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### A XANTHINE DERIVATIVE INHIBITS PROLIFERATION OF LUNG EPITHELIAL CELLS BY cGMP-DEPENDENT PDE INHIBITION ACTIVITY: INVOLVEMENT OF p21 EXPRESSION

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Proliferation of lung epithelia cells is a key event to develop an obliterative bronchiolitis. KMUP-1, a xanthine derivative, has been demonstrated to stimulate eNOS/sGC/cGMP and to inhibit phosphodiesterase (PDE) in vascular and tracheal smooth muscle cells. This study is designed to identify whether KMUP-1, possibly with anti-proliferation activity, has the ability to affect the expression of eNOS/sGC/cGMP in H441 lung epithelial cells. In the normoxic condition, Western blotting analysis demonstrated that KMUP-1, dose-dependently initiated an increase of eNOS expression at $10^{-8}$M. The maximal eNOS expression of KMUP-1 was achieved at 18 hr. The expression of eNOS was attenuated by a NOS inhibitor L-NAME. Using flow cytometry techniques to analyze cell cycles, KMUP-1 produced both time- and concentration-dependent inhibitions on H441 cell at S phase, arrested the cell cycle at $G_0/G_1$ phase. Notably, the effects were dramatically significant after 72 hr exposure to KMUP-1, in comparison with YC-1. In the hypoxic model, H441 cells decreased the percentage of $G_0/G_1$ phase and increased the percentage of S phase during cell growth. After treatment with KMUP-1, the DNA synthesis was reduced and the cell cycle was arrested in $G_0/G_1$ phase. Western blotting analysis showed that KMUP-1 caused a significant decrease of HIF-1α and VEGF expression, but did not show significant change of eNOS. Although expression of a cyclin-dependent kinase (CDK)-inhibitory protein p21 is associated with anti-proliferation activity by exogenous NO, eNOS gene transfer, and iNOS overexpression. However, expressions of p21, MAPK (p38) and MAPK (p42/p44), together evaluated as protein markers in associated suppression of cell proliferation by KMUP-1, indicating suppression of proliferation by KMUP-1 on H441 cells through NO-independent signaling in a cGMP-dependent manner under normoxia and hypoxia condition.

References: 1. JPET. 316: 709-717, 2005; 2. BJP: 146:862-871, 2005; 3. BJP: 142, 1105-1114, 2004.

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### DOXAZOSIN IN PROSTATE CANCER

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Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in men, following lung cancer. Despite the benefits of local therapy with radical prostatectomy and radiation, many patients with prostate cancer develop hormonally resistant disease. No single chemotherapeutic agent or regimen has been demonstrated to provide a survival advantage in this disease. Doxazosin is a quinazoline based α1-adrenergic receptor antagonist which has been shown to induce apoptosis in prostate cancer cell lines via an α1-adrenergic receptor-independent mechanism. Recently, we performed cDNA microarray analyses to better understand the mechanism of action of doxazosin in prostate cancer cells. We found that doxazosin induces deregulation of genes implicated in DNA replication and repair, such as XRCC5 (Ku80) and PRKDC (DNA-PKcs). Toghether with the fact that doxazosin is able to bind DNA, our results allowed us to postulate a novel mechanism for doxazosin action in prostate cancer cells that implies DNA-damage mediated apoptosis. In these studies, the antitumor effect of the combination of doxazosin and chemotherapeutic agents, was investigated in human prostate cancer cells. The androgen-independent prostate cancer cell line PC-3 was used as *in vitro* model system to study the cytotoxic effect of doxazosin combined either with etoposide, cisplatin, doxorubicin or captothecin. Our results show that doxazosin is able to sensitize prostate cancer cells to chemotherapeutic agents, such as etoposide and captothecin. These findings may have therapeutic significance in the development of novel treatment approaches for patients with hormone refractory disease.

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### DNA DAMAGE-INDUCED INHIBITION OF THE ONCOGENIC KINASE AURORA A LEADS TO MITOTIC ARREST

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Transition through mitosis is an obligatory step in the cell cycle of normal and transformed cells. Mitotic kinases are the ultimate target of pathways sensing genotoxic damage and impinging on the cell cycle machinery. In this study we provide evidence that Aurora A (AurA) is inhibited upon induction of DNA damage. Inactivation of AurA was observed specifically upon generation of double-strand breaks in DNA and was not merely the consequence of cell synchronization or stress responses caused by the drug employed. We demonstrate that AurA was not downstream of CDK1 and that inhibition of AurA and CDK1 by DNA damage occurred independently. Using a cell line functionally deficient in CHK2, a selective CHK1 inhibitor and siRNA to CHK1, we show that DNA damage signals were delivered to AurA through a CHK1-dependent pathway. With regard to the molecular mechanism of AurA inhibition, we found that the point mutation $Ser_{342}$>Ala rendered AurA resistant to inhibition by DNA damage. By means of two distinct approaches we examined the impact of reconstitution of AurA activity in DNA damaged cells: (i) Transient expression of wild-type and $Ser_{342}$>Ala mutant, but not kinase-dead, AurA led to bypass of the DNA damage block; (ii) Direct transduction of highly active wt-AurA into G2 arrested cells precisely after induction of DNA damage resulted in mitotic entry. We show that the mechanism through which AurA allowed entry into mitosis was reactivation of CDK1, thus indicating that AurA plays a key role upstream of CDK1. Building on this knowledge, we have initiated a new study aimed at exploring whether some cancer cell lines and tumours *in vivo* may be inherently more resistant to DNA damaging agents by virtue of their documented AurA overexpression. A model depicting the possible role of AurA at the onset of mitosis and upon DNA damage is presented.

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### DISRUPTION OF PROTEIN-PROTEIN COUPLING BETWEEN PTEN AND SEROTONIN 5-HT2C RECEPTOR SUPPRESSES MARIJUANA- AND NICOTINE-INDUCED REWARDING EFFECTS

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The widespread distribution of the tumor suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10) in adult brain suggests its important role in a broad range of brain functions. Here we showed evidence supporting a physical interaction of PTEN with a region in the third intracellular loop (3L4F) of serotonin 5-HT2C receptor (5-HT2cR, formerly 5-HT1c receptor) in cell cultures. PTEN limits agonist-induced 5-HT2cR phosphorylation via its protein phosphatase activity. We next showed the likely existence of PTEN:5-HT2cR complexes in putative dopaminergic neurons in the rat ventral tegmental area (VTA), a brain region in which virtually all abused drugs exert rewarding effects by activating its dopamine neurons. We then synthesized the interfering peptide Tat-3L4F, which is able to disrupt PTEN coupling with 5-HT2cR. Systemic application of Tat-3L4F or the 5-HT2cR agonist Ro600175 suppressed the increased firing rate of VTA dopaminergic neurons induced by delta9-tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana. Using the conditioned place preference and Morris water maze paradigms, we further found that Tat-3L4F or Ro600175 blocked the rewarding effects of THC and nicotine without detectable effects on learning and memory. Other behavioral tests showed that Ro600175, but not Tat-3L4F, produced anxiogenic effects, penile erection, hypophagia and motor functional suppression. These results suggest a novel universal strategy for treating drug addiction with the Tat-3L4F peptide.