Indian Journal of Animal Sciences **87** (12): 1495–1496, December 2017/Short communication

Gene expression profile of anti-inflammatory action of rutaecarpine with microarray module on *in-vitro* inflammatory model of porcine chondrogenic satellite cells isolated from femoro-petallar chondular regions of piglets

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Received: 23 February 2017; Accepted: 13 June 2017

Key words: Chondrogenic satellite cells, Inflammation, Microarray, Porcine, Rutaecarpine

Osteoarthritis (OA) is a major health issue which invariably tends to pain in the joints in tissue peripheral to cartilage, including the joint capsule, ligaments, periosteum and subchondral bone due to the presence of nociceptors. However, whether the inflammation leading to painful condition interfere with the gene responsible for maintaining tissue stability has not been largely investigated. Rutaecarpine (quinazolinocarboline alkaloid) has variety of biological activities (Cai *et al.* 2016) and therefore, in order to evaluate its anti-inflammatory function and to identify the modulation of the genes during inflammatory conditions, we explored the molecular interaction networks from gene expression data, using ingenuity pathway analysis (IPA) software.

We performed the experiments upon the isolation of chondrogenic satellite cells (Oseni *et al.* 2013) and treated them with rutaecarpine at a concentration of 2 and 4 μM after exposing them first to 2 μg/ml lipopolysaccharide (LPS) for 24 h. All the treated cells were subsequently analysed based on microarray using Gene Spring GX 12.5 referred as Agilent bioanalyzer 2100 (Affymetrix, Santa Clara).

The treated cells showed total genes upregulation (i.e. 1,387,408 genes transcripts), out of which known genes were only 30,198. On the other hand, from the abundant gene transcript (781, 536), the actual downregulated were 264, 182 genes, respectively with rutaecarpine at 2 μM and/or 4 μM. Two fold and four fold decrease in the genes were observed in the expression of 61 gene and 69 genes upon treatment with rutaecarpine (Table 1).

Table 1. Significant expression of highly down-regulated genes upon rutaecarpine treatment

Gene	Gene symbol	Control (LPS-treated)	Exp. Ratio (Rutaecarpine treated)	
			2 μM	4 μM
Alveolar macrophage-derived chemotactic factor-II	AMCF-II	287.948	3.040	2.093
Interleukin 8	IL8	129.836	2.359	2.263
Chemokine (C-X-C motif) ligand 2	CXCL2	46.543	2.794	4.271
Superoxide dismutase 2, mitochondrial	SOD2	338.326	22.335	28.815
B-factor, properdin	SBAB-707F1.11	9.039	2.694	2.420
Aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	AKR1C4/AKR1CL1	109.577	28.761	34.862
Interleukin 6 (interferon, beta 2)	IL6	21.862	5.554	8.123
Similar to cyclophilin D	LOC100152612	17.703	5.775	6.964
Similar to putative ISG12(a) protein	LOC100153902	306.597	149.356	204.856

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Inflammation of chondrocytic cells produced phosphatidylinositol 3-kinase (PI-3K)/protein kinase B (Akt), mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) leading to activation of the multiple pro-inflammatory signaling pathways (Ahmed 2016). On the other hand, the use of rutaecarpine as a treatment module prevented the rise of IL-1β, TNF-α,

IL-6, cyclooxygenase-2 (COX-2) and matrix metalloproteinases (MMPs) during inflammatory reaction.

Rutaecarpine presumed to have worked by down-regulating increased cytosolic calcium (Ca^{2+}) to inhibit phospholipase C activity and to bring relief from pain. Rutaecarpine also supposedly increased properdins, influenced AKR1C4 and also declined CXCR1 and CXCR2 expressions and rutaecarpine appeared to have controlled inflammation by preventing ROS elevation and activation of ERK ½ and P38 signalling pathways. By influencing other upregulated genes upon inflammation, i.e. long-chain acyl-CoA synthetase 1 (ACSL1), HMOX 1 along with Cyclophilin D and STAT3 on PCSC's, rutaecarpine brought drastic down regulatory effects directly or indirectly prevented the chondrocyte from inflammation. Rutaecarpine had moderately effected SRPK1 and ASF/SF2 gene expression which reflected that it works by destabilizing the unstable complex of ASF/SF2 that reaches the cytoplasm of the cells. Therefore, it was further observed that rutaecarpine not only prevented inflammatory episodes in these cells but also protected nuclear dissipation of ASF/SF2 complex and kept the cell nucleus intact (Thomas *et al*. 2016).

SUMMARY

Rutaecarpine have been studied in the past particularly for inflammatory reactions to overcome side effects of conventionally used chemicals for the purpose. However, the molecular mechanisms of rutaecarpine have not so far been addressed particularly on osteoarthritis. Therefore, we carried out gene expression profiling in an effort to understand the effect of rutaecarpine on porcine chondrocytes using microarray. The present study provides novel insights into the gene mechanisms possibly involved in cellular metabolism, apoptosis leading to anti-inflammation.

ACKNOWLEDGEMENT

S R Park and N K Singh has contributed equally to this paper as first author. S J Lee acted as corresponding author of the manuscript. This study was supported by research grant from Kangwon National University, Republic of Korea. The study was also partially supported from research startup grant under XII plan (No. R/Dev/D/XII Plan/Recurring/Startup grant/95941, Dated: July 29, 2015), Banaras Hindu University, Varanasi, Uttar Pradesh, India.

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