Evaluation of Alterations in Behaviour, Cognition, and Neuronal Excitability Induced by Administration of QTracker® 800 Quantum Dots

B. Salvetti[1], S. Becchi[2], F. Schio[1], E. Moscardo[1], G. Bertini[1], P.F. Fabene[1], F. Osculati[1]

[1] *Department of Neurological, Neuropsychological, Morphological and Movement Sciences, University of Verona, Verona, Italy. beatrice.salvetti@gmail.com*

[2] *Brain and Mind Research Institute, University of Sydney, Sydney, NSW, Australia.*

Abstract

Studies of Quantum Dots biodistribution have shown that these nanoparticles accumulate in various organs, including brain parenchyma, possibly causing functional alterations. To investigate its potential neurotoxicity, we administered QDs to young mice and studied their behaviour during the following weeks. Spontaneous locomotor behaviour in the home cage was not significantly altered in treated mice, the Rotarod and Grip Strength Meter tests were normal, whereas memory deficits were found with the Novel Object Recognition test up to three weeks after the injection. We also found a significant increase in the neuronal excitability of QDs-treated mice, following the administration of a Pentylenetetrazol challenge.

Keywords: Nanoparticles, Behaviour, Neurotoxicity, Novel Object Recognition.

Introduction

Quantum Dots are nanometre-sized particles generally composed of a heavy metal core made of cadmium-selenium (CdSe) or cadmium-telluride (CdTe) and a shell made of zinc-sulphide (ZnS) or zinc-selenide (ZnSe) to render QDs' bioavailability [1]. These semi-conductors, fluorescent nanocrystals are gaining increasing attention because of the large numbers of potential applications in biomedical research and neuroscience. However, the assessment of biocompatibility and biosafety of QDs is a critical issue for further applications as diagnostic and imaging tools on humans and still little is known about their potential toxicity. Previous studies in our laboratory have shown that QDs accumulate in the liver, spleen, and lungs. Moreover, we found that QDs can cross the blood brain barrier and can be observed by means of transmission electron microscopy in different cell types (both neurones and glia), both in the nucleus and in the cytosol.

We hypothesized that nanoparticles in the brain parenchyma may elicit an inflammatory response caused by an alteration in proinflammatory and signalling molecule expression, inducing fever and profound psychological and behavioural changes generally called ''sickness behaviour'' [2]. To such aim, we investigated possible alterations in basal locomotor behaviour, cognitive functions, and neuronal excitability in QDs treated mice.

Materials and Methods

BALB/c male mice received a single i.v. treatment (via tail vein), of 10µl/g 40pM QTracker® 800 QDs solution (Invitrogen). Animals were housed in PhenoTyper® chambers (45 cm W x 45 cm L x 60 cm H, Noldus Information Technology, Wageningen, The Netherlands) from the day of their arrival, under a reversed 12h light/12h dark cycle (lights off at 7 AM). Basal locomotor activity of the animals was recorded both before and after the treatment. Recognition memory was assessed using the NOR task [3] before the injection (baseline), and then 24 hours, 1 week, 2 weeks, and 3 weeks after the injection. Pentylenetetrazol (PTZ), a CNS stimulant, was injected 3 weeks after QDs injection.

Animal activity and behaviour during the NOR test was automatically recorded in the PhenoTyper®. Each chamber was divided into two portions with a white plastic divider, so that it accommodated two animals.

Automated tracking was performed using the EthoVision XT® software (Noldus Information Technology, Wageningen, The Netherlands).

The distance moved by each animal in the cage and its mean velocity (cm/s), mobility, and immobility durations were continuously recorded for 24 hours at different time points: before treatment (baseline), and 48 hours, 1 week, 2 weeks, and 3 weeks after treatment.

The NOR test consists of two phases, a sample trial (10 min duration) and a choice trial (5 min duration) with 60 min retention interval between the two (see Figure 1). During the sample trial two identical objects were placed in the PhenoTyper cage 10 cm away from the sidewalls and 7 cm from each other. The animal was left undisturbed for the delay period and then presented with a third copy of the previously seen objects and with a new one. To place and remove objects mice were confined at the end of the cage by the use of a mobile separator made of paper, which allows placing objects in the correct position without being seen by the animal. Ten different objects (in triplets) made of plastic, wood or glass that differed in terms of height, colour, shape and surface texture are used. The objects are fixed to the floor of the arena with tape to ensure that the mice could not displace them, they had no known ethological significance for the mice, and had never been paired with a reinforcer. Pilot studies ensured that mice of the BALB/c genetic background strain could discriminate between the objects, and there was no per se preference for one of these objects. The position and the identity of the novel object and familiar object were randomized between animals. The time spent exploring the objects was recorded. Exploration was defined as touching and sniffing the objects or directing the nose to them at a distance ≤ 2 cm. Discrimination ratio was used as an index of recognition memory, i.e. percentage of time spent exploring the novel object, relative to the total time spent exploring both objects ($(t_{novel}/t_{novel}+t_{familiar})*100$). Values higher than 50% suggest preference for the novel object.

