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Inhibition of Angiogenesis by Potential Cancer Chemopreventive Agents -Establishment of a Human *in vitro* Anti-Angiogenesis Assay and Mechanistic Evaluation of Potent Inhibitors

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The aim of this study was to identify novel angiopreventive agents by means of the human *in vitro* anti-angiogenic assay followed by the mechanistic elucidation of selected compounds. Representative targets included effects at the transcriptional levels of pro-angiogenic factors, anti-gelatinolytic and anti-endothelial activity. Also the anti-tumour potency *in vivo* was investigated in the dorsal skinfold chamber model.

Establishment of a human *in vitro* anti-angiogenic assay

For the identification of novel inhibitors of angiogenesis, a human *in vitro* anti-angiogenic assay was established, optimised by several growth studies and validated using known anti-angiogenic compounds. These included hydrocortisone, suramin, and indomethacin as well as the chemopreventive agents resveratrol, curcumin, and (−)-epigallocatechin gallate, which inhibit angiogenesis by various pathways. A series of twelve chemopreventive lead compounds, belonging to the chemical classes of phloroglucinol derivatives, anthraquinones, isothiocyanates, flavanones, diterpenes, bibenzyl derivatives of lunularic acid and chalcones was successfully tested. The compounds were selected based on their potential to prevent carcinogenesis by multiple mechanisms, including the modulation of carcinogen metabolism, anti-oxidant, anti-inflammatory, anti-hormonal and anti-tumour promoting activities. No single chemopreventive activity was able to predict the outcome of the anti-angiogenic screening and most likely a combination of inhibitory mechanisms contribute to the angiopreventive potential of the compounds tested (Bertl *et al*., 2004).

Investigations on the angiopreventive potential of xanthohumol and sulforaphane

Two chemopreventive compounds, xanthohumol (XN) from *Humulus lupulus* L. (hop) and sulforaphane (SFN), an isothiocyanate derived from cruciferous vegetables (*Brassicaceae*), were selected for more detailed investigation of their angiopreventive potential and mechanisms involved in inhibition of angiogenesis.

- In 264.7 Raw macrophages the effect on endotoxin-mediated angiogenic stimulators was investigated using RT-PCR. SFN reduced the maximum levels of c-Myc mRNA and strongly inhibited iNOS mRNA expression. Its effects on Cox-2, Hif-1α and VEGF mRNA levels were less pronounced. On the other hand, XN strongly inhibited c-Myc and iNOS mRNA induction and reduced levels of Hif-1α and VEGF mRNA. Lowest effects were seen on ODC and Cox-2 induction.
- Using gelatine zymography, XN and SFN treatment strongly suppressed MMP-9 activity in a time- and concentration-dependent manner and exerted inhibitory effects on MMP-2 activity. RT-PCR experiments revealed that XN delayed and strongly suppressed maximal MMP-9 mRNA expression at nanomolar concentrations, whereas SFN was less active. Interestingly, TIMP-1 and -2 mRNA was transiently induced with a maximum at 2-4h after LPS-stimulation, and the influence of XN and SFN was less pronounced. AP-1 binding was prevented by XN treatment at 6 μM concentration.
- Studies investigating the NF-κB and AP-1 binding to the respective DNA consensus motif exhibited potent inhibitory effects by XN treatment at 12.5 to 50 μM concentrations. The inhibition of NF-κB DNA binding by SFN has been demonstrated previously (Heiss *et al*., 2001).
- Under hypoxic conditions using human microvascular endothelial cells (HMEC-1), strongest inhibitory effects were seen with XN, which potently inhibited c-Myc, Hif-1α and MMP 2 mRNA induction and completely prevented the induction of VEGF mRNA at a 10 μM concentration over a period of 24 h. SFN reduced mRNA induction of c-Myc, KDR, iNOS and MMP 2, but was generally less potent than XN. When the concentration-dependent inhibition of mRNA expression was investigated after keeping the cells for 12 h under hypoxic conditions, both compounds most potently inhibited the expression of iNOS, VEGF, and MMP 9 at low micromolar concentrations.
- XN and SFN were identified as potent anti-endothelial agents inhibiting endothelial proliferation, migration and differentiation at low halfmaximal inhibitory concentrations.
- Intravital microscopy was used as an *in vivo* model to monitor the effects of XN on tumour growth and tumour-induced angiogenesis of breast tumour xenografts implanted in dorsal skinfold chambers in SCID mice. S.c. application of XN for 7 and 14 days potently inhibited tumour growth and reduced the size of the established tumours as well as of functional vessel density. These findings strongly suggest that these chemopreventive agents represent attractive lead structures that should be further explored for prevention of angiogenesis and tumour development in animal models and pre-/clinical trials.