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Original Article

Characterization of tannic acid- and gallic acid-functionalized single- and multiwalled carbon nanotubes and an *in vitro* evaluation of their antioxidant properties

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الملخص

أهداف البحث: الأنابيب النانوية الكربونية هي مؤكسدات قوية تؤثر على تطبيقاتها الطبية الحيوية. ويتأثر دورها الفاعل كمضادات للأكسدة بوظيفتيها، كمثبط للسمية وكمحفز للتأثيرات المضادة للأكسدة. تختص هذه الدراسة بالقدرة الكامنة للأكسدة لكل من الأنابيب النانوية الكربونية أحادية الجدار والأنابيب النانوية الكربونية متعددة الجدر بتفعيلهما بواسطة حمض التانيك وحمض القاليك وتقييم خصائصهم المضادة للأكسدة مخبريا.

طرق البحث: تمت عملية التفعيل بواسطة جهاز "فوريه" لتحويل طيف الأشعة تحت الحمراء وتقييم المحتوى الفينولي الكلي. أما تحليل مضادات الأكسدة فتم بواسطة كل من كسح أيمينو أزينيوم-ثنائي الفينول- (٦.٤.٢) ثلاثي نايترات الفينول) وأكسدة الدهون بالبيروكسيد، والتحديد الكمي لأنواع الأكسجين التفاعلي وإخماد الهيدروكسيل والجذور فائقة الأكسدة المتولدة خارجيا.

النتائج: يتوافق توظيف مضادات الأكسدة على الأنابيب النانوية بواسطة جهاز "فوريه" لتحويل طيف الأشعة تحت الحمراء وتقييم المحتوى الفينولي الكلي. لوحظ كسح الجذور العليا بالنسبة للأنابيب النانوية الكربونية أحادية الجدار المفعلة بواسطة حمض التانيك مقارنة بغيره من التفعيلات، وكذلك بالأنابيب النانوية الكربونية متعددة الجدر. أظهرت نتائج أكسدة الدهون بواسطة البيروكسيد أن توظيف الأنابيب النانوية بمضادات أكسدة حمض التانيك خفضت أكسدة الدهون بشكل ملحوظ (٣٦٪) مقارنة بالثابت المتعري (٨٥٪) والثابت الإيجابي (٩٤٪). إضافة إلى ذلك فإن الأنابيب النانوية المفعلة بواسطة مضادات الأكسدة أظهرت إنتاجا لا يُذكر من أنواع الأكسجين التفاعلي تحت ظروف مختلفة من الإشعاع وجذور الهيدروكسيل وفائقة الأكسدة المتولدة خارجيا.

الاستنتاجات: أوضحت هذه الدراسة أنه باستخدام النماذج المخبرية فإن التوظيف الفاعل للأنابيب النانوية الكربونية باستخدام حمض التانيك وحمض القاليك يؤدي إلى خصائص مضادة للأكسدة مذهلة. وأبدت الأنابيب النانوية الكربونية المفعلة كمضادات للأكسدة انخفاضا في نسبة موت الخلايا مصحوبا بإنتاج لا يُذكر من أنواع الأكسجين التفاعلي بتأثير الحالات الإشعاعية المختلفة، وانخفاضا في جذور الهيدروكسيل وفائقة الأكسدة المتولدة خارجيا. إضافة إلى ذلك فإن الأنابيب النانوية الكربونية المفعلة كمضادات للأكسدة كان لديها توافقا أكبر مع جدار الخلية.

الكلمات المفتاحية: مضادات الأكسدة؛ التوافق مع الحياة؛ الأنابيب النانوية الكربونية؛ التوظيف؛ الكسح الجذري

Abstract

Objectives: Carbon nanotubes (CNTs) have powerful oxidative properties that influence their biomedical applications. This study addresses the oxidative potential of both single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) by functionalizing them with tannic acid (TA) and gallic acid (GA), and an *in vitro* evaluation of their antioxidant properties is presented. Their effective role as antioxidants is influenced by their dual functions of reducing toxicity and inducing antioxidant effects.

Methods: Functionalization was confirmed by Fourier transform infrared spectroscopy (FTIR), and the total phenolic content was assessed. The antioxidant properties

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were analyzed by scavenging di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium, lipid peroxidation, reactive oxygen species (ROS) quantification and quenching externally generated hydroxyl and superoxide radicals.

Results: The functionalization of nanotubes with antioxidants was conformed via FTIR and measurement of total phenolic compounds. Higher radical scavenging was observed for TA-functionalized SWCNTs than for other functionalizations and MWCNTs. The lipid peroxidation results revealed that the functionalization of nanotubes with the antioxidant TA significantly decreased lipid peroxidation (36%) compared with naked nanotubes (85%) and the positive control (94%). Furthermore, antioxidant-functionalized nanotubes showed negligible production of ROS after being irradiated under different conditions, and externally generated hydroxyl and superoxide radicals were quenched.

