ABSTRACTS

Abstracts for Posters:

CP3.

INHIBITION OF NUCLEOSIDE TRANSPORTERS IN ENDOTHELIAL CELLS BY EMODIN

SYT Lin,[1] RWS Li,[1] ASM Sit,[1] YW Kwan,[2] GPH Leung[1]
[1]Department of Pharmacology and Pharmacy, The University of Hong Kong, [2]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

Nucleoside transporters play critical roles in endothelial cell functions. Nucleosides are precursor molecules for ATP, nucleic acids, coenzymes and signaling molecules. However, it is known that epithelial cells have a low capacity for de novo synthesis of nucleosides, and they therefore also depend on nucleoside absorption from the extracellular fluid (i.e. salvage pathway). In addition, nucleoside transporters are important in fine tuning the extracellular concentration of adenosine (a vasodilator and anti-inflammatory agent which is generated in ischemia and inflammation) in the vicinity of adenosine receptors. In endothelial cells, the major nucleoside transporters are equilibrative nucleoside transporter (ENT) 1, ENT2 and nucleoside/nucleobase transporter.

Emodin is a natural anthraquinone compound present in Chinese herbs and vegetables. Emodin possesses anti-cancer, antiviral, antimicrobial, hepatoprotective, anti-inflammatory and anti-angiogenic properties. The relaxing and anti-proliferative effect of emodin on vascular smooth muscle cells has also been recently reported. The objective of this study was to investigate the effects of emodin on nucleoside transporters.

The [^{3}H]adenosine uptake was measured in human umbilical vein endothelial cells (HUVECs). The results showed that emodin inhibited [^{3}H]adenosine uptake with and IC_{50} of 17.1±2.3 μM. Since adenosine is a substrate of ENT1, ENT2 and nucleobase/nucleoside transporters, further experiments were performed to differentiate the effects of emodin on these three transporters. [^{3}H]uridine uptake was measured instead because uridine is a substrate for ENT1, ENT2 but not nucleobase/nucleoside transporter. The data showed that emodin inhibited [^{3}H]uridine uptake in HUVECs with an IC50 of 51.4±5.6 μM. Besides, [^{3}H]uridine uptake was measured in the nucleoside-transporter-deficient PK15NTD cells that stably express cloned human ENT1 and ENT2. Emodin could inhibit ENT1 and ENT2 with IC50 of 33.8±1.7 μM and 18.5±3.0 μM, respectively. The inhibitory effects of emodin on nucleoside transporters can be washed out. Taken together, our data indicates that emodin is a general inhibitor which can block ENT1, ENT2 and nucleobase/nucleoside transporter reversibly. The inhibition of nucleoside transporters may account for the vasodilatory and antiangiogenic effects of emodin. Kinetic studies will be carried out to study whether emodin is a competitive or non-competitive inhibitor.

CP4.

INVOLVEMENT OF NADPH OXIDASE AND RENIN-ANGIOTENSIN SYSTEM IN TISSUE INFLAMMATION OF THE RAT ADRENAL MEDULLA DURING INTERMITTENT HYPOXIA

Y Liu, M L Fung
Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong

We have shown that intermittent hypoxia (IH) associated with recurrent apnea induces oxidative stress and inflammation in the adrenal medulla. However the pathogenic mechanism is not clear at present. We hypothesized that the expression of NADPH oxidase induced by renin-angiotensin system (RAS) plays a role in the tissue inflammation of the rat adrenal medulla in chronic IH. Adult Sprague-Dawley rats were exposed to air (normoxic (Nx) control) or IH treatment (8 hrs/day) which mimicked a severe recurrent sleep apneic condition for 14 days. Injections of apocynin, an inhibitor of NADPH oxidase, (25 mg/kg i.p.) or vehicle were performed daily before the IH treatment. The mRNA levels of NADPH oxidase subunits $p22^{phox}$, NOX-2 and NOX-4 were examined by RT-PCR, the protein expressions of IL-6, TNF-α and COX-2 were examined by the ELISA kit and the protein expressions of NOX-4 and RAS components (AGT, AT1 and AT2) were examined by Western blot. Our results showed that the protein expression of IL-6, TNF-α and COX-2 were significantly higher in the IH group than that of the Nx and apocynin-treated hypoxic (AIH) group, suggesting that inhibition of NADPH oxidase attenuates IH-induced local inflammation in the rat adrenal medulla. The mRNA levels of $p22^{phox}$, NOX-2 and NOX-4 were also increased significantly in the IH group, when compared with that of the Nx control and AIH group. In addition, the protein expression of NOX-4 was significantly more in the IH group than that of the AIH group. Furthermore, the protein expressions of AGT, AT2 and AT2 were increased in the IH group, indicating that the upregulation of NADPH oxidase may be induced by the increased RAS expression. In conclusion, we have shown that NADPH oxidase plays a pathogenic role in the IH-induced local inflammation in the rat adrenal medulla.