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Toxicity of cobalt oxide nanoparticles to normal cells; an in vitro and in vivo study

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ABSTRACT

The aim of this study was to find out the intracellular signaling transduction pathways involved in cobalt oxide nanoparticles (CoO NPs) mediated oxidative stress in vitro and in vivo system. Cobalt oxide nanoparticles released excess Co^{++} ions which could activated the NADPH oxidase and helps in generating the reactive oxygen species (ROS). Our results showed that CoO NPs elicited a significant ($p < 0.05$) amount of ROS in lymphocytes. In vitro pretreatment with N-acetylene cystine had a protective role on lymphocytes death induced by CoO NPs. In vitro and in vivo results showed the elevated level of TNF-α after CoO NPs treatment. This TNF-α phosphorylated the p38 mitogen-activated protein kinase followed by activation of caspase 8 and caspase 3 which could induce cell death. This study showed that CoO NPs induced oxidative stress and activated the signaling pathway of TNF-α-caspase-8-p38-caspase-3 to primary immune cells. This study suggested that bare CoO NPs are a toxic for primary human immune cells that deals directly with human health. Surface modification or surface functionalization may open the gateway for further use of CoO NPs in different industrial use or in biomedical sciences.

1. Introduction

Metal nanoparticles (MNPs) exhibit unique properties in terms of optical, magnetic and electrical activity [1]. Inorganic nanoparticles are promising materials for applications in drug/gene delivery, cell imaging, biosensing, and cancer therapy [2,3]. In recent times, nanoparticles (NPs) have received much attention for their implications in cancer therapy [4]. Studies have shown that NPs induce cytotoxicity in a cell specific and proliferation-dependent manner. In particular, iron, nickel, and cobalt NPs are destined to find their place in medical biotechnology because of their magnetic properties. Magnetic NPs, in fact, have been proposed for cancer treatment [5]. Furthermore, they are emerging as a class of novel contrast agents for medical imaging [6]. In particular, when used for magnetic resonance imaging (MRI), magnetic NPs are very efficient as relaxation promoters, enhancing tissue contrast and helping to form sharper images of the area of interest [7]. More recently cobalt NPs have been suggested as an alternative to iron due to their greater effects on proton relaxation [1].

The toxicity of cobalt based nanoparticles, may be characterized either to the direct uptake of the NPs by cells or to the dissolution of the NPs leading to the increased level of Co^{++} ions in the media, with subsequent effects on cells. There are a number of studies related to the potential toxicity of cobalt based nanoparticles. Cobalt oxide nanoparticles were found to exert an oxidative stress in cells [8], DNA damage [9,10], and inflammatory response [11], in human mononuclear cells [12], and neutrophils [13].

Cytotoxicity, morphological transformation, and genotoxicity induced by Co-NPs were demonstrated in Balb3T3 cells [3,10]. In addition, it was shown that Co-NPs induce genotoxic effects in human peripheral leukocytes [14]. Azaria et al. in 2011 hypothesis that the toxic effects of cobalt NPs are mainly due to cobalt ion dissolution from the nanoparticles [15]. This group also proved that the cobalt oxide nanoparticles induced toxicity depends on concentration and exposure time. Beside these they also proved that primary DCs were the least sensitive cells among the used other cell lines (A549, MDCK, NCIH441, Caco-2, and HepG2) [15].

However, considering, on the one hand, the future developments of cobalt-based NPs, the adverse effects of nanomaterials, accurate studies regarding the possible interactions of NPs with

Abbreviations: CoO NPs, cobalt oxide nanoparticles; IL-10, Interleukin 10; LDH, lactate dehydrogenase; MAPK, mitogen activated phosphokinase; NPs, nanoparticles; PI, propidium iodide; ROS, reactive oxygen species; SGOT, serum glutamate oxalate transaminase; TNF-α, tumor necrosis factor alpha.

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cells or tissues and the consequences of these interactions are desirable. Therefore, in the present study, we have focused our interest on the study of toxicity of synthesized cobalt oxide (CoO) nanoparticles in bothe in vitro and in vivo system and try to find out the probable mechanism of the toxicity.

1.1. Chemicals and reagents

Cobalt oxide nanoparticles, Histopaque 1077, propidium iodide (PI), RNaseA, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT reagent), ethidium bromide, acheridine orange, acetylsalicylic acid (ASA), indomethacin, pentoxifylline (POF) were procured from Sigma (St. Louis, MO, USA). Minimum Essential Medium (MEM), RPMI 1640, fetal bovine serum (FBS), penicillin, streptomycin, sodium chloride (NaCl), sodium carbonate (Na_2CO_3), sucrose, Hanks balanced salt solution, and ethylene diamine tetra acetate (EDTA), dimethyl sulfoxide (DMSO) were purchased from Himedia, India. Tris–HCl, Tris buffer, KH_2PO_4, K_2HPO_4, HCl, formaldehyde, alcohol and other chemicals were procured from Merck Ltd., Mumbai, India. All other chemicals of the highest purity grade were purchased from Merck Ltd., SRL Pvt., Ltd., Mumbai.