In order to exclude a decreased object exploration due to impairments in locomotor functions animals were tested immediately after the NOR test for coordination and strength. Coordination and balance of the animals were measured with the Rotarod test (Ugo Basile, Comerio, Italy). Forelimb strength was assessed with the Grip Strength Meter test (Ugo Basile, Comerio, Italy).

To test further a possible alteration in neuronal excitability we injected PTZ i.p., a CNS stimulant with pro-convulsivant properties, 3 weeks after QDs injection. Latency from PTZ injection to 5 different convulsive stages was measured [4].

- Stages 1-3: hypoactivity and abdomen on bottom of the cage; twitches; mouse falls but recovers.
- Stage 4: falling and forelimbs parallel to body axis and stiff.
- Stage 5: major convulsion resulting in death.

Animals were sacrificed after a period of 30 minutes elapsed from stage 4. However, all animals reached stage 5 before this cut off.

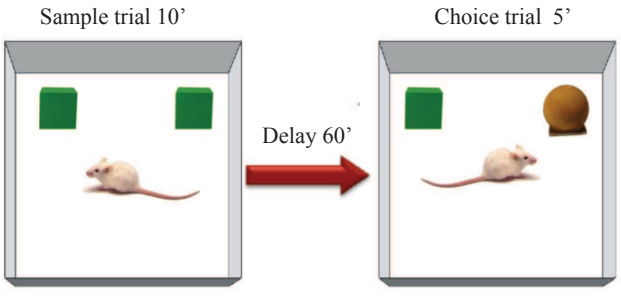

Figure 1. Schematic representation of novel object recognition test.

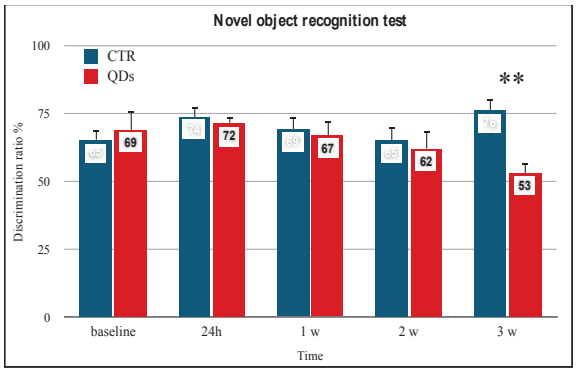

Figure 2 Novel Object Recognition results.

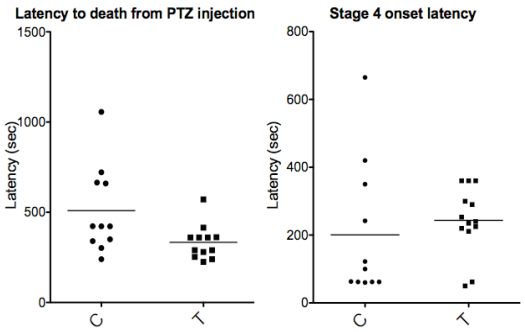

Figure 3. PTZ challenge results, latency from injection to death and stage 4.

Results and Discussion

Analysis of mice locomotor activity inside the chambers revealed no differences between QDs treated mice and controls in any of the considered parameters and time points. Rotarod and Grip Strength Meter tests revealed no differences between control and treated animals at any of the five time points. The NOR test revealed no differences between control and treated mice at baseline, 24 h, 1 week, 2 weeks but a difference was found in the discrimination ratio at 3 weeks after the treatment (t_{14}=4.283, p<0.01, see **Error! Reference source not found.**). In the PTZ challenge significant differences emerged between controls and treated mice in latency to death from injection (t_{21}=2.331; p=0.0298), and latency to stage 4 onset from the injection (t_{21}=2.508; p=0.0204, see **Error! Reference source not found.Error! Reference source not found.**). Data indicates that exposure to QDs induced a decrease in NOR performance over time, with an impairment at 3 weeks. Decrease in NOR performance might be caused by the presence of exogenous compound in the brain parenchyma, which can cause inflammatory response resulting in functional alterations. Data from the PTZ challenge show an increased duration of the seizures after the injection in control mice, that is, QDs treated mice died sooner than control ones after the treatment indicating an increased neuronal excitability possibly related to the presence of QDs in brain parenchyma.

Ethical statement

The experiments received authorisation from the Italian Ministry of Health, and are conducted following the principles of the NIH Guide for the Use and Care of Laboratory Animals, and the European Community Council (86/609/ EEC) directive. Mice are housed and used according to current European Community rules. Experiments on mice are approved by the research committees from the University of Verona and Italian National Institute of Health.

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