The role of elevated autophagy on the synaptic plasticity impairment caused by CdSe/ZnS quantum dots

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1. Introduction

Autophagy is a highly conserved lysosomal degradation pathway which plays an important role in maintaining cytoplasmic homeostasis [1,2]. It is characterized by formation of double-membraned autophagosomes which engulf cytosolic components into lysosome for degradation and recycling [3]. During autophagy, light chain protein 3 (LC3) is conjugated to phosphatidylethanolamine (PE), thereby converting the inactive LC3-I to the active PE-conjugated LC3 (named LC3-II). This activation triggers initiation of autophagosomes formation [4]. In addition, P62, as an autophagy substrate, is degraded during autophagy activation [5].

It is well known that autophagy protect neurons from nutritional starvation [6]. Indeed, a growing body of evidence has shown that constitutive autophagy is responsible for neuronal survival [7], and dysfunction of autophagy causes many neurodegenerative diseases and behavioral deficits [8,9]. In addition, few reports have indicated that autophagy may contribute to synapse function. Abnormal autophagic vesicle disrupts pre-synaptic terminals and causes axonal dystrophy [10]. Mammalian target of rapamycin (mTOR) inhibitor rapamycin induces autophagy in dopaminergic axons with associated decreased dopamine release [11]. N-methyl-D-aspartate (NMDA) application also causes up-regulation of LC3-II in cultured neuron coincident with degradation of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid receptor (AMPAR) [12].

Abbreviations: QD, quantum dot; LC3, light chain protein 3; PE, phosphatidylethanolamine; mTOR, mammalian target of rapamycin; LTP, long-term potentiation; I/O, input/output; PPF, paired-pulse facilitation; IPI, inter-pulse interval; DG, dentate gyrus; 3-MA, 3-methyladenine; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; HFS, high frequency stimulation; TEM, transmission electron microscopy; ROS, reactive oxygen species.

2. Results

2.1. Elevated autophagy is caused by CdSe/ZnS quantum dots (QDs).

We have demonstrated that CdSe/ZnS quantum dots (QDs), which are widely applied in vitro for diagnostics and cellular imaging, can impair synaptic transmission and synaptic plasticity in the dentate gyrus (DG) area, but the mechanism is still unclear. Here we show that elevated autophagy is at least partly responsible for this synaptic dysfunction induced by QDs in vivo. QDs elicited autophagy in the HeLa cells and cultured hippocampal neurons as well, accompanied with GFP-light chain protein 3 (LC3) puncta dots and autophagosome formation, extensive conversion of LC3-I to LC3-II and a significant decrease of p62. Furthermore, we found that autophagy inhibitors (wortmannin, 3-MA or chloroquine) suppressed QDs-induced autophagic flux, partly blocked LTP impairment, coincident with down-regulation of synapsin-I and synapse deficits by QDs in the hippocampal CA1 area. Our studies have important implications in providing a potential clinical remedy for brain damage caused by nanomaterials and in designing safer nanoparticles.

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spectrum, and good photostability [23–26]. However, growing concerns over biosafety of QDs have emerged. In fact, the cytotoxicity of QDs has been well studied previously, including DNA damage [27], oxidative stress [28], mitochondrial damage [29], autophagy induction [30] and cell death [31].

We have previously reported the neurotoxicity of QDs in the central nervous system (CNS) [32,33], and found QDs-induced synaptic plasticity impairment in the dentate gyrus (DG) area of the hippocampus [34], but the mechanism is not well known. To determine the molecular mechanism of QDs-mediated synaptic dysfunction, in the present study, we investigated the ability of QDs to cause autophagy and long-term potentiation (LTP) impairment in CA1 area in vivo, and we further assessed the role of autophagy involved in QDs-induced synaptic plasticity impairment. Such studies might have important implications for developing functional nanomaterials for biomedical applications and providing a potential clinical remedy for nanoparticles-induced brain damage.

