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CYTO-TOXICITY RESPONSE TO CARBON NANO TUBES BY LYMPHOCYTE CELLS OF HEALTHY & ALLERGIC PATIENTS

Madhuri Sharon[1]*, Maheshwar Sharon[1] and Amol Kakde[2]

[1] N.S.N. Research Centre for Nanotechnology and Bionanotechnology, SICES College, Jambhul Phata, Ambernath (W), 421 505, India

[2]Sir Hurkisondas Hospital and Research Center, Mumbai, India

MAIL:sharonmadhuri@gmail.com

ABSTRACT

In the present paper we present our attempts to study whether carbon nano tubes (CNT) are safe materials to be administered in the living system. For this purpose effect of CNTs on the lymphocyte of peripheral blood sample of healthy persons as well as allergic patients were studied. Allergic patients were considered because CNT can be allergic to certain individuals. Cell growth, viability, apoptosis and metabolic activity were evaluated in proliferating human peripheral blood lymphocytes. The concentrations of CNTs tested were 1, 2.5, 7.5 and 10 µg/ml; and treatment duration of 72 h, in accordance with the biological target investigated. A statistically significant decrease in cell growth was found in cells treated with 7.5 and 10 µg/ml CNT in healthy individuals but not in the allergic patients.

KEYWORDS: **C**arbon Nano Tubes, CNT, Cytotoxicity, Peripheral blood lylphocytes, Allergic patients

INTRODUCTION

In the past decade, two blossoming technologies have been hot research topics of the world. The powerful utility of concerted application of Nanotechnology and Biotechnology has been recently exemplified by breakthroughs in bio-directed Nano-synthesis /assembly and Nano-aided biological recognition. Drug delivery promise using carbon nano materials (CNM) have been shown [1]. However, use of CNM has posed the grave question whether they are safe material to be used; especially when it is to be administered in the living system. Moreover, publication referring to antimicrobial activity of CNM due to photocatalysis [2] has also shown capability of carbon nano tubes (CNT) to kill microbial cells in presence of light. There have been a reports declaring CNM both as safe [3, 4] and unsafe [5] for it's used in living system. Also posed a question whether it is toxic to animal cells or not. There have been findings that [6] indicate that the action of carboxy-fullerene on Gram-positive bacteria is achieved by insertion into the cell wall and destruction of membrane integrity

The complexity of use of nanotechnology and living system go hand in hand. Hence, in this case answers give rise to new questions. One of such critical problem is the toxicity of CNM. CNT affect human epidermal kerotinocyte cells and human kin fibroblast by triggering the proteins involved in response to oxidative stress. Toxicity of CNM may also be due to structural manipulations. Thus study of cytotoxicity of CNT and its allergic impact needs to be looked into

In this paper, we present are efforts to study the effect of multiwalled CNT (MWCNT) on the lymphocyte of peripheral blood sample of healthy persons. Since CNT can be allergic to certain individuals it was decided to take lymphocyte sample from allergic patients also.

Allergic rhinitis, an inflammatory nasal disorder in which a range of cells and mediators contribute in a coordinated complex network that underlies clinical disease expression. Since the known drug therapies as well as steroidal treatment invariably load the patient with heavier dose than actually required. We have a vision that if nano carbon is not toxic to human cell it can be safely used as steroid or drug delivery vehicle. It must be said here that therapies like anti-IgE monoclonal antibody to develop allergen-specific immune system would remain another important aspect of allergy treatment

MATERIALS AND METHODS

MWCNT – These MWCNTs were synthesized by CVD method using Fe/Ni as catalyst having 95% purity. It has a diameter of 30-40nm and length of 5-50μm. It has < 2% amorphous carbon and < 0.2% ash. The surface area of MWCNT was 300m^2/g, where as its thermal conductivity was 1812$\pm$300 W/m.K. It was procured from Monad Nanotech Pvt Ltd.