2. Methodology

2.1. Synthesis of cobalt oxide nanoparticles

Cobalt oxide NPs were prepared by the thermal decomposition method. The starting materials were $CoCl_2{\cdot}3H_2O$, Na_2CO_3. 2 g of starting material ($CoCl_2{\cdot}3H_2O$) was taken in a beaker and mixed with Na_2CO_3 at a molar ratio 1:1. Then the solution was being rotated 1 h at room Temperature. After that the precipitations were collected by centrifugation to obtain the nanoparticle precursor. The precursor was calcinated at 300 °C in air in a porcelain crucible for 2 h to get cobalt oxide NPs [16].

2.2. Characterization

2.2.1. Dynamic light scattering (DLS)

Dynamic light scattering (DLS) analysis was done in Zetasizer Nano ZS (Malvern Instruments) according to the method of Chattopadhyay et al. [16], with some modifications. The concentration of the cobalt oxide nanoparticles was 100 μg/ml and was sonicated for 2 min and dynamic particles size were measured suspending two drops of aqueous suspension of nanoparticles in 10 ml of Millipore water. When the particle was completely dispersed in water then particle was analyzed with a dynamic light scattering analyzer. The experiments were repeated several times to get average size of nanoparticles [16].

2.2.2. Scanning electron microscopy (SEM)

The particle size and the microstructure were studied by high resolution scanning electron microscopy with a JEOL (Japan) 3010 high-resolution scanning electron microscope operating at 200 kV according to the method of Chattopadhyay et al., 2013 [17], with some modifications. In brief, CoO NPs were suspended in deionized water at a concentration of 1 mg/ml, and then the sample was sonicated by using a sonicator bath until the sample formed a homogeneous suspension. For size measurement, sonicated stock solutions of all CoO NPs (0.5 mg/ml) were diluted 20 times. Scanning electron microscopy was used to characterize the size and the shape of the CoO NPs. A drop of aqueous CoO NP suspension was placed onto a carbon-coated copper grid and this was dried in air to obtain scanning electron microscopy images.

2.2.3. Fourier transformed infrared spectroscopy (FT-IR)

The surface chemistry of the CoO NPs was investigated by Fourier transform infrared (FTIR) spectroscopy with a PerkinElmer Spectrum RX I FTIR system according to the method of Chattopadhyay et al. [16], with some modifications. In brief, 1.0 mg sticky mass of CoO NPs with 100 mg KBr medium and a thin film was prepared under atmosphere separately. The FTIR spectra were recorded between 500 and 4000 cm^{-1}.

2.2.4. Zeta potential

The zeta potential of the $CoFe_2O_4$ NPs was measured by using a Zetasizer-Nano ZS (Malvern, Malvern Hills, U.K.). 1 mg/ml $CoFe_2O_4$ NPs salutation was prepared in Milli-Q water. Then the salutation was filtered by using woman No 1 filter paper and the filtrate was used to experiment [18].

2.2.5. Dissolution study

CoO NPs were suspended in medium (without FBS and antibiotics) and incubated for 1 week at 37 °C. After the incubation period, the supernatant was used for the estimation of free cobalt ions in the medium by atomic absorption spectroscopy (AAS) using different concentrations of $CoCl_2{\cdot}6H_2O$ as a standard [18].

2.3. Selection of human subjects for collection of lymphocytes

Six healthy subjects were chosen to collect the blood sample for separation of lymphocytes. All subjects enrolled in this study were asymptomatic and none of them showed any abnormality on physical examinations and routine laboratory tests. All the subjects are from same geographical area and same economic status, nonsmokers and non-alcoholic, and having same food habit. These subjects received no medication, including vitamin E and vitamin C. All subjects gave informed consent. The selection not only excluded individuals with acute infections or chronic diseases, but also excluded healthy individuals undergoing supplementation with antioxidative substances. The study protocol was in accordance with the declaration of Helsinki, and was approved by the ethical committee of Vidyasagar University.

2.4. Separation of lymphocytes

Fresh blood samples were collected from all groups of individuals satisfying the Helsinki protocol. The lymphocytes were isolated from heparinized blood samples according to the method of Hudson and Hay [19]. Blood was taken and diluted with phosphate-buffered saline (pH 7.0) in equal ratio and then layered very carefully on the density gradient (histopaque) in 1:2 ratio, centrifuged at 500*g* (1400 rpm) for 40 min and the white milky layer of mononuclear cells, i.e., lymphocytes were carefully removed. The layer was washed twice with the same buffer and then centrifuged at 2400 rpm for 10 min to get the required pellet of lymphocytes.

2.5. Cell culture

The normal human lymphocytes were cultivated for in vitro experiments. It was cultured in RPMI 1640 medium and minimal essential medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, 4 mM L-glutamine under 5% CO_2, and 95%, humidified atmosphere at 37 °C.

2.6. Preparation of drug

Several doses of CoO nanoparticles and doxorubicin (1–50 μg/ml) were prepared using sterile phosphate buffer saline (pH 7.4).

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