Abstract

Nanotechnology in the intensive care: Intravascular biocompatibility of carbon nanomaterials – effect of carbon nanotubes on blood platelets.

EFFECT OF CARBON NANOTUBES ON BLOOD PLATELETS

Carbon nanotubes (CNTs) are among the principal materials currently used in biomedical nanotechnologies. CNTs possess superior mechanical and chemical characteristics including enormous tensile strength, elasticity and conductivity. As a result they are very popular and attractive for use in various biomedical applications. Many of these applications may lead ultimately to contact of carbon nanomaterials and blood. Furthermore, CNTs may also be present intravascularly as a result of environmental or occupational exposure. Therefore, the investigation of the intravascular biocompatibility of CNTs is a critical safety issue.

We studied the effects of structurally different purified CNT materials from different manufacturers on human platelets and compared their effects to amorphous carbon black nanoparticles (ACB), fullerene C_{60}, fullerenol $C_{60}(OH)_{24}$ and NIST standard polystyrene nanobeads (PNBs). Using light transmission aggregometry of human platelet rich plasma, we found that various CNTs induce PLT aggregation and this occurs in a concentration dependent manner. In contrast to CNTs, ball-like shaped fullerene did not cause platelet aggregation. Flow cytometry analysis showed that CNTs induce platelet activation, demonstrated by the detection of surface exposure of CD62P and CD63 and the release of $CD62P^+$ and $CD63^+$ platelet membrane microparticles (MPs). Field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) confirmed that the platelets in contact with CNTs undergo morphologic changes from resting discoid to activated state with pseudopodia, membrane budding and microparticle shedding. CNTs induce an increase in intracellular Ca^{2+} concentration in platelets loaded with Ca^{2+}-sensitive probe FURA-2AM, as detected by ratio fluorometry. Rapidly occurring CNT-induced extracellular Ca^{2+} influx could be inhibited by calcium channel blockers SKF 96365 and 2-APB. Investigating this phenomenon further, we observed that CNTs penetrate the platelet plasma membrane without any discernible damage but then interact with the dense tubular system (DTS) causing depletion of platelet intracellular Ca^{2+} stores as shown by electron (FESEM, TEM) and immunofluorescent microscopy. This process is

accompanied by the clustering of stromal interaction molecule 1 (STIM1) co-localized with Orai1 protein, indicating an activation of the store-operated Ca^{2+} entry (SOCE) mechanism.

To investigate the effect of size and charge of nanosized materials we used the PAMAM dendrimer model.

In conclusion, we were able to prove that CNTs induce platelet activation and aggregation and thus possess highly prothrombotic properties. Furthermore we revealed the underlying molecular mechanism of CNT-induced platelet activation. These findings are critical in the evaluation of the biocompatibility of carbon nanomaterials with blood.