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Screening the Pharmacological Activity of Cerium Oxide Nanoparticles *In vitro*

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ABSTRACT

In microbiological and clinical studies, cerium oxide (CeO_2) nanoparticles have been shown to protect cells in culture from lethal stress. Cardiac-specific expression of monocyte chemo-attractant protein-1 (MCP-1) in mice causes ischemic cardiomyopathy associated with activation of endoplasmic reticulum (ER) stress. CeO_2 nanoparticles protect against the progression of cardiac dysfunction and remodeling by attenuation of myocardial oxidative stress, ER stress and inflammatory processes probably through their auto regenerative antioxidant properties. In the present study we have examined the effect of cerium oxide (CeO_2) nanoparticles *in vitro*. For this purpose we have choosen frog as an experimental animal and the tissue was frog isolated heart. Cerium oxide (CeO_2) nanoparticles elicited dose-dependent cardiac depressant activity. Atropine (ATP) a muscarinic blocker could not antagonize the effects of cerium oxide nanoparticles which indicate that the activities are not mediated through muscarinic receptors.

Key words: Cerium oxide, Heart rate, Cardiac output.

INTRODUCTION

Nanotechnology has gained a great deal of public interest due to the needs and applications of nanomaterials in many areas of human endeavours such as industry, agriculture, business, medicine, public health amongst many others. Nanotechnology includes the integration of these nanoscale structures into larger material components and systems, keeping the control and construction of new and improved materials at the nanoscale[1].

Cerium oxide (CeO_2) nanoparticles have been shown to protect cells in culture from lethal stress. Cardiac-specific expression of monocyte chemo-attractant protein-1 (MCP-1) in mice causes ischemic cardiomyopathy associated with activation of endoplasmic reticulum (ER) stress. The aim of this study was to assess the effects of CeO_2 nanoparticles on cardiac function and remodeling as well as ER stress response in this murine model of cardiomyopathy. MCP-1 transgenic mice (MCP mice) and wild-type controls were administered intravenously 15 moles of CeO_2 nanoparticles or vehicle only twice a week for two weeks. Cardiac functions, myocardial histology, nitrotyrosine formation, expression of cytokines and ER stress-associated genes were evaluated. Treatment with CeO_2 nanoparticles markedly inhibited progressive left ventricular dysfunction and dilatation in MCP mice and caused a significant decrease in serum levels of MCP-1, C-reactive protein and total nitrated proteins. The infiltration of monocytes/macrophages, accumulation of 3-nitrotyrosine, apoptotic cell death and expression of proinflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 in the myocardium were markedly inhibited by CeO_2 nanoparticles. Expression of the key ER stress-

associated genes, including glucose-regulated protein 78 (Grp78), protein disulfide isomerase (PDI) and heat shock proteins (HSP25, HSP40, HSP70) were also suppressed by CeO_2 nanoparticles[2]. CeO_2 nanoparticles protect against the progression of cardiac dysfunction and remodeling by attenuation of myocardial oxidative stress, ER stress and inflammatory processes probably through their autoregenerative antioxidant properties. On this back ground in the present study an attempt was made to screen the pharmacological activity of cerium oxide nanoparticles *in vitro.*

MATERIAL AND METHODS

Preparation of Cerium Oxide Nanoparticles

Step 1

Transfer 10 g of cerium chloride into a 100 ml 2 neck flasks and add 20 ml of 2-Methoxy ethanol solvent and stir for 20 min.

Step 2

Weigh 3.5 g of Cetyl Trimethyl Ammonium Bromide (CTAB) and transfer into a 100 ml beaker, add 10 ml of 2-Methoxy ethanol solvent and stir the contents for 10 min. If the solution is not clear add few drops of HCl. Add Step 2 contents to step 1 and stir for 20 min. Add ammonia solution (12.5 ml) drop wise to the above solution under constant stirring and leave the final contents stirring for overnight. Filter the contents and wash several times with distilled water. Collect 2 ml of filtrate after 8^{th} wash and add few drops of ammonia solution, if white precipitate was found further wash with distilled water. Dry the precipitate in the oven at 125^0C for 24hrs and carefully remove final product from the filter paper. Heat treats the final product in the furnace at 200^0C for 48hrs.

Physiological solution

Frog Ringer's solution

Weigh 9 gm NaCl, 0.42 gm KCl, 0.12 gm $CaCl_2$, 0.50 gm $NaHCO_3$, 1 gm dextrose and dissolve in sufficient amount of distilled water. After complete dissolution make the volume upto 1 litre with distilled water. Either $CaCl_2$ or $NaHCO_3$ should be added at the end inorder to prevent the formation of $CaCO_3$ which forms a precipitate.

Frog Heart perfusion by Syme's technique[3]

The effect of cerium oxide nanoparticles on isolated frog heart was done by Syme's technique. Frog (Rana tigrina) was stunned by head-blow using a steel rod and pithed. The skin and abdomen were cut and opened. The pectoral girdle was cut using a bone cutter and pericardium was removed. Syme's cannula was connected to the reservoir of frog Ringers solution and introduced immediately into the sinus venosus of the heart. The connecting blood vessels were cut and heart was isolated from the animal and mounted on to a stand. Heart was then covered with thin layer of cotton wool to prevent drying. Frog Ringer solution was used to wet the heart frequently to prevent drying. Heart was connected to Starling's lever and adjusted to mark on the smoked drum for recording the responses of the heart.

The level of frog Ringer solution in the Syme's cannula was maintained by fixing a glass tube into the cork fixed to the reservoir (Marriott bottle) tightly. The heart was allowed to stabilize and when the heart rate and cardiac output were taken, the recordings were made on a slow rotating soothed drum, to which a soothed kymograph paper was affixed. The effect of cerium oxide nanoparticles was studied on isolated perfused frog heart. The parameters studied include the force of contraction, heart rate and cardiac output. Minimum 5 mins time was allowed between the additions of samples *per se* (in frog Ringers solution) and its fractions. When a blocker was used, it was diluted with known amount of frog Ringer solution in the syringe itself and added slowly. The Heart rate (HR), Cardiac output (CO) and Force of contraction were the parameters used for the study. The dilutions were prepared in frog Ringers solution. No suspending agents were used. The heart was moistened with frog Ringers solution from time to time.

RESULTS AND DISCUSSION

In microbiological and clinical studies, CeO_2 nanoparticles protect against the progression of cardiac dysfunction and remodeling by attenuation of myocardial oxidative stress, ER stress, and inflammatory processes probably through their auto regenerative antioxidant properties[3].

Cerium oxide (CeO_2) nanoparticles elicited dose-dependent cardiac depressant activity. Atropine (ATP) a muscarinic blocker could not antagonize the effects of cerium oxide nanoparticles which indicate that the activities are not mediated through muscarinic receptors.

Further work in this direction might reveal the mechanism involved in these pharmacological actions and it is hoped that a systematic and exhaustive work is likely to yield some agents of therapeutic value. This work is first of its kind so we wish that more attention be paid in order to conclude the activity of the new category of compound.

REFERENCES

1. Ju-Nam, Y. *Sci Total Environ,* **400**: 396 (2008).
2. Jianli Niu, Asim Azfer, Linda Rogers, Xihai Wang and Pappachan Kolattukudy. *Cardiovascular Research*, 73:549 (2006).
3. Burn J.H., Practical Pharmacology. Oxford. Black Well Scientific Publication (1952).