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Mecanismos e Efeitos da Internalização de Nanotubos de Carbono de Parede Simples Sobre o Ciclo Celular

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RESUMO

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O presente trabalho teve por objetivo avaliar alterações devido à exposição a Nanotubos de Carbono de Parede Simples (NTCPS) em duas linhagens celulares epiteliais (BBnt e HK2) e em uma linhagem celular monocítica (THP-1), enfocando os mecanismos de internalização e os efeitos sobre o ciclo celular. Foi avaliada a ação dos receptores *scavenger* na internalização dos NTCPS nas células HK2 e THP-1 e a interferência de duas concentrações de NTCPS sobre os elementos do citoesqueleto e no ciclo celular, nas células HK2 e BBnt. As concentrações utilizadas foram equivalentes as permitidas pelo *The National Institute for Occupational Safety and Health*: 2,4 e 24 $\mu g/cm^2$. A análise de expressão de mRNA por RT-PCR para receptores *scavenger*, mostrou que a internalização do NTCPS pode ocorre por endocitose. Sendo que os receptores SCARA5 e SRA são os responsáveis pela internalização nas células THP-1, enquanto MARCO e SRA realizam o processo de internalização nas células HK2. Observou-se que em ambas as concentrações, as células BBnt apresentaram amplificação centrossômica, com a ocorrência de 25,38% e 28,46% de mitoses alteradas para cada concentração, respectivamente. Não houve interferência significativa na progressão do ciclo celular em ambas as linhagens. O estudo da interação dos NTCPS com vesículas lipídicas não apresentou evidencias de alterações ou danos na membrana das vesículas, porém as vesículas apresentaram-se associadas umas às outras após o tratamento com 24 $\mu g/cm^2$.

Palavras-chave: Nanotubos de carbono. Internalização. Receptores *scavenger.* Amplificação centrossômica

ABSTRACT

Souza MM Mechanisms and effects of internalization of single wall carbon nanotube in cell cycle. [Master thesis (Science)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2014.

This study aimed to assess changes due to exposure to of Single-wall Carbon Nanotubes (SWCNT) in two epithelial cell lines (BBnt and HK2) and a monocytic cell line (THP-1), focusing on the mechanisms of internalization and effects on the cell cycle. The action of scavenger receptors in the internalization of SWNTC in HK2 and THP-1 cells and the interference of two concentrations of SWNTC about elements of the cytoskeleton and the cell cycle, in BBnt and HK2 cells was evaluated. The concentrations used were equivalent to those allowed by The National Institute for Occupational Safety and Health: 2,4 to 24 $\mu g/cm^2$. Analysis of mRNA expression by RT-PCR for scavenger receptors showed that the SWNTC internalization can occurs by endocytosis. Being that SCARA5 and SRA receptors are responsible for internalization in THP-1 cells, while MARCO and SRA perform the process of internalization in HK2 cells. It was observed that at both concentrations, the cells showed centrosome amplification in BBnt cells, with the occurrence of 25.38% and 28.46% of mitosis changed for each concentration, respectively. There was no significant interference with cell cycle progression in both strains. The study of the interaction of lipid vesicles with SWNTC showed no evidence of change or damage the membrane of the vesicles, but the vesicles were associated with each other after treatment with 24 $\mu g/cm^2$.

Keywords: Carbon nanotubes. Internalization. Scavenger receptors. Centrosome amplification

1 INTRODUÇÃO

Nanociência diz respeito a todas as estruturas de tamanho nanométrico (um bilionésimo de metro). Uma das primeiras implicações nesta área é o papel dominante da superfície. O aumento da área da superfície é muito útil em muitos campos como, por exemplo, na entrega da droga ou na catálise. Esta é apenas uma das muitas características da nanoescala, de fato nanoestruturas possuem muitas outras propriedades peculiares, dando origem a uma ampla gama de aplicações em física, química, engenharia, biologia e medicina. Várias novas subáreas foram recentemente iniciadas, tal como, por exemplo, "nanomedicina" e "nanoengenharia". A descoberta e as aplicações subsequentes de nanotubos de carbono (NTC) têm permitido o desenvolvimento de todo um ramo da nanotecnologia com base neste material versátil. NTCs têm sido considerados um material versátil com potencial para aplicações em diversos produtos, como: dispositivos eletrônicos, biomédicos, catalisadores e adsorventes. Grande parte destas aplicações são possíveis devido ao tamanho nanométrico e a elevada área superficial dos NTCs alterarem de maneira significativa as suas propriedades químicas e físicas. Apesar das grandes possibilidades, do interesse e investimentos em NTCs, os efeitos toxicológicos desse material ainda não estão completamente avaliados. Tem sido relatado que as características dos NTCs os tornam em potenciais agentes toxicológicos. Entretanto, na tentativa de contornar o problema da citotoxidade e genotoxicidade e tornar os NTCs úteis para este tipo de aplicações, tem sido utilizada a estratégia para modificar os NTCs por meio de reações que adicionam grupos funcionais sobre a superfície dos NTCs, tais como polímeros, proteínas ou ácidos nucléicos para torná-los biocompatíveis. Porém para que as aplicações se tornem realidade ainda é necessário esclarecer quais mecanismos as células internalizam os NTCs.

