[1]The Third Department of Internal Medicine, Nara Medical University, Kashihara, Japan

Introduction: We have previously reported that the renin-angiotensin system (RAS) plays a pivotal role in hepatocarcinogenesis via augmentation of angiogenesis, and that clinically-used several RAS inhibitory agents i.e. angiotensin converting enzyme inhibitor (ACE-I) or angiotensin 2 receptor 1 blocker (ARB) suppressed hepatocarcinogenesis via attenuation of neovascularization. The aim of our current study was to elucidate an anticancer effect of a recently developed direct renin inhibitor (DRI), Aliskiren, in the rat hepatocarcinogenesis model.

Methods: Early hepatocarcinogenesis was induced in male F344 rats by feeding choline-deficient L-amino acid-defined (CDAA) diet for 18 weeks. The rats received 50 and 100 mg/kg per day of DRI additionally for 12 weeks starting from week 6 to the end of experiment by gavage, respectively. Hepatocarcinogenesis was detected by glutathione-S-transferase placental form (GST-P) stain. Angiogenesis was assessed by RT-PCR of CD31 and VEGF.

Results: Administration of CDAA diet induced multiple GST-P positive pre-neoplastic lesions, which were markedly attenuated by DRI treatment in a dose-dependent manner. Also, expression levels of RAA systems- and neovascularization- related genes were reduced by DRI treatment.

Conclusion: Our *in vivo* results suggested a possibility of anticancer effect of DRI by inhibition RAA-angiogenesis pathway. Since DRI is widely used in the clinical practice without serious side effects, DRI could represent a potential new strategy against hepatocarcinogenesis in the future.

Disclosure of Interest: None Declared

P-041 PPARγ ENHANCES THE CELL GROWTH INHIBITION BY HONOKIOL IN HUMAN HEPATOMA CELLS

H.-T. Hsu [1,*], H.-C. Chen [1], C.-W. Chi [2]

[1]Department of Pharmacology, National Yang-Ming University, [2]Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan

Introduction: Hepatocellular carcinoma (HCC) is one of the malignant cancers having high morbidity and mortality worldwide, especially in Asia and Africa. Till now, surgical resection is the best treatment for HCC. It has been reported honokiol, a natural biphenolic compound, exerts inhibitory effects on the cell growth and migration in hepatoma cells via inhibiting STAT3 activation. Most recently, it was found that honokiol functions as not only an RXR agonist but also a PPARγ agonist, and is capable of potentiating the activation of PPARγ in the presence of PPARγ agonist rosiglitazone in 3T3-L1 adipocyte and HLE human hepatoma cells. Moreover, PPARγ exhibited inhibitory role in hepatocarcinogenesis *in vitro* and *in vivo*. However, whether PPARγ overexpression could enhance the cell growth inhibition by honokiol is not known.

Methods: In this study, Mahlavu and SK-Hep1 human hepatoma cells with overexpression of human PPARγ by retroviral transduction method were treated with honokiol. MTT assay and sulfurhodamine B assay (SRB) were used to examine the cell growth inhibition by honokiol. Flow cytometry analysis was used to observe the effect on cell cycle progression by honokiol. Western blot was used to examine the expression of cell growth-related proteins.

Results: First, different doses of honokiol (0, 10, 20, 30, 40 μM) inhibited growth in a dose- and time-dependent manner from 24 to 72 hours in Mahlavu and SK-Hep1 cells. Further, 40 μM honokiol treatment led to the cytostatic effect in both cell lines. Secondly, decreased cyclin D1/2 and increased p21 were observed in a dose-dependent manner after treatment with honokiol. Thirdly, in SK-Hep1 cells with overexpression of PPARγ the cells had long shape as compared to vector control cells. However, Mahlavu cells with PPARγ overexpression showed no morphological changes as compared to vector control cells. Fourthly, Mahlavu-PPARγ and SK-Hep1-PPARγ cells exhibited about 20% and 30% growth inhibition after treatment with 40 μM honokiol at 48 hour as compared to vector control cells by MTT assay, respectively. Moreover, both Mahlavu-PPARγ and SK-Hep1-PPARγ cells showed cytotoxic effect after treatment with 40 μM honokiol as compared to control which showed cytostatic effect from 24 to 72 hours. Similarly, both Mahlavu-PPARγ and SK-Hep1-PPARγ cells showed about 20% growth inhibition by SRB assay under same condition compared to vector control cells. Finally, 40 μM honokiol treatment induced G0/G1 arrest from 24 to 72 hours in SK-Hep1 cells. In addition, SK-Hep1-PPARγ cells showed about 15% increase in G0/G1 phase after 20 μM honokiol treatment at 24 hour compared to vector control cells. However, Mahlavu-PPARγ cells did not show significant change in the cell cycle phase after honokiol treatment up to 72 hours.

Conclusion: Honokiol-induced cell growth inhibition with cytostatic effect mediated through regulation of cyclin D1/2 and p21. Overexpression of PPARγ in human hepatoma cells enhanced honokiol-induced cytotoxic effect, and G0/G1 cell cycle arrest.