2. Experimental section

2.1. Reagents and antibodies

Streptavidin-conjugated CdSe/ZnS QD was purchased from Molecular Probes Inc (QDots 525, Eugene, OR, USA, Q10141MP). Wortmannin (S1952) was from Beyotime (China). Rapamycin (R8781), chloroquine (CQ, C6628), l- Glutathione reduced (GSH, C4251) and 3-methyladenine (3-MA, M9281) were from Sigma–Aldrich. Synapsin-I primary antibody (D12GS) was from Cell Signaling Technology. 2′-7′-Dichlorofluorescin diacetate (DCFH-DA, D399) was from Invitrogen. Lc3 primary antibody (NB100-2220) was from Novus. GAPDH (MAB374) antibody was from Millipore. P62 primary antibody (ab56416) was from Abcam. Rhodamine Red-X-Affini Pure Goat Anti-Rabbit IgG (111-295-003) was from Jackson ImmunoResearch Laboratories. FITC-goat anti-mouse IgG was from Sigma–Aldrich.

2.2. Characterization of QDs

To evaluate the characteristics of QDs, we took a transmission electron microscopy (TEM) image using JEOL-2010 high-resolution transmission electron microscopy (HRTEM). TEM images were captured at 200 kV using a device from Tecnai. The emission and absorption spectra were obtained using a spectrophotometer (RF-5301, Shimadzu).

2.3. Cell and cell culture

GFP-LC3/HeLa stable cell lines were established from HeLa cells transfected with GFP-LC3 plasmid followed by selection [18]. GFP-LC3/HeLa and HeLa cells were grown as a monolayer at 37 °C in 5% CO2. To detect QDs cellular uptake, HeLa cells were preincubated with 2 μl of Opti-MEM medium at 37 °C and then incubated with QDs (2 nm and 10 nm) for 6 h, followed by washing with PBS (pH 7.4) for three times to remove any free QDs. The cells with internalized QDs were observed under fluorescence microscopy (Olympus IX71, Olympus, Japan) with excitation at 488 nm and emission at 530 nm, and were also analyzed by a fluorescence activated cell sorting (FACS) caliber flow cyrometer (Becton Dickinson, San Jose, CA, USA). To detect ROS generation, HeLa cells, treated with or without 10 nM QD for 6 h, were washed with PBS (pH 7.4) for three times and then incubated with 10 μM ROS fluorescent probe (DCFH-DA) for 15 min at 37 °C. Subsequently, cells were washed twice with PBS and observed under fluorescence microscopy.

2.4. Cellular uptake and intercellular reactive oxygen species (ROS)

HeLa cells were seeded in a 6-well plate (1 × 10⁶ cells/well) and cultured at 37 °C in 5% CO2. To detect QDs cellular uptake, HeLa cells were preincubated with 1 μl of Opti-MEM medium at 37 °C and then incubated with QDs (2 nm and 10 nm) for 6 h, followed by washing with PBS (pH 7.4) for three times to remove any free QDs. The cells with internalized QDs were observed under fluorescence microscopy (Olympus IX71, Olympus, Japan) with excitation at 488 nm and emission at 530 nm, and were also analyzed by a fluorescence activated cell sorting (FACS) caliber flow cyrometer (Becton Dickinson, San Jose, CA, USA). To detect ROS generation, HeLa cells, treated with or without 10 nM QD for 6 h, were washed with PBS (pH 7.4) for three times and then incubated with 10 μM ROS fluorescent probe (DCFH-DA) for 15 min at 37 °C. Subsequently, cells were washed twice with PBS and observed under fluorescence microscopy.

2.5. Animals

Animal care and use were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication NO.80-23, revised in 1996) after approval from the Institutional Animal Care and Use Committee of University of Science and Technology of China. Every effort was made to minimize animal suffering and number of animal used.