A compreensão dos mecanismos de internalização pode ser útil em aplicações de *drug delivery* (para entrega drogas ou biomoléculas), terapia para câncer, agente de contraste para diagnóstico por imagem, entre outras. Estudos têm apontado que as células podem internalizar os NTCs por dois mecanismos diferentes, são eles: processos dependente de energia (fagocitose e endocitose) e a internalização por processo independente de energia (difusão passiva) [1]. A internalização por fagocitose tem sido observada quando os NTCS na forma de agregados com tamanho que varia de algumas centenas de nanômetros até alguns micrômetros [2]. Quando os NTCs apresentam dimensões elevadas o processo de fagocitose pode não ser bem sucedido. Esse processo é conhecido como fagocitose "frustrada" e tem sido associado à respostas inflamatórias [3–5]. Nas células não especializadas em fagocitose, o processo de internalização dos NTCs ocorre por via endocítica, mediada por receptores de membrana. O conhecimento sobre o papel de receptores de superfície de membrana na internalização, motivou a utilização NTCs conjugados como agentes quimioterápicos e com ligantes específicos a receptores superexpressados em células cancerosas. Um exemplo é o uso de receptores de EGF ou de ácido fólico para realizar uma internalização seletiva e aumentar a eficiência dos quimioterápicos [6, 7].

Uma família de receptores de superfície da membrana plasmática bem conhecida, são os receptores scavenger [8]. Esses receptores podem ser expressos por células mieloides, endoteliais e algumas células epiteliais e são capazes de reconhecer diferentes ligantes, como patógenos bacterianos e lipoproteínas. Eles têm sido relacionados também com a internalização

6. CONSIDERAÇÕES FINAIS

Ao tratar as linhagens celulares estudadas com NTCPS, observou-se que em baixas concentrações ($2,4\ \mu g/cm^2$ e $24\ \mu g/cm^2$) os nanotubos interagem e são internalizados. A expressão relativa de mRNA evidenciou que receptores de membrana plasmática membros da família *scavenger* estão relacionados com a internalização endocítica dos NTCPS. Os dados obtidos demonstram que diferentes receptores desta família podem atuar durante o processo de internalização e um aumento da expressão de mRNA é estimulado pelo aumento na concentração de NTCPS. Por meio da microscopia Raman ficou evidente a interação dos nanotubos de carbono com células epiteliais derivadas de carcinoma de pulmão humano. Embora os nanotubos de carbono tenham sido internalizados, os efeitos observados sobre a interferência no ciclo celular não foram estatisticamente significativos. Esses resultados indicam que os efeitos dos NTCPS nas células BBNT e HK2 se apresentaram menos tóxicos do que aqueles observados para células primarias e normais do epitélio pulmonar humano [19] ou para células de provenientes de rato ou camundongo [56, 57]. Enquanto que, para a linhagem celular BBNT houve alterações no padrão das mitoses, levando a células dessa linhagem a gerarem mitoses multipolares. O que pode ser um efeito resultante da interação dos NTCPS com os centrossomos durante o processo de divisão. A análise da interação dos nanotubos de carbono com vesículas unilamelar gigante não apresentou alterações que indicassem uma interação entre os nanotubos e as vesículas. Entretanto, foi visualizado uma interação vesícula–vesícula não usual que, pode indicar uma influência dos nanotubos de carbono sobre o comportamento das vesículas. Contudo esses estudos devem ser aprofundados para determinar se esse comportamento é resultante da ação dos NTCPS. Conjuntamente os efeitos da exposição a longo prazo e a efetividade da internalização devem ser exploradas de maneira a elucidar como os nanotubos de carbono podem influenciar a integridade celular.

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