SOURCE OF LYMPHOCYTES - Blood samples were collected from 7 healthy persons (as controls) who were neither on medication nor had a history of any illness in the past. Moreover as mentioned above from 4 Patients of Allergic Rhinitis (recruited by a well knowledgeable Allergologist on the basis of specific allergic symptoms), blood was collected when they were off treatment with antihistamine. Prior to collecting the blood sample, Skin scratch test was performed for different allergens viz. Pollen, fungi, Insects, Mites, Dust, Animal Dander, Feathers, Non vegetarian food, Vegetarian food. Total IgE by Capture Assay Radim Liquid Allergen (C.A.R.L.A.) System (RADIM)

Medium Used for Culturing Lymphocytes: was Ficoll Hapaque, RPMI- 1640 Medium, Foetal Bovine Serum (FBS from Sigma-Aldrich) and Gentamycine (from Nicholas Piramal).

Reagent tried for Lymphocyte Proliferation assay was Alamar Blue (AB) (from Biosource).

Lymphocyte Proliferation assay was done to test the Cytotoxicity of CNT

Patients and healthy human blood were collected in Heparin tubes. Peripheral blood (PB) T lymphocyte was isolated Ficoll h-paque separation method ( ). Cells were then culture in RPMI- 1640 media supplemented with FBS and gentamycine making the concentration of cells 1 million/ml. Incubation of cells was done by adding 20µl of nanomaterials to 180µl of cell suspension at 37^0C in 5% CO_2 atmosphere for 72 hours. The final concentration of added nanomaterials was 10, 25, 75, 100 mg/ml. After 72 hours of treatment with MWCNT, 20 µl of Alamar Blue was added in each well and incubated for 24 hours. Reduction in Alamar Blue showed the percentage proliferation of lymphocyte. The proliferation of lymphocyte was calculated by formula

$$\text{Percentage reduction in Alamar blue} = \frac{(O2 \times A1) - (O1 \times A2) \times 100}{(R1 \times N2) - (R2 \times N1)}$$

Where: O1 = molar extinction coefficient (E) of oxidized Alamar Blue (Blue) at 540nm; O2= E of oxidized Alamar Blue at 630nm; R1 = E of reduced Alamar Blue at 540nm; R2= E of reduced Alamar Blue at 630nm; A1 = absorbance of test wells at 540nm; A2 = absorbance of test wells at 630nm; N1 = absorbance of negative control well (media plus Alamar Blue but no cells) at 540nm and N2 = absorbance of negative control well (media plus Alamar Blue but no cells) at 630nm

Effect of MWCNT on Lymphocyte proteins by SDS PAGE

Pellet of the suspension of cells by centrifugation at 2500 x g for 10 minutes. Supernatant were discard. 1ml of Mammalian Protein Extraction Reagent (M-PER)(Thermo scientific) was added to each pellet. After shaking for 10 minutes debris were remove by centrifugation at 14000 x g for 15 minutes. The supernatant were then analyzed on 8% SDS PAGE.

Data analysis:

Total IgE serum level in both the healthy and patients were analyzed by Mann-Whitney Test. Cell growth data were analyzed by applying the paired sample t-test. In all cases, P value lower than 0.01 were considered as statistically significant. The statistical tests were performed by SPSS 15 software.

RESULTS AND DISCUSSIONS

The total IgE serum level in healthy control were 184.57±31.61 and in allergic rhinitis patients were 2487.50±471.86, it shows the significant difference, P= 0.006.

The viability of freshly isolated lymphocyte suspension assessed by trypan blue staining was 96±6%.

Figure 1 demonstrates the dynamics of reduced AB (from healthy individuals) accumulation in PB lymphocytes (from by1, 2.5, 7.5, 10 µg/ml of MWCNT. It shows that there is a significant decrease in the all PB lymphocyte with increasing concentration of MWCNT as compare to control.

As compare to the result obtained with the lymphocytes of healthy patients, there is comparatively less impact of MWCNT on the proliferation of PB T lymphocytes of Allergic Rhinitis patients (Fig.2). It must be stated here that the percentage proliferation of untreated (control) lymphocytes of both healthy and patients showed about 10% variation that is being less in patients.

Time dependent and dose dependent toxicity of SWCNT has been shown (7) on proliferation of kidney epithelial cell; similar findings were obtained by MWCNT on lung epithelial cells (8), but upto 10µg/ml SWCNT was found to induce DNA damage and higher concentration like 25 and 50 µg/ml caused decreased cell growth in cultured human blood cells [9]. In another interesting finding has shown that human mast cells and pheripheral basophil which are involved in allergic response were inhibited by fullerene and Carbon nano materials.