All the experiments were performed in adult male Wistar rats weighing 240–300 g. Animals were placed in a rearing room at a constant temperature (23 ± 0.5 °C) on a 12 h light–dark cycle (8:00 a.m–8:00 p.m.), with free access to food and water. They were used for electrophysiological recordings in vivo during the same periods (between 9:00 a.m. and 9:00 p.m.) at the age of 60 (±10) days.

2.6. Intrahippocampal infusion and field potential recording in vivo

Intrahippocampal field potential recordings and drug infusion were performed as described in previous studies [36,37]. Briefly, the rats were anesthetized with 10% urethane (1.8 g/kg, i.p.) and maintained at a surgical level with supplemental injections as needed, and then positioned in a stereotoxic apparatus. Rectal temperature was monitored and maintained at 37 ± 0.5 °C by an automatic heating pad. A tungsten bipolar stimulating electrode was placed into the Schaffer collateral (SC, coordinates with the skull surface fat at Bregma: 4.2 mm posterior, 3.8 mm lateral, 2.8 mm ventral). A simultaneous electrode-guide combination (Plastics One, Roanoke, VA, USA) was used to record field excitatory postsynaptic potential (fEPSP) from the CA1 area at a rate of 250 Hz/min. Electrophysiological recordings were performed after drug administration.

The electric signals from the CA1 area were amplified by a thousand times, digitized at 10 kHz, and band-pass filtered at 1–3 kHz. The Input/Output (I/O) curves were generated by a series of field potentials evoked by various stimuli (100 μA, 0.1–1.5 mA). The strength of a field potential was evaluated by the slope of IEPSP.
During the recording, the stimulus intensity was adjusted to elicit 40–50% of the maximal fEPSP. For each recording session, a 20 min stable baseline (< 10% change) was acquired before the application of drugs or conditioned stimulus. The paradigm of high frequency stimulation (HFS) was used to induce LTP consisting of five trains at 1 Hz, and each train was composed of 100 pulses at 200 Hz. The HFS was repeated 6 times at intervals of 1 min. The intensity of HFS was the same as that used in the baseline recordings. We also recorded paired field potentials, which elicited by paired pulses of stimuli with interpulse intervals (IPI) ranging from 10 to 400 ms. Paired-pulse ratio (PPR) was calculated with the fEPSP of the second field potential divided by those of the first one.

2.7. Western blotting

Hippocampal tissues and cells were homogenized with Teflon glass homogenizer in Lysis Buffer (P0013, Beyotime, China) as soon as they were removed. The homogenate was centrifuged at 17,000 × g for 30 min at 4 °C, the resulting supernatant was collected and the protein concentration was determined by BCA. Western blotting for LC3, p62 and synapsin-I proteins was performed as described previously [18,20].

2.8. GFP-LC3 dot observation

GFP-LC3/HeLa cells were grown in the 96-well plates, and treated with rapamycin (200 nm), QDs (2 or 10 nm) and QD plus GSH (1 μM) for 24 h. GFP-LC3 dots were observed under fluorescent microscopy (Olympus IX71, Olympus, Japan).

2.9. Immunofluorescence

Cultured hippocampal neurons were fixed by 4% paraformaldehyde for 10 min at room temperature, followed by permeabilization in 0.3% Triton X-100 for another 10 min. After preblocking for 1 h in PBS containing 10% fetal bovine serum (v/v), 3% BSA (w/v) and 0.1% Triton X-100 (v/v), cells were incubated with LC3 and synapsin-I antibodies overnight at 4 °C and then with Rhodamine Red-X-Affini Pure Goat Anti-Rabbit IgG or FITC-goat anti-mouse IgG for 1 h at room temperature. Neurons were observed under fluorescent microscopy (Olympus IX71, Olympus, Japan).

2.10. Electron microscopy

Cultured neurons and hippocampal tissues were fixed in suspension with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 h, and then postfixed for 1 h at room temperature in 1% OsO4. After dehydration with graded ethanol and embedded in epoxy resin, areas containing neurons or tissues were block mounted and cut into ultrathin sections. Thin sections were stained with uranyl acetate and lead citrate, and then observed on transmission electron microscope (TEM, JEOL-1230).

