QuSpez extracts (12.5 and 50 μg/ml), the cell growth and bi-dimensional organization were analyzed after 24h.
In vivo angiogenic assay: In vivo angiogeneic assay was performed in female Balb/C mice (6–8 weeks old) by analyzing the growth of blood vessels from subcutaneous tissue into a Matrigel plug. Matrigel was mixed with or without VA extracts and was injected into the abdominal subcutaneous tissue. The mice were also injected with VA preparations intraperitonially (IP) (20 μg/day). Mice were sacrificed after 7 days, and the Matrigel plugs were excised and processed for histological analysis.
Apoptosis assay: EA-hy926 cells were incubated for 24 hrs with varying concentrations of VA extracts (12.5 and 50 μg/ml). The induction of apoptosis by VA extracts was analysed by Annexin V labeling that recognizes exposed phosphotidyl serine on apoptotic cells and PI that binds to DNA.
Results: Treatment of the cells with VA Qu Spez was associated with a reduction in capillary network, in a dose dependant manner. VA Qu Spez at 50 μg/ml induced a nearly complete disruption of the capillary tube formation. The area of angiogenesis network was also reduced by 33%. In our in vivo studies, there was a dramatic reduction in the vascular density in the matrigel treated with VA Qu Spez at the time of the implantation (intra-matrigel treatment) and followed by systemic (IP) treatment as compared to control untreated mice. VA QuSpez also induced apoptosis (upto 60%) of EA-hy926 cells as analysed by Annexin V and PI staining.
Conclusions: Our results show that VA QuSpez reduces angiogenesis in vitro and in vivo and the induction of apoptosis is the one of the underlying mechanisms. The anti-angiogenic properties of VA extracts may explain at least in part to their efficacy as adjuvant therapy in cancer patients.

524 POSTER

Stimulatory effect of eucalyptus essential oil on macrophage/ granulocyte phagocytic activity: in vitro and in vivo evidences

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Background: many species of the genus *Eucalyptus* from the Myrtaceae family are used in folk medicine for a variety of pathologies. Monoterpenoid oil components of aromatic constituents are traditionally used as analgesic, anti-inflammatory, and antipyretic remedies and are commercially available for the treatment of the common cold and other symptoms of respiratory infections. Phytochemical analysis have shown, that the profile of the monoterpenoids changes among the *Eucalyptus* species with potential variations in medicinal properties. In *Eucalyptus globulus* the major monoterpenoid component is eucalyptol, constituting the 60–90%. Macrophages constitute one of the primary cellular mechanisms of the immune response playing a pivotal role in the detection and elimination of foreign body such as pathogenic microorganisms. To our knowledge, in literature actually there is no available data, concerning the influence of *Eucalyptus* essential oil in the cell components of the immune system, the only exception is for the effect of some cytokine production. In this study we investigated whether essential oil from *Eucalyptus globulus* (EO) is able to affect the phagocytic activity of human monocyte-derived macrophages (MDMs) *in vitro* and of rat peripheral blood monocytes/granulocytes *in vivo*.
Materials and Methods: analysis of morphological changes, characteristic of activated MDMs, was performed by scanning electron microscopy. The evaluation of phagocytic activity was carried out: a) in EO treated and untreated MDMs *in vitro* with confocal microscopy after fluorescent beads administration; b) in monocytes/granulocytes from peripheral blood of BDIX rats, after *in vivo* EO administration, with cytofluorimetric analysis using the phagotest kit from ORPEGEN Pharma. Immuno-suppression in BDIX rats was induced by administration of the chemotherapeutic agent 5-fluorouracil (5-FU).

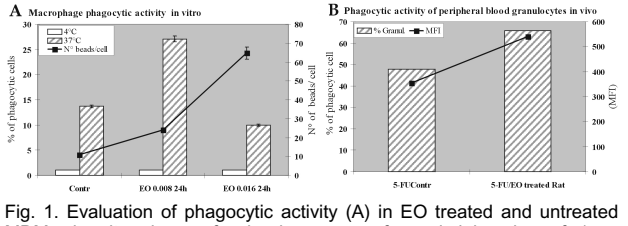

Fig. 1. Evaluation of phagocytic activity (A) in EO treated and untreated MDMs *in vitro*, by confocal microscopy, after administration of 1 μm fluorescent beads; and (B) in BDIX rat peripheral blood granulocytes, after *in vivo* EO treatment, in absence or in presence of 5-FU administration, by cytofluorimetric analysis.