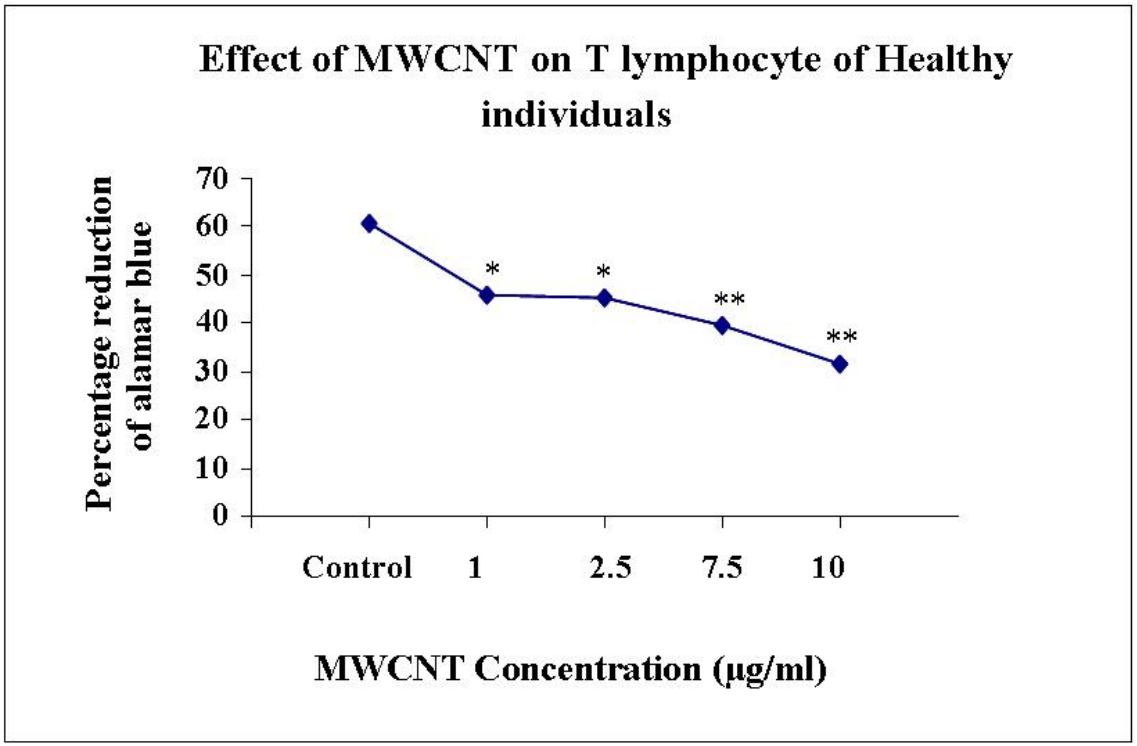

Fig. 1: Effect of different concentration of MWCNT on the proliferation of T lymphocyte of healthy individuals (as indicated by reduction of Alamar Blue). Data presented is mean of 7 readings obtained from 7 healthy donors. Significant difference for treated cells with MWCNT vs. untreated cells (control) group; *P<0.05, **P<0.01; pair sample t-test.