2.11. Statistical analysis

The baseline values of fEPSP slopes were obtained from the first 20 min recordings. Then, fEPSP slopes in every 1 min were averaged and were normalized to baseline values. All data were shown as means ± standard error of mean (SEM). For electrophysiological data, comparison between groups was made using two-way ANOVA with Turkey test. The other data were analyzed with one-way ANOVA or two-tailed Student’s t test. The p values less than 0.05 were considered statistically significant.

Fig. 1. Characterization of streptavidin-conjugated CdSe/ZnS QD. A) TEM images of QDs. The right panel showed a higher magnification image of the indicated portion. B) The emission spectrum of QDs with an emission wavelength at 461 nm.

3. Results

3.1. QDs characterization

Streptavidin-conjugated CdSe/ZnS QD was employed in this work. The particle size of QDs, analyzed by TEM, was about 5 nm (Fig. 1A). As the size of QDs was tightly controlled, the resulting emission spectrum, near 534 nm, excited at 461 nm, was narrow and symmetric (Fig. 1B).

3.2. Cellular uptake of QDs

To qualify and quantify the intracellular uptake of the QDs, we monitored cellular fluorescence using fluorescence microscopy (Fig. 2A) and flow cytometry (Fig. 2B) respectively. HeLa cells incubated with 2 nM QD and 10 nM QD, with significantly increase in uptake as relative to the autofluorescence of cells were observed for both. Furthermore, according to the results of FACS analysis, the cellular uptake by QD reached 35.5% at 10 nm in HeLa cells, while only 2.83% of uptake showed at 2 nM of QD. These results suggested that QDs were internalized into the cell in a dose-dependent manner.

3.3. Autophagy induction by QDSs

LC3 is a specific marker to monitor autophagy. The cytoplasmic form LC3 (LC3 I) is lipidated and recruited to the autophagosomes during the autophagy activation. The resulted lipidated form of LC3, known as LC3 II, is attached to the autophagosome membrane [2]. To examine the autophagy–inducing ability of QDs, we incubated a HeLa cell line (HeLa-LC3), stably expressing GFP-LC3 fusion protein, with 2 nM or 10 nM QD for 24 h (Fig. 3A). As the autophagy up-regulated, diffusely presented GFP-LC3 accumulated on the autophagosome membrane from cytoplasm, resulting green punctuate...
dots in cells. Compared to evenly distributed green fluorescence in untreated control cells, a large number of green punctuate dots were observed in cells treated with 10 nM QD for 24 h. As a positive control, rapamycin, a classic autophagy inducer, also led to GFP-LC3 dots formation. The proportion of GFP-LC3 positive cells to total cells was counted. Nearly 49.8 ± 2.3% of cells showed the positive effect at 2 nM QD treatment, while 79.7 ± 2.4% cells at 10 nM QD (Fig. 3B; p < 0.01; 10 nM Vs 2 nM), suggesting a dose-dependent GFP-LC3 dots formation induced by QDs. These results demonstrated that QDs caused autophagy in HeLa cells, but it’s still unclear the autophagy-inducing ability of QDs in neurons. To solve this problem, we detected endogenously-expressed LC3 protein in cultured hippocampal neurons. After treatment with 10 nM QD for 24 h, conversion of LC3 I to LC3 II was determined in primary hippocampal neurons by immunofluorescence (Fig. 4A) and Western blot (Fig. 4B and C). As shown in Fig. 4A, multiple LC3 red puncta were observed in the QDs-treated neurons, indicating the accumulation of the endogenous LC3 protein on the autophagosomes by QDs. Autophagy is a dynamic flux with complete process to degrade the cargo. P62, an autophagic substrate, degraded by selective autophagy, was monitored in the neurons treated by QDs. Subsequently, we revealed that QDs could elicit an LC3-II conversion and down-regulation of P62 by Western blot (Fig. 4B and C). TEM further demonstrated many autophagosomes containing partially degraded cytoplasmic materials formation in neurons treated with QDs for 24 h (Fig. 4D). To determine autophagy-inducing ability of QDs in vivo, we performed intrahippocampally infusion without or with 20 nM QD (5 μl) for 2 h. QDs-induced autophagy of the hippocampus was confirmed by elevation in the conversion of LC3 I to LC3 II and reduction of the P62 protein level as seen by Western blotting and revealed quantitatively by the normalized LC3 II/GAPDH and P62/GAPDH ratio (Fig. 5A and B). These data altogether proved the induction of autophagy by QDs both in vitro and in vivo.

