SYNOPSIS

In vitro Toxicological Evaluation of Hydroxyapatite micro and nano Particles: A Comparative Analysis.

The applications of nano sized particles for biological applications are increasing enormously in various fields such as medicine, cosmetology etc. These particles have unique properties like smaller size, larger surface area to unit mass ratio and may cause adverse effects in biological systems. These are able to translocate from the site of deposition to distant organs through different routes. Since these particles are widely used for biological applications they should be thoroughly screened for toxicological evaluation.

Hydroxyapatite (HA) is chemically similar to the mineral component of bone and teeth and nano sized and bulk forms of hydroxyapatite are extensively used to repair bones and teeth. Nano sized hydroxyapatite is also used for coating other biomaterials such as metallic implants to improve their biocompatibility. Moreover nano sized HA particles can be used to prepare HA nano composites for drug delivery applications as well as to prepare bone cement. In the bulk form it is biocompatible but because of the unique properties of nano sized particles the behaviour may vary from its bulk form. Hence the hydroxyapatite nano sized particle for biological application has to be put under scrutiny for its toxicity. The present study entitled ***"In vitro Toxoicological evaluation of hydroxyapatite micro and nano particles: a comparative analysis"*,** is an attempt to discriminate the toxicological nature of hydroxyapatite micro and nano particles by *in vitro* methods.

The main objectives put in the study were: Synthesis and characterization of HA nano and micro particles (NHA and MHA); Toxicological/biocompatibility screening of NHA and MHA by *in vitro* methods viz., Lipid peroxidation, Antioxidants and ROS generation, Cytotoxicity and apoptosis assay, Genotoxicity evaluation by chromosome aberration, Immunological screening by splenocyte proliferation, Blood compatibility by hemolysis, cell aggregation and clotting time

and comparative analysis of toxicology/biocompatibility of NHA and MHA on the basis of the above parameters.

The micro and nano particles were synthesized by wet chemical method and characterized by transmission electron microscopy for particle size analysis, XRD spectrum for phase purity and Infra Red spectrum for chemical confirmation were studied.

The effect of hydroxyapatite micro and nano particles on lipid peroxidation, antioxidants property, DNA adduct formation in the rat liver homogenate and reactive oxygen species generation in L 929 cell lines were conducted. The antioxidants and lipid peroxidation were assayed by standard biochemical methods. DNA adduct formation was evaluated by quantifying 8 OHdG by competitive ELISA method. ROS generation was done by fluorimetric analysis.

MHA up to a dose of 500µg/mL is neutral in terms of lipid peroxidation while NHA is nonreactive only up to 100µg/mL. Lipid peroxidation induced by NHA (from 250µg/mL) may be due to the smaller size and larger surface area, characteristics of nano material. GSH level was maintained comparable to a control level up to 500 µg/ml, but at higher doses GSH was decreased. GR activity was also showed a similar behaviour, lower concentrations of MHA and NHA appeared to be safer for biological applications. A dose dependent effect of the materials on GPx activity was available in this evaluation. MHA and NHA were not inducing oxidative stress in terms of SOD up to 1000µg concentrations, appears to be safer up to this dose level. No significant elevation of 8OHdG level as an index of oxidative DNA damage was reported by this study which suggests that the material is not genotoxic in nature. *In vitro* studies on fibroblast also proved that these materials are not capable of inducing oxidative stress as evidenced by failure in ROS generation at lower concentrations suggesting these materials as biocompatible at least to this concentration.

Cytotoxicity was evaluated by well accepted MTT assay and caspase, marker for apoptosis, was evaluated by fluorimetry. Both the assays were done on

L 929 fibroblast cell line as test system. The study showed that the morphology of the cells was preserved and the percentage viability was greater than 80% with MHA up to 400µg/mL. While in NHA treated cells the morphology was preserved and the percentage viability was greater than 80% up to 600µg/ml concentrations. Caspase assay revealed that MHA and NHA could not induce apoptosis in L 929 fibroblast cells up to a concentration of 500µg/mL.

Chromosome aberration study was done as per international standards by culturing human peripheral blood lymphocytes and preparing metaphase chromosome spreads. Karyotyping was also done on chromosome spreads of human peripheral blood lymphocytes by G banding technique. Genotoxic evaluation made by chromosome aberration and karyotyping proved that hydroxyapatite micro and nano particles are non genotoxic/biocompatible in nature up to 250µg/mL.

Splenocyte proliferation was done on mouse splenocytes by incorporating tritiated thymidine for evaluating the immunological response of hydroxyapatite micro and nano particles. Decrease in proliferation of splenocytes was obtained at higher concentrations of NHA and MHA compared to positive control and at medium concentrations it was increased.

The blood compatibility of hydroxyapatite micro and nano particles was done on human peripheral blood by evaluating the hemolysis, RBC, WBC and platelet aggregation and whole blood clotting time. Hemolysis was done by evaluating the hemolytic index/ percentage hemolysis as per international standards. RBC, WBC and platelet aggregation was assessed microscopically by standard methods. Whole blood clotting time was done by clinical methods. The present study on MHA and NHA proved that the materials are hemocompatible.

The present investigations conclude that MHA and NHA are comparatively safer biocompatible material suitable for biological applications at lower concentrations. The size difference between the MHA and NHA in the present context did not influence the toxicity of the particles except in certain situations like cytotoxicity, splenocyte proliferation and LPO.