Multifunctional Anti-angiogenic Activity of the Cyclic Peroxide ANO-2 with Antitumor Activity

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Angiogenesis plays a significant role in both initial tumor development and tumor metastasis. The system that comprises urokinase-type plasminogen activator (u-PA) and its specific receptor (u-PAR) has been a target for anti-angiogenic agent research, since many reports have shown that either the inhibition of u-PA enzymatic activity or the disruption of the u-PA/u-PAR system by small molecules results in decreased metastasis and angiogenesis *in vivo*. However, these u-PA-targeting compounds have not yet been used clinically. Although promising, this approach is thought to have several drawbacks. Many kinds of solid tumors and their surrounding stromal cells constitutively produce u-PA; therefore, direct, continuous inhibition of u-PA is thought to be difficult *in vivo*. Moreover, tumor cells and stromal cells express several kinds of proteinases including PAs (u-PA and tissue-type PA), matrix metalloproteinases, and cathepsins. Additionally, these proteinases might act on each other's substrates complementarily; consequently, even if a specific enzyme inhibitor successfully blocks the function of the target proteinase, such inhibitors alone might not be enough to inhibit angiogenesis or metastasis. Therefore, the aim of our study was to develop a novel anti-angiogenic agent that inhibits u-PA production and also other protease cascades in both endothelial and tumor cells.

During preliminary screening the effects of 13 ozonides on the inhibition of u-PA production in human fibrosarcoma HT-1080 cells and on the inhibition of angiogenesis on chicken embryonic chorioallantoic membranes were determined. Of the ozonides tested, 9 inhibited *in vitro* u-PA production of HT-1080 cells and 7 of these 9 showed strong anti-angiogenic activity. Interestingly, 6 of the 13 ozonides also inhibited cathepsin B activity. 1-Phenyl-1,4-epoxy-1*H*,4*H*-naphtho[1,8-de][1,2]dioxepin (ANO-2) potently inhibited cathepsin B ($IC_{50} = 0.47\ \mu M$) as well as u-PA production. ANO-2 inhibited tube formation by human umbilical vein endothelial cells cultured on Matrigel while exhibiting no cytotoxicity. Additionally, *in vivo* administration of ANO-2 inhibited angiogenesis induced by mouse Sarcoma-180 cells tested using the mouse dorsal air sac assay. Moreover, ANO-2 also suppressed primary tumor growth and reduced the number of pulmonary metastases caused by Lewis lung carcinoma cells in mice. These *in vitro* and *in vivo* activities indicate that ANO-2 has considerable potential as a new and potent anti-angiogenic drug that inhibits both u-PA production and enzymatic activity of cathepsins, indicating that ANO-2 may be multifunctional inhibitor of angiogenesis.