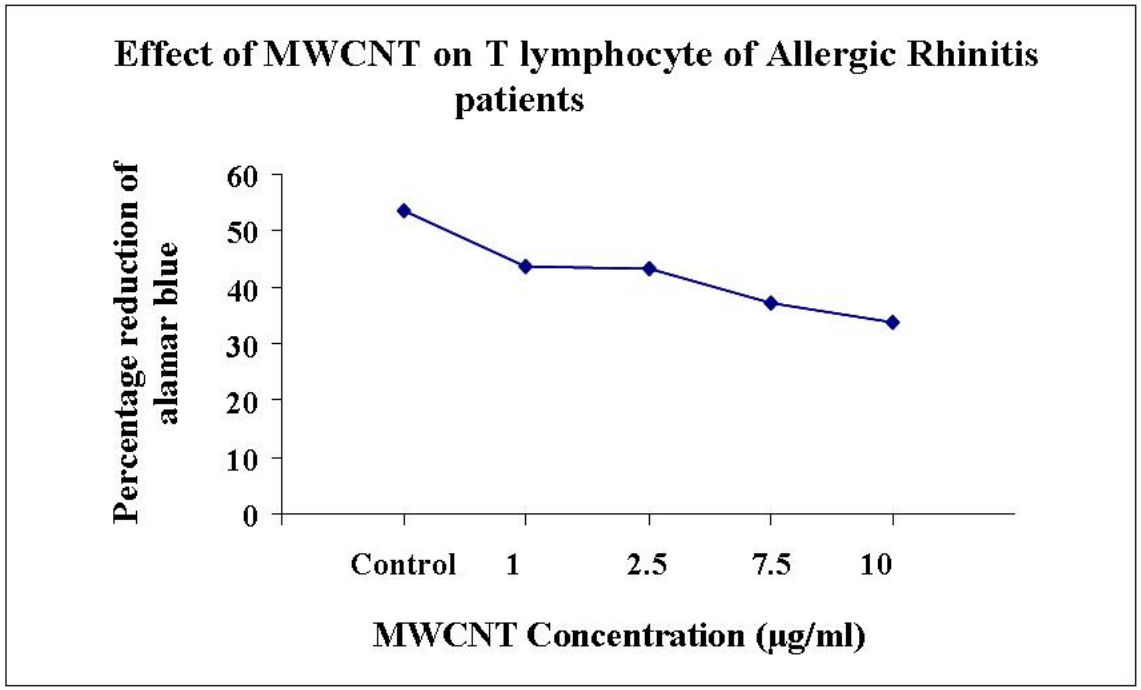

Fig. 2. Effect of different concentration of MWCNT on the proliferation of T lymphocyte of allergic patients as indicated by reduction of Alamar Blue. Data presented is mean of 4 readings obtained from 4 patients. Significant difference for treated cells with MWCNT vs untreated cells (control) group; *P<0.05, **P<0.01; pair sample t-test.

Lymphocytic protein are expected to be affected more by the exposure to pathological protein hence it was decided to assessed lymphocytic protein by SDS-PAGE analysis of lymphocyte treated with different doses of MWCNT(5).

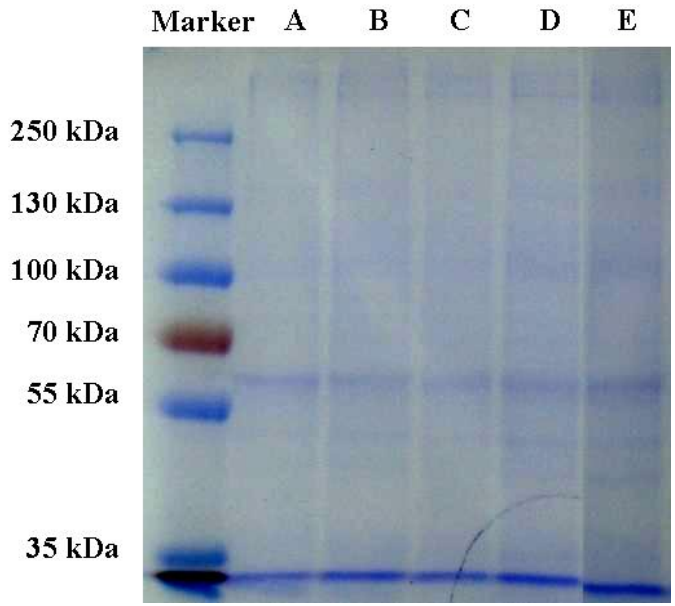

Fig. 3. Effect of different concentration of MWCNT on the protein of the T lymphocyte

(A) 10µg/ml, (B) 7.5 µg/ml, (C) 2.5 µg/ml. (D) 1 µg/ml and (E) Control (no MWCNT)

CONCLUSION

Our experiments demonstrate that MWCNTs at different concentration showed significant change in healthy control but there was no significant change in allergic rhinitis. The comparative study of healthy control and patients of allergic rhinitis not showed any significant change. It will give the opportunity to use MWCNTs as a carrier in drug delivery in allergic patients.

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