### 3.4. Intracellular oxidative stress involvement in QDs-induced autophagy

To illustrate the mechanism underlying in QDs-mediated autophagy, we measured ROS formation in HeLa cells, as determined by DCF fluorescence. ROS production was significantly increased by QD (10 nM) with compared to the control group. Treatment with the antioxidant GSH (1 mM) reduced the fluorescence of QDs-treated cells (Figure S1 A and B). We further determined the role of ROS in QDs-induced autophagy in the HeLa-LC3 cell line. Compared to the control cells, 1 mM GSH had no effect on GFP-LC3 dots formation, but significantly reduced the ability of QD-induced GFP-LC3 dots formation. Nearly 81.1 ± 1.6% of cells showed the positive effect at 10 nM QD treatment, while 16.1 ± 3.6% cells at QD plus GSH treatment (Figure S1 C and D; p < 0.005; QD Vs QD plus GSH). Thus our results suggested that increasing oxidative stress was involved in QDs-induced autophagy in the HeLa cells.

### 3.5. Autophagy involvement in synaptic impairment by QDs

To further test whether acute treatment of QDs alters synaptic function through autophagy modulation, we measured the I/O function, paired-pulse reaction (PPR) and LTP induction in the...
presence of autophagy inhibitor. After acute intrahippocampally applications of PBS ($n = 10$), QD (20 nM, $n = 12$), QD plus wortmannin (100 μM, $n = 10$) and QD plus 3-MA (100 μM, $n = 10$), I/O curves with a range of stimulus intensities (0.1–1.0 mA) revealed that QDs application significantly decreased the fEPSP slope when compared with the control (Fig. 6B, $p < 0.05$). Wortmannin and 3-MA, as PI3K-dependent autophagy inhibitors, partly abrogated the QDs-induced depression of fEPSP slope ($p < 0.05$). This suggested...
that QDs impaired the basic synaptic transmission in the hippocampal CA1 area through autophagic pathway. Using the double-pulse test with IPI ranging from 10 to 400 ms, we demonstrated that QDs also decreased the average peak facilitation at IPI = 80 ms (Fig. 6C and D, p < 0.05) contrary with the control, while there were no significant differences among the control, QD group and QD plus CQ group. Similarly, QD could significantly block QD-induced LC3-II conversion (Fig. 8C) and down-regulation of P62 (Fig. 8D) by Western blot as well. These results suggested that autophagy could play a crucial role in QDs-induced impairment of synaptic plasticity in the CA1 area in vivo.

3.5. Synaptic plasticity impairment by QDs

To investigate whether QDs have an effect on synaptic physiology, we assessed synapse density in the CA1 area of hippocampus using TEM. QDs treatment resulted in a reduction of synapse density compared to the control (QD, 0.307 ± 0.026; Control, 0.423 ± 0.018; p < 0.05), although the addition of wortmannin (QD, 0.307 ± 0.024; QD plus wortmannin, 0.413 ± 0.026; p < 0.05), although the addition of wortmannin alone decreased synapse density (Control, 0.423 ± 0.018; wortmannin, 0.286 ± 0.024; p < 0.05). In addition, the volume and cellular density of the hippocampus did not differ (data not shown). These results indicated that autophagy was associated with QDs-induced deficits of synapse density in the hippocampal CA1 area.

