Anti-Inflammatory Implications of Vagus Nerve Stimulation in Colon Carcinogenesis

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The present study was undertaken to study the effect of transcutaneous auricular vagus nerve stimulation (taVNS), α7nAChR agonist (PNU) alone and in combination against DMH induced colorectal cancer (CRC) and role of cholinergic anti-inflammatory pathway (CAP) in same. Chemotherapy and surgery are the available treatment strategies for the management of CRC. Chemotherapy is non- specific method which affects non-cancerous tissues and results in lethal side effects. Surgical treatment is invasive and painful. Therefore, non-drug and non-surgical therapy for management of CRC is need of the day.

Previous studies also claimed effectiveness of vagus nerve stimulation in regulation of inflammation associated complications such as lipopolysaccharide induced inflammation and colitis. Recently taVNS also proved its effectiveness in management depression which is a non-invasive method.

In the present study *albino wistar* rats were used as experimental animal. CRC was induced by administration of 1,2- dimethyl hydrazine (DMH) at the dose of 30 mg / kg, weekly for 6 weeks. taVNS with variable stimulation parameters and PNU were used for treatment. After completion of scheduled treatments, animals were subjected for hemodynamic changes [electrocardiogram (ECG) and heart rate variability (HRV)]. Next day of ECG recordings, animals were subjected for blood collection through retro-orbital plexuses and sacrificed using light ether anaesthesia. Physiological [(weight variation, pH, total acidity and aberrant crypt foci (ACF)], biochemical [thiobarbituric acid reactive substances (TBAR's), glutathione (GSH), catalase and protein carbonyl (PC)], morphological [(methylene blue staining, H & E staining and scanning electron microscopy(SEM)], western blotting, qRT-PCR and metabolomics studies were performed to affirmed the effectiveness of treatments against DMH induced colon carcinogenesis.

As per results of our study, DMH administration was very well apparent for autonomic dysfunction as observed through distorted hemodynamic profile (ECG and HRV). taVNS as well as PNU were recorded to have significant effect upon the ECG. Mono and combination therapy also up- regulated the time and frequency domain parameters for HRV. It would be appropriate to remark that decrease in HRV has emerged as a non-invasive marker for autonomic dysfunction in cancer sufferers

Morphological characterization is one of the affirmative methods for cancer diagnosis. When considered morphologically, the colonic mucosa of the DMH treated animals were observed for the presence of ACF. The presence of ACF was confirmed by using methylene blue staining, SEM and H&E staining, and found in line with previous literature. When observed through higher magnification, small neoplastic lesions occurred in the colonic tissue. However, taVNS markedly diminished the ACF count. All in all, the proposed taVNS therapy restored the colonic architecture towards the normal. It would not be out of place to mention that the neoplastic lesions as observed after DMH administration were also subsided after taVNS.

DMH administration was also recorded for per-oxidative damage, supporting the involvement of reactive oxygen species in colon cancer development. Monotherapy as well as combination therapy of taVNS and PNU resulted in significant reduction of lipid and protein peroxidation as confirmed by decrease in TBAR's and PC.

In present study, the levels of SOD and catalase were decreased in response to DMH treatment. This decrease in enzymatic activity of SOD and catalase could be attributed to increased utilization in response to oxidative stress. taVNS resulted in restoration of the SOD and catalase, confirming the say of taVNS in colon carcinogenesis.

DMH application conspicuously inhibited the mitochondrial apoptosis which was

restored back after taVNS and PNU application, when scrutinized through immunoblotting and qRT-PCR studies. taVNS application up-regulated the CAP as perceived through increased expression for α7nAchR and decreased expression for NFκB, TNF-α and HMGB-1 at protein and mRNA levels. Effect of taVNS in combination with PNU also studied through metabolomics studies. Metabolomics studies affirmed the effectiveness of combination in positive regulation of metabolic profiling against DMH induced CRC.

All in all, taVNS up-surged the CAP to counteract DMH induced colon carcinogenesis. Among all the stimulation parameters used, taVNS 3 (pulse width-1 ms, frequency-6Hz, voltage-6v, duration- 240 min.) was observed to be most effective and these stimulating parameters were used to studied the effect of taVNS in combination with PNU.

Considering anti-inflammatory, non-invasive properties of taVNS and receptor specificity of PNU, the present work hold as upper edge over currently available invasive, painful and non-specific therapeutic regimen for clinical management of CRC. Considering the effectiveness of taVNS alone as well as in combination with PNU, it could be potential therapeutic alternative for clinical management of CRC.

Moreover, the use of taVNS alone and in combination with PNU as a complimentary therapy for colon carcinogenesis is under investigation at our laboratory. Monotherapy as well combination therapy of PNU and taVNS could be further explored as strategy in the management of colon carcinogenesis; however the same needs to be validated through clinical studies.

Authors would like to conclude that taVNS alone as well as in combination with PNU imparted a significant effect upon DMH induced colon carcinogenesis mediated through CAP.