Conclusion: This study showed, using *in vitro* models, that effective functionalization of CNTs with TA and GA leads to remarkable antioxidant properties. Antioxidant-functionalized nanotubes showed a reduction in cell lethality correlated with negligible ROS production under different irradiation conditions and quenching of externally generated hydroxyl and superoxide radicals. Further, antioxidant-functionalized nanotubes were more compatible with the cell membrane.

Keywords: Antioxidants; Biocompatibility; Carbon nanotubes; Functionalization; Radical scavenging

Introduction

Carbon-based nanomaterials, especially carbon nanotubes (CNTs), have opened new doors for a wide range of biological applications. Because of their unique electronic, chemical and mechanical properties, CNTs have potential applications in biological systems, including as biosensors,[1] biofilms,[2] drug carriers[3] and vaccine delivery vehicles.[4] Published reports show that application of CNTs in living system appears to be an elusive goal, as they have been found to be toxic and incompatible with biological systems.[5] Relevant to their toxicity, CNTs are oxidants that produce an excess of reactive oxygen species (ROS), causing deleterious effects, such as destruction of biological cell membranes and adenosine triphosphate and deoxyribonucleic acid fragmentation.[6,7] Studies have shown that purified single-walled carbon nanotubes (SWCNTs) were toxic to the PC12 cell line, directly or indirectly destroying cellular integrity and affecting gene regulation and cellular signaling pathways.[8,9] Further, time- and dose-dependent adverse effects on cell viability, morphological changes, DNA damage, increased apoptosis and damaged redox homeostasis have also been reported for CNTs with different functionalizations.[10,11] The available literature on the state of the art of CNTs reveals that their metal catalyst, mass, surface chemistry, purity and aspect ratio have a strong impact on their nature of toxicity.[5,6,12] However, from a biological perspective, functionalized CNTs are more compatible with the cell environment than naked ones. For instance, upon functionalization, CNTs were rendered biocompatible by inducing the formation of collagen in osteoblast cultures.[13] Functionalized CNTs favored ideal bone regeneration through mesenchymal stem cell proliferation *in vitro*.[5] Thus, targeted functionalization reduces the toxic nature of CNTs and favors their use in biological materials for multiple applications.[6]

To date, very few reports exist on the functionalization of CNTs with antioxidants. As antioxidants are effective radical scavengers of reactive oxygen species (ROS), their incorporation might reduce CNT toxicity. Recently, Lucente-Schultz, R. M. et al.[14] investigated antioxidant properties of SWCNTs functionalized with butylated hydroxytoluene (BHT). Follow-up studies showed that gallic acid (GA) functionalization of MWCNTs improved their free radical scavenging ability.[15] Lin, D. and Xing, B.[16] functionalized MWCNTs (multiwalled carbon nanotubes) with tannic acid (TA) for better dispersion.

In the present study, an attempt was made to functionalize the surface of SWCNTs and MWCNTs with water-soluble antioxidants, such as GA or TA, by a simple chemical approach. Our investigation revealed that naked CNTs were effective ROS generators, but that oxyradical generation was greatly suppressed in TA-/GA-functionalized ones. Further, through various *in vitro* assessments, such as measurement of the phenolic content, di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) assay, lipid peroxidation, and superoxide and hydroxyl radical scavenging activities, we found that TA-functionalized SWCNTs and MWCNTs had higher antioxidant properties than GA-functionalized and naked CNTs. These results may open a new area in a wide range of future biomedical applications.

Experimental

Antioxidant functionalization

Information regarding CNTs and the chemicals used for experiments is available at S1 in Appendix A. For antixodiant functionalization, a suspension of CNTs (1 mg/mL) and antioxidant (TA or GA) (1 mg/mL) was incubated for 3 days on a shaker at 120 rpm.[16] The resulting mixture was then sonicated (Rivotek, Mumbai, India) for 1 h and centrifuged at 3000 rpm for 15 min to remove excess unreacted antioxidants using a poly(tetrafluoroethylene) membrane filter (pore size: 0.2 μm). The filtered membranes were dried in oven at 80 °C for 24 h, and the dried samples then collected. The dried samples were re-dispersed (1 mg/mL) in water for antioxidant evaluation. They were then subjected to further analysis and assays. The antioxidant functionalization was assessed using Fourier transform infrared (FTIR) spectroscopy (Bruker, Tensor 47) in the range of 2000−700 cm^{-1}.