Results: Our results demonstrate that EO is able to activate MDMs and peripheral blood monocytes/granulocytes both *in vitro* (Fig. 1A) and *in vivo*, stimulating their phagocytic activity. EO is also able to induce a dramatic recovery of granulocyte phagocytic activity after bone marrow suppression induced by 5-FU (Fig. 1B).
Conclusion: Our results suggest that the components of essential oil extracts from eucalyptus represent a possible new class of immunoregulatory agents useful in chemotherapy.

Prodrugs

525 POSTER

Targeting Doxorubicin to LHRH-receptor positive tumors by the cytotoxic hybrid ZEN-008 (AN-152)

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ZEN-008 (AN-152) is a cytotoxic analog of the luteinizing hormone releasing hormone (LHRH) in which doxorubicin (DOX) is linked to [D-Lys^6]LHRH. ZEN-008 binds to LHRH-receptors, which are found on a variety of tumors including breast, prostate, ovarian and endometrial cancers. After binding, ZEN-008 is internalized and transported to the nucleus where it induces apoptosis upon release of DOX. The activity of this compound has been demonstrated in experimental models of a variety of human cancers. Here we report on the antitumor activity of ZEN-008 in experimental human endometrial cancers.
LHRH receptors were determined in the HEC-1A human endometrial cancer cell line. The efficacy of ZEN-008 was evaluated and compared to its cytoxic radical DOX in athymic nude mice bearing HEC-1A tumors. The safety and tolerability of ZEN-008 was evaluated in series of studies including safety pharmacology studies and acute and subchronic toxicity studies.
43 days after the injection of ZEN-008 HEC-1a tumor growth was significantly inhibited by 54.2%, while treatment with an equimolar dose of DOX only resulted in a nonsigificant tumor inhibition by 23.4%. WBC 8 days after application was signifcantly suppressed by DOX, but not by ZEN-008.
The good safety profile was confirmed in safety pharmacology studies evaluating the effects of ZEN-008 on respiratory and cardiovascular parameters in the dog as well as in the Irwin and Rotarod test. In the cardiovascular safety study in beagle dogs, no evidence of QT prolongation was seen at any dose administered. Superior tolerability of ZEN-008 as compared to DOX was confirmed in acute and subchronic toxicity studies in mice, rats and dogs, respectively. In contrast to DOX, where lymphohistiocytic myocarditis with intramuscular fibrosis was observed, ZEN-008 did not induce any cardiotoxicity.
Targeted chemotherapy with ZEN-008 is significantly more effective than DOX itself. ZEN-008 is less toxic than DOX as reflected by a consistently higher LD50 and reduced cardiotoxicity. Due to the attractive mechanism of action and the overall promising safety and toxicity profile, ZEN-008 was selected to be evaluated in clinical phase I trials. ZEN-008 is available as a red powder (50 mg) lyophilisate for i.v. application as a solution.

526 POSTER

A Prostate-Specific Antigen (PSA) activated channel-forming toxin as therapy for prostatic disease

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Background: While the exact physiologic function of the prostate is unknown, it is a gland associated with significant morbidity in the aging male. The prostate is the most common site of non-skin cancer diagnosed in American men, with one in six developing the disease during their lifetimes. In addition, approximately 80% of men will present with a symptomatic benign overgrowth of the prostate known as benign prostatic hyperplasia (BPH) by age 80. Prostate-specific antigen (PSA) is a serine protease that is secreted at high levels (micro to mg/ml) by the normal and diseased prostate. To develop effective prostate tissue-selective therapy for localized prostatic disease we modified proaerolysin (PA), the inactive precursor of a bacterial cytolytic pore-forming protein, to produce a PSA-activated protoxin (PRX302).
Materials and Methods: PRX302 was generated by replacing the wild type furin protease activation site within PA with a 6 amino acid PSA-selective activation site. PRX302 was tested for in vitro toxicity against PSA positive and negative prostate cancer cell lines. Intratumoral efficacy of PRX302 was evaluated in PSA-producing xenograft models. Since the PSA gene