Fig. 5. QDs induced autophagy in vivo. The level of LC3-II (A) and P62 (B) in hippocampus of rats intrahippocampally infused with PBS or QD (20 nM) for 2 h was determined by Western blotting with LC3 antibody, P62 antibody and GAPDH served as loading control. The right panel of (A) and (B) showed the relative level of LC3-II/GAPDH and P62/GAPDH with the average value for control (PBS) set at 1. All data shows Mean ± SEM, n = 3 from three samples of different animals, ** indicates p < 0.01; *** indicates p < 0.001.

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**Fig. 6.** Effects of QDs on I/O curves, PPF in CA1 area. A) Photomicrographs illustrated the location of recording electrodes. A typical fEPSP evoked at the SC-CA1 synapse was shown at the right panel. B) I/O curves after acute intrahippocampally applications of PBS, QD (20 nM), QD plus wortmannin (100 μM) and QD plus 3-MA (100 mM). C) Paired-pulse ratio (PPR) of fEPSP slope after acute infusion of PBS, QD, QD plus wortmannin and QD plus 3-MA. D) PPR of fEPSP slope at inter-pulse intervals (IPI) of 80 ms after infusion of PBS, QD, QD plus wortmannin and QD plus 3-MA. * Indicates significant difference compared with control, \( p < 0.05 \). All data shows mean ± SEM.

**Fig. 7.** Effects of PI3K inhibitor on QDs-induced LTP impairment in the CA1 area. A) LTP was induced by HFS (5 trains of 20 pulses at 200 Hz separated by 1 s, repeated six times at intervals of 1 min) after treated with PBS, QD, QD plus wortmannin and QD plus 3-MA. Upper arrow indicates the application of HFS. B) Comparison of averaged fEPSP slope in 60 min after HFS after treated with PBS, QD, QD plus wortmannin and QD plus 3-MA. All data show mean ± SEM. ** indicates \( p < 0.01 \). C) Representative traces at special time point 1, 2 and 3. Bar indicates 2 mV and 10 ms respectively. LC3 (D) and P62 (E) level in the hippocampus infused with PBS, QD, wortmannin and QD plus wortmannin, were determined by Western blotting. The right panel of (D) and (E) showed the relative level of LC3-II/GAPDH and P62/GAPDH with the average value for control (PBS) set at 1. Mean ± SEM, \( n = 3 \) from three different samples, * indicates \( p < 0.05 \); *** indicates \( p < 0.001 \).

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4. Discussion

QDs, as powerful inorganic fluorescent probes, are now widely considered as materials for electronics, photonics and biomedical applications [43]. Due to its long-term, multiplexed imaging and detection, QDs have been developing into a promising platform for cancer detection and therapy, drug or micromolecular tracing [44]. However, a great deal of concerns about its potential safety have led to the need for comprehensive examination to elucidate whether and how QDs can induce cytotoxicity. Although cadmium-based QDs toxicity is mainly ascribed to metal ions release into the cell, mounting studies have demonstrated physico-chemical properties of QDs involved in its toxicity, including core/shell, size, shape, surface charge and coatings [45,46]. For instance, water-soluble InP/ZnS QDs were showed a much lower toxicity by in vitro and in vivo assessment [47,48]. Liu et al. showed that CdTe QDs coated with surfactant were more toxic than CdTe/ZnS core/shell QDs capped with polyethylene glycol (PEG) [29]. Other studies have shown that QDS surface charge can affect cytotoxicity, with positive QDs being more toxic than negative QDs [49]. In this work, we revealed the ability of streptavidin-coated CdSe/ZnS quantum dots (QD525) to induce autophagy in hippocampal neurons and synaptic impairment in the hippocampal CA1 area, and more importantly, we found that elevated autophagy participated in the process of synaptic impairment by QDs.

