

Results: Theobromine injected subcutaneously as well as preincubated with cancer cells showed antiangiogenic activity in TIA test. We showed statistically significant inhibition of IL-8, bFGF and VEGF production by ovarian cancer cells after preincubation with theobromine in therapeutic concentration (20 μg/ml). Lack of transcript of tPA and uPA was shown in theobromine treated cell.

Conclusions: Theobromine is a potent angiogenesis antagonist. The mechanism of its action is complex and includes inhibition of proangiogenic factors production (bFGF, IL-8, VEGF) as well as of uPA and tPA transcript.

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Unwanted effects of evening primrose oil on tumor angiogenesis and blood granulocyte number

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Purpose: Evening primrose oil (EPO) has been investigated as a potential additional treatment for various diseases. It contains high levels of polyunsaturated fatty acids (PUFAs). However excessive amount of PUFAs in human and animal diet have been accounted with increased incidence of tumors. The aim of the study was to estimate the influence of EPO on 1) angiogenic activity of human lung cancer cells and 2) number and activity of mice blood granulocytes.

Methods: Cells from tumors of 18 lung cancer patients were grafted intradermally into 108 Balb/c mice. For the 3 consecutive days 20 mg of EPO was applied on the sites of implantation. After 72 hours mice were sacrificed and the new vessels were counted. Paraffin oil was used as a control drug. In the other experiments 48 mice were fed for 4 weeks with 250 mg/kg day EPO.

Results: The data demonstrated that EPO enhanced neovascular response (mean number blood vessels 19.31 ± 0.61 for EPO; 15.0 ± 0.61 in control group). The number of mice blood granulocytes significantly decreased after EPO treatment but their activity increased.

Conclusions: Angiogenesis – enhancing and granulocyte lowering activities of EPO should be taken into consideration in diet of cancer patients.

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CD44-isoforms promote adhesion to endothelial cells through recognition of chondroitin-4-sulfate

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Purpose: The transmembrane glycoprotein CD44 has been implicated in a wide range of adhesion-dependent cellular processes. Alternative splicing events can give rise to a large number of differentially expressed CD44 isoforms. The aim of the present study was to define the involvement of such isoforms in cellular adhesion to non-activated endothelium.

Methods: The CD44-negative murine lymphoma cell line TIL-1 was transduced with the human CD44H (90 kD) and CD44R1 (130 kD) cDNA by retroviral-mediated gene transfer. Adhesion of fluorescently labelled lymphoma cells to a murine endothelial cell line (SVEC) was evaluated using a fluorescence plate reader.

Results: Lymphoma cells expressing the common CD44 isoform CD44H or the alternatively spliced isoform CD44R1 containing exons v8-v10 can both bind to immobilised and soluble hyaluronan. Cells expressing CD44R1 and to a lesser extent those expressing CD44 H but not the CD44-negative parental cell line were also able to bind the murine endothelial cell line SVEC. This adhesive interaction is mediated by recognition of chondroitin-4-sulfate on SVECs as chondroitinase pretreatment abrogates the cellular interaction. Evidence was obtained that among the proteins that can present chondroitin-sulfate to CD44 is CD44 itself.

Conclusion: We conclude that modification of CD44 on endothelial cells with chondroitin-4-sulfate plays an important role in regulating cellular adhesion to vascular endothelium.

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Examination of secretory phospholipase A_2 ($sPLA_2$) as a maker of metastasis

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Purpose/Methods: $sPLA_2$ is a marker of inflammatory disease. It can be induced by the proinflammatory cytokines IL-1, IL-6 and TNFα. These cytokines are also increased in patients with metastatic cancer diseases. To examine if $sPLA_2$ can serve as a common marker of metastasis too, its amount have been quantified in sera of different groups of cancer patients (breast cancer (121), ovarian cancer (31) and gastrointestinal cancer (37)) as well as in healthy individuals (172) using a commercially available immunoassay.

Results/Conclusions: Significantly increased levels of $sPLA_2$ have been measured in all groups of cancer patients compared to healthy individuals