of cathepsin A in vitro, a lysosomal peptidase, which is widely distributed within mammalian cells and tissues, including tumors.

Methods: In the present study we describe the inhibitory effect of the proteasome inhibitor, lactacystin, on cathepsin A activity in murine melanoma cell lines (B16F10, MmB18 and B78) both in vitro and in vivo. Cathepsin A activity was assayed at pH 5.5 using its specific substrate Cbz-Phe-Ala by ninhydrin method.

Results: We have found that lactacystin metabolite, b-lactone, at concentration of 1mM, significantly suppressed cathepsin A activity in B78 melanoma cell lysates by about 50%. Also exposure of three murine melanoma cell lines with different metastatic potential to lactacystin at concentration of 5 mM for 6 hours caused a significant reduction of carboxypeptidase activity of this enzyme, and the inhibitory activity remained unchanged for at least 12 hours. Other proteasome specific inhibitors, e.g. epoxomicin and PSI at concentration of 1mM did not affect cathepsin A activity in melanoma cell line lysates.

Conclusions: The data presented herein support our previous hypothesis that lactacystin is not a specific inhibitor of the proteasome. Since cathepsin A is also tumor-associated enzyme, further research is needed to clarify its role, including the significance of its inhibition by lactacystin, in tumor biology.

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Soluble plasma P-selectin is elevated in patients with advanced carcinoma

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Purpose: Platelet activation occurs in a variety of disease states and is associated with increased levels of both circulating and platelet expressed P-selectin. In cancer patients activated platelets may release angiogenic factors that in turn may promote tumour growth and metastasis. The aim of this study was to investigate platelet expressed and soluble P-selectin levels in patients with advanced and local cancer.

Methods: Twenty-eight subjects were recruited prospectively. Twelve had disseminated malignancy, 6 had locally contained disease and 10 were controls without cancer. Platelet expressed P-selectin (pP-selectin) was measured using a double stained, whole blood, flow cytometry method. Soluble plasma P-selectin (sP-selectin), was investigated using a specific Enzyme Linked Immunosorbent Assay (ELISA) kit, (R&D systems, Minneapolis, Minnesota).

Results: Soluble P-selectin (ng/ml) was elevated in the disseminated malignancy group compared to the locally contained group (66.2 vs. 35.6; $p<0.02$) and the control group (66.2 vs. 38.3; $p<0.05$). There was no statistical difference between the groups for pP-selectin. The platelet count (x109/l) was significantly higher in the disseminated cancer group compared to the control and local cancer groups (337 vs. 212; $p<0.005$ and 337 vs. 253; $p<0.05$ respectively).

Conclusions: Platelet activation occurs in patients with advanced cancer as demonstrated by elevated plasma sP-selectin levels. Therefore, this potential cause for cancer progression should be investigated by further research.

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The effects of intravenous bisphosphonate treatment on the bone microenvironment in patients with breast cancer and bone metastases

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Purpose: Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption and hence may limit bone-derived growth factors available to breast cancer cells in vivo. This pilot study aimed to investigate the effects of bisphosphonate treatment on cytokines and growth factors in the serum and bone marrow of patients with advanced breast cancer.

Methods: 17 patients with breast cancer and bone metastases were recruited to the study along with 13 patients with primary breast cancer. Samples of serum, urine and bone marrow were taken from the advanced group before and three days after intravenous treatment with pamidronate (90mg) and zoledronate (4mg and 8mg) and from the primary breast cancer group on a single occasion. Serum and bone marrow samples were assayed for the presence of a panel of cytokines and growth factors, including TGFb-1, IGF-1, FGF-2, IL-6 and soluble IL-6 receptor (sIL-6R). Urine samples were assayed for markers of bone resorption.

Results: Samples from patients with advanced breast cancer had significantly higher levels of TGFb-1, IL-6 and soluble IL-6 receptor than those from patients with primary breast cancer ($p<0.05$). In the advanced breast cancer group, no changes were seen in IGF-1, IL-6 or TGFb-1 levels 3 days after bisphosphonate treatment. However, treatment did result in significantly lowered levels of serum FGF-2 and sIL-6R ($p<0.05$).

Conclusion: There appear to be differences in certain cytokines and growth factors between patients with advanced and primary breast cancer. The beneficial effects of bisphosphonates experienced by patients with breast cancer and bone metastases may be due to alterations in cytokines and growth factors, such as FGF-2, that are important in bone. Further studies at different time points are required to confirm these observations.

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Enhancement of WY-14,643-induced transactivation of peroxisome proliferator-activated receptor alpha by green tea extract and its components

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Purpose: Recently green tea was reported to increase the number of peroxisome and its specific enzyme activity in rats. In this study, to determine whether these increases are exerted through activation of peroxisome proliferator-activated receptor alpha(PPARalpha), we investigated the interaction of Wy-14,643, tea extracts, and major tea components with PPAR alpha, cloned from mice using a cell transient transfection assay.

Method: 24 hrs after the transfection, cells were treated with four freshly prepared tea extracts (green tea, oolong tea, black tea, and doongule tea) or tea components in the presence of Wy-14,643. Also, rats received green tea extract (2.5%, W/V) and/or Wy-14,643 (0.5%, W/W) for 2 weeks. Thereafter, RT-PCR was done for acyl-CoA oxidase mRNA.

Result: Activation of PPARalpha was 1.5-2 times increased by green tea extract (0.2%), compared with control. Wy-14,643-induced PPARalpha activation was 4-10 fold enhanced by 0.0001% green tea extract, compared with Wy-14,643 treatment only. Whereas black tea was similar to green tea, semi-fermented oolong tea had little effect on PPARalpha. Even though (-)-epigallocatechin gallate (EGCG) showed the highest activation of PPARalpha and enhancement of Wy-14,643-induced PPAR alpha activation among the components of green tea, its concentration (10 uM) is too higher than expected concentration value of EGCG (<0.9 uM) contained in 0.0001% green tea extract. Oolong tea and doongule tea extract neither increased the activation of PPARalpha nor enhanced Wy-14,643-induced PPARalpha activation. Regarding acyl-CoA oxidase mRNA in the liver of rats fed with Wy-14,643 and/or green tea extract, the combination of Wy-14,643 and green tea extract showed more intensified band compared to that of Wy-14,643 treatment alone.

Conclusion: These results suggest that green tea possess an potent regulatory role in activation of PPAR alpha by peroxisome proliferators, as well as a direct effect through PPAR alpha. In addition, some chemicals like EGCG may have a role in the regulation and be present in the full-fermented black tea.

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BCL-2 down regulation is associated with GO/G1 phase accumulation in 13-CIS-retinoic acid treated HL-60 cells

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Purpose: Retinoic acid and its derivates have been found to cause differentiation of various leukemic cells and therefore are used as a potent therapeutic agent in treatment of acute promyelocytic leukemia. It has been reported that retinoids down regulate the expression of antiapoptotic bcl-2 protein. The aim of this study was to investigate 13 cis retinoic acid regulation of bcl-2 and the possible consequences on cell cycle distribution.

Methods: HL-60 (human promyelocytic cell line) cells were treated with 10-5 M and 10-6 M concentrations of 13-cis-retinoic acid for the period of 24, 48 and 72 hours. DNA content was measured by FACS analysis of propidium iodide stained cells. Cell cycle distribution was estimated using ModFit software. Percentage of bcl-2 protein positive cells was detected