As many reported nanoparticles, QDs can induce autophagy in different cells in vitro [22,50,51]. Here we demonstrated it could elicit autophagy in the HeLa cells, hippocampal tissue and cultured neurons as well, accompanied with GFP-LC3 puncta and autophagosome formation, LC3-II expression and p62 degradation, (Figs. 3–5). We further observed that the intracellular uptake of QDs could disturb the oxidative balance and cause oxidative stress in HeLa cells. GSH was shown to eliminate ROS and block GFP-LC3 puncta formation by QDs (Figure S1). Thus our results implicated that oxidative stress involved in QDs-mediated autophagy.

The role of autophagy in synaptic plasticity is indeed intriguing. Autophagy protects the brain against the development of a number of neurodegenerative diseases. In Drosophila, it positively regulates synapse development [52]. The local pre-synaptic autophagy degrades synaptic vesicles and rapidly inhibits neurotransmitter release [10]. Recently, NMDAR-dependent autophagy has been shown to elicit AMPAR degradation through PI3K-Akt-mTOR pathway [12]. For assessing the role of autophagy in the QDs-induced synaptic dysfunction, we introduced three autophagy inhibitors, wortmannin, together with 3-MA and CQ in our experiments. Wortmannin and 3-MA, suppresses autophagy via inhibition of class III PI3K, which plays a crucial role at initiation of autophagy. We observed that both could inhibit QDs-induced autophagy and partly block QDs-induced LTP impairment (Figs. 6 and 7). Whereas, wortmannin alone could not only impair basic synaptic transmission (Figure S2 A and B) but suppress hippocampal LTP (Figure S2 C). This may be reflected from wortmannin-induced reduction of basal class I PI3K activity, which has been shown to participate in synaptic plasticity [39,40]. The other autophagy inhibitor, CQ, can inactivate lysosomal hydrolases through raising the lysosomes pH values, thus inhibit the autophagosome-lysosome fusion and cause accumulation of autophagosomes in cells [41]. Similarly, we also found that CQ inhibited autophagy induced by QD in hippocampus and alleviated QDs-induced LTP impairment (Fig. 8), while CQ alone had no effect on LTP expression due to its specially inhibition of autophagosome-lysosome fusion. In order to further verify the role of autophagy in synaptic dysfunction by QDs, we detected the synapsin-I level and spine synapse density (Figs. 9 and 10). Synapsin-I is associated with pre-synaptic neurotransmitter released by regulating synaptic vesicles for exocytosis, and LTP expression as well [53]. Spine synapse density is used to monitor the synapse number in a brain area. After wortmannin treatment, decreased level of synapsin-I and reduced number of spine synapse density by QDs in the CA1 subregion of the hippocampus could be partially recovered. These
results indicate that autophagy plays a part in the process of QDs-induced synaptic dysfunction. Additional studies are needed to clarify precisely how autophagy regulates dysfunction of synaptic activity. Our study may have significant implications for the biosafety of this classic engineered nanomaterial and provides a recommendation for designing QDs-based fluorescent labels that avoid autophagy induction.

5. Conclusions

In this study, we have explored the in vivo synaptotoxicity of QDs, and the role of autophagy in QDs-induced synaptic impairment. Increased autophagosome formation in the hippocampal CA1 area, accompanied by depressed synaptic plasticity and synapse density by QDs, may be involved in synaptic dysfunction. In brief, QDs induce ROS-mediated autophagy and trigger hippocampus-dependent synaptic impairment. Autophagy inhibition significantly reduces autophagic flux and ameliorates synaptic dysfunction by QDs in vivo. Therefore, this study may add new mechanisms for toxicity of nanoparticles, especially neurotoxicity, and suggest that autophagy-blocking reagents may serve as a potential remedial agent for synaptic impairment induced by QDs.

Conflict of interest statement

All authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.09.048.

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