References: Atanasov AG, Wang JN, Gu SP, et al. Honokiol: a non-adipogenic PPARgamma agonist from nature. Biochim Biophys Acta 2013;1830:4813-9.
Shen B, Chu ES, Zhao G, et al. PPARgamma inhibits hepatocellular carcinoma metastases in vitro and in mice. Br J Cancer 2012;106:1486-94.

Disclosure of Interest: None Declared

P-042 THE EFFECT OF ACYCLIC RETINOID ON CELL PROLIFERATION AND APOPTOSIS IN HEPATOCELLULAR CARCINOMA CELLS UNDER INSULIN-RESISTANCE CONDITION

N. Norihisa [1,*], Y. Hitoshi [1], R. Noguchi [1], T. Namisaki [1], K. Moriya [1], M. Kitade [1], Y. Aihara [1], A. Douhara [1], K. Takeda [1], Y. Okura [1], H. Fukui [1]

[1]The third department of internal medicine, Nara medical university, Kashihara, Japan

Introduction: It is widely known that a large number of patients with chronic liver diseases tend to suffer diabetes mellitus (DM), and that risks of hepatocarcinogenesis are increased significantly under insulin resistance (IR) condition represented by DM. Acyclic retinoid (ACR) has recently been focused on as a new anti-HCC reagent via augmentation of apoptosis. However, less is known whether ACR can augment pro-apoptotic effect on HCC under IR condition or not. In this study, we evaluated anti-cancer effect of ACR under IR-mimic condition in *in vitro*.

Methods: Huh-7 and HepG2 cells were used as HCC line. To assess the effect of glucose and insulin on HCC line, These cells were incubated under low-glucose (100mg/dl), low-glucose/insulin (200nM), high-glucose(275mg/dl), and high-glucose/insulin(200nM) conditions. Also, to examine pro-apoptotic effect of ACR, both cells were treated with ACR (10uM) under high-glucose/insulin (200nM) condition, which was deemed to mimic IR condition. Under these conditions, cell proliferation was examined by WST-1 assay. Apoptosis was evaluated by expression of p21 and caspase3.

Results: High-glucose or insulin supplemented conditions augmented cell proliferation compared with low-glucose condition. In high-glucose and insulin condition, cell proliferation was augmented additively. Under this IR-mimic condition, ACR-treatment inhibited cell proliferation with increase of p21 and caspase3 expression suggesting effective pro-apoptotic effect of ACR.

Conclusion: Our dataset suggested ACR is effective reagent against HCC even under IR condition. This finding may support wide use of ACR on HCC in chronic liver disease patients.

Disclosure of Interest: None Declared

P-043 ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS OF THE BIOACTIVE INGREDIENTS FROM THE SEDUM MEXICANUM BRITT. ON HEPATOCELLULAR CARCINOMA HEP 3B CELLS

T.-Y. Chin [1,*], S.-L. Lee [2], H.-Y. Huang [3]

[1]Department of Bioscience Technology, Center for Nanotechnology, Center for Biomedical, Chung Yuan Christian University, Chung-Li, [2]Department of Biological Science and Technology, China Medical University, Taichung, [3]Department of Chemistry, Chung Yuan Christian University, Chung-Li, Taiwan

Introduction: *Sedum mexicanum* Britt., a traditional Chinese herbal medicine, has a wide range of clinical effects, including analgesic, anti-inflammation, and hypolipidemic properties. The aim of the present study was to investigate the anticancer activity of *Sedum mexicanum* extract (SME) in hepatic cancer cells.

Methods: This study focuses on the effect of SME using two different solvents, ethanol and water on hepatocellular carcinoma (Hep 3B) cells *in vitro*. The active components were extracted from SME using water or ethanol and were separated by HPLC prior to use in cell assay. The cytotoxic extracts and fractions were also subjected to morphological assessment, MTT, trypan blue assay and Hoechst staining.

Results: In cell culture, the water extraction of SME significantly reduced Hep 3B cells viability. Based on the MTT assay, the IC_{50} concentration of the crude extract was 0.76 mg/ml whereas HPLC-separated mixtures obtained at different time points had IC_{50} concentrations of 0.23 mg/ml (16 – 35 mins) and 0.39 mg/ml (36 – 55 mins). Besides, upon using ethanol as solvent, the crude extract obtained after 19 days of soaking yielded a larger amount and lower IC_{50} concentration (0.20 mg/ml) than extracts obtained after 3 (0.25 mg/ml) and 7 days (0.33 mg/ml). TLC purification (EA: MeOH = 5:4) of the extract with the lowest IC_{50} yielded mixtures 1, 2, and 3, with mixture 1 conferring the most decrease in cell viability with an IC_{50} of 0.037 mg/ml. Further purification (EA: Hexane = 3:4) of mixture 1 generated mixtures 1-1, 1-2, 1-3, 1-4, and 1-5, which were used for MTT and trypan blue assays to assess their effect on cell activity. Immunofluorescence experiments using both 1-3 and 1-4 mixtures revealed the formation of apoptotic bodies among treated Hep 3B cells. Chemical characterization in order to partially identify the class of the mixture suggests that 1-3 may possibly contain flavanones.

Conclusion: These results suggest that the active ingredients in the SME has potential in preventing and inhibiting effects on hepatocellular carcinoma, which is associated with apoptosis of the cancer cells.

Disclosure of Interest: None Declared