INHIBITORY EFFECTS OF NADPH OXIDASE INHIBITOR APOCYIN ON EHEN-INDUCED RAT RENAL CARCINOGENESIS

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Aim: Our previous comparative proteome analysis showed that accumulation of oxidative stress plays a key role in N-ethyl-N-hydroxyethylnitrosamine (EHEN)-induced rat renal carcinogenesis. The aim of the present study was to examine the chemopreventive effects of apocynin, an internal NADPH oxidase inhibitor, on EHEN-induced rat renal carcinogenesis.

Methods: Thirty-two male Wistar rats were divided into three groups as follows: group 1, non-treatment group; group 2, EHEN alone group; group 3, EHEN+apocynin group. Groups 2 and 3 were administered 500 ppm EHEN in their drinking water for the first 2 weeks. After the EHEN treatment, group 3 were treated with 15 mg/kg BW apocynin 5 times a week for 30 weeks. At the end of experiment week 32, histopathological and molecular analyses were carried out using the rat kidney tissues.

Results: Incidence and multiplicities of atypical tubule hyperplasia (ATH, a well known preneoplastic lesion) and renal cell tumor (RCT) and tumor volumes were significantly decreased in group 3 compared with group 2 with apocynin treatment, accompanying with significant decreases in reactive oxygen species, cell proliferative activity and microvessel density in RCT. The findings that apocynin inhibited both ATH formation and growth of RCT, suggested that it exerted inhibitory effects throughout multiple stages of the rat renal carcinogenesis. We also examined the effects of apocynin on the activities of PI3K/Akt/mTOR signaling pathway by comparing the RCT of groups 2 and 3 by immunohistochemistry and western blot. However, none of the transcription factors, such as pS6 and p4EBP1 in RCT, were affected by apocynin treatment. Therefore, we established four cell lines from EHEN-induced RCT to elucidate mechanisms underlying the inhibitory effects of apocynin. In vitro cell viability assays shows that apocynin inhibited the growth of all four cell lines in a dose-dependent manner, suggesting these cell lines are useful for determining the mechanisms of inhibitory effects of apocynin on renal carcinogenesis.

Conclusions: These data indicated that apocynin exerts chemopreventive effects against EHEN-induced rat renal carcinogenesis via a mechanism independent of the PI3K/Akt/mTOR signaling pathway.

Disclosure: All authors have declared no conflicts of interest.