Reversion of asthmatic complications and mast cell signalling pathways in BALB/c mice model using Quercetin nanocrystals

Kriti Gupta\(^1\), Sandeep Kumar\(^2\), Rinkesh Gupta\(^1\), Akanksha Sharma\(^1\), Alok Verma\(^1\), K. Stalin\(^3\), Bhushan P. Chaudhari\(^1\), Mukul Das\(^1\)

\(^1\)CSIR-Indian Institute of Toxicology Research, Lucknow, India; \(^2\)Roswell Park Cancer Institute, Buffalo NY, USA; \(^3\)CSIR-National Physical Laboratory, New Delhi, India

Aims: Evaluation of Anti-asthmatic Potential of Quercetin Nanocrystals in BALB/c Mice.

Methods:

1. Preparation and Lyophilisation of Quercetin Nanocrystals: The preparation of nQ was carried out under high energy transfer using ultrasonicator.

2. Physical Characterization of Quercetin Nanocrystals: The Physical Characterization of Quercetin Nanocrystals was done by the FTIR and UV spectra. The shape and size of nQ were seen by TEM measurements. The average hydrodynamic size, polydispersity index and zeta potential of nQ was analyzed by dynamic light scattering (DLS) and phase analysis, light scattering, using a Zetasizer Nano-ZS.

3. Solubility and Stability of nQ: Sedimentation kinetics of equivalent quantities of bulk quercetin and nQ suspended in PBS was observed at different time points. The stability of nQ with varying temperature and pH was also study using high performance liquid chromatography (HPLC).

4. In-Vivo Pharmacokinetics of nQ: Animal studies were carried out to understand the pharmacokinetics of delivering bulk quercetin and nQ to female BALB/c mice. Serum was isolated from the blood samples of treated mice and the concentration of delivering bulk and nQ were determined by HPLC.

5. Total Serum IgE Assay OVA Specific IgE and IgG1 Assay: Total IgE (tIgE) was estimated with the Optia mouse IgE kit. Specific IgE (sIgE) and sIgG1 levels against OVA were estimated by enzyme-linked immunosorbent assay (ELISA).

6. Real Time PCR for IL-4, IL-5 and Foxp-3 Expressions: The total RNA from the lung tissues of control, OVA+nQ and OVA groups was isolated using use of TRI-Reagent and cDNA were prepared using high capacity cDNA reverse transcriptase kit.

7. Measurement of Allergic Mediators in the Serum: Mediators of allergic asthma like Prostaglandin D2 (PGD2), cysteinyl leukotriene (CysL), mouse mast cell protease-1 (mMCPT-1) and mouse thymic stromal lymphopoietin (TSLP) levels were determined in the sera of control, OVA + nQ and OVA mice using commercially available EIA and ELISA kits.

8. Western Blot Analysis: The levels of Th1/Th2 transcription factors T-bet, GATA-3, c-maf, NfAT and SOCS-3 were studied in the lungs of control, OVA+nQ and OVA treated groups.

Results: The nQ was found to be more stable and soluble in PBS, and sera of BALB/c mice compared to bulk quercetin. Dose dependent experiments with nQ on OVA sensitized asthma mice exhibited significant anti-asthmatic potential of nQ at much lower dose (1 mg/kg body weight) compared to bulk quercetin. The treatment of nQ remarkably resulted in reduced OVA specific immunoglobulin E (sIgE) production, anaphylaxis signs and type 1 skin test. The nQ also significantly modulated the expression of Th2 cytokines like IL-4 and IL-5, which are responsible for IgE class switching and suppressed the degranulation/secretion of different chemical mediators (PGD2, mMCPT-1 Cys-L and TSLP) from activated mast cells. The levels of FceR1, Syk, c-Yes, PI-3, p-PI-3, PLC-γ2, and p-PLC-γ2 were found to be reduced in the OVA sensitized BALB/c mice treated with nQ compared to those treated with OVA only.
**Discussion:** In this study, we have successfully demonstrated the enhanced bioavailability, solubility and counteractive efficiency of quercetin by synthesized quercetin nanocrystals (nQ). Further, effect of nQ has been established by studying the pharmacokinetics, stability as well as anti-asthmatic property in the OVA induced allergic asthma mice. More importantly, mast cells, one of the significant pathological features of asthma, were found to be decreased by nQ leading to diminished asthma symptoms. The present study indicate that nQ alleviate pulmonary inflammation and airway hyporesponsiveness in allergic asthma at much lower dose compared to bulk quercetin and may be considered as a potential drug for the treatment of asthmatic patients.

**Conclusion:** In conclusion, the prepared water soluble quercetin nanocrystals have shown potential as promising nanovehicle with in vivo and ex vivo stability, excellent bioavailability, and therapeutic efficacy in asthma murine model. It has also been found to be effective in preventing anaphylaxis in murine model of allergic asthma. In totality, the nQ has potential to substantially reduce the symptoms of asthma and may play an important role in providing the most effective cure for asthma that is currently lacking.

*Figure 1*