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Toxicity of combined exposure of ZnO nanoparticles (NPs) and myricetin to Caco-2 cells: changes of NP colloidal aspects, NP internalization and the apoptosis-endoplasmic reticulum stress pathway

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Phytochemicals as typical food components may significantly influence the toxicity of nanoparticles (NPs) in intestinal cells, indicating a need to evaluate the toxicological effects of NPs in a complex situation. Previous studies suggested that the anti-oxidative properties of phytochemicals were important to elicit cytoprotective effects against NP exposure. However, we recently found that the changes of signaling pathways may be more important for cytoprotective effects of phytochemicals. In this study, we investigated the influence of myricetin (MY) on the cytotoxicity of ZnO NPs in Caco-2 cells and the possible mechanism. MY at 50 μ M showed minimal impact on the solubility and colloidal aspects of ZnO NPs, but protected Caco-2 cells from NP exposure as it increased the EC50 value. For comparison, dihydromyricetin (DMY; chemical analog of MY) increased the EC50 value to a much lesser extent. Exposure to ZnO NPs significantly induced intracellular Zn ions, whereas MY or DMY did not significantly influence the internalization of NPs. However, ZnO NPs significantly promoted the ratio of caspase-3/pro-caspase-3, which was inhibited by the presence of MY. Exposure to ZnO NPs did not significantly promote the biomarkers of endoplasmic reticulum (ER) stress, but co-exposure to ZnO NPs and MY significantly lowered the levels of a panel of ER stress biomarkers. In conclusion, these results suggested that MY could protect Caco-2 cells from ZnO NP exposure, which may not be related to the changes of colloidal stability or internalization of NPs but could be alternatively related to the reduction of ER stress leading to lower cleaved caspase-3.

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Introduction

Since nanoparticles (NPs) are increasingly used in the food industry for purposes of *e.g.* nutrition supplements,¹ food packaging² and anti-microorganisms,³ oral exposure to NPs has become a relevant issue in modern society and therefore the possible toxicity of NPs *via* oral exposure should be carefully evaluated.⁴ Recently, it has been suggested that nanotoxicological studies should consider the interactions between food components and NPs to better reflect the toxicity of NPs *via* oral exposure.^{5,6} Typical food components, such as proteins,⁷ lipids⁸ and sugars,⁹ have been shown to influence the colloidal stability of NPs and define the biological effects of

NPs *via* oral exposure both *in vivo* and *in vitro*. Still, since food varies largely in its components, more work is still needed to further investigate the influence of a specific food component on the toxicity of NPs.⁵

Recent studies suggested a need to further understand the influence of phytochemicals on the biological effects of NPs.¹⁰ Phytochemicals are small molecules derived from plants and have been used as food components in relatively large amounts due to their well-documented health effects.¹¹ For this reason, phytochemicals in food are likely to interact with NPs used in food products, and therefore it may be necessary to evaluate the influence of phytochemicals on biological effects of NPs.¹⁰ For instance, because phenolic compounds are anti-oxidants, Martirosyan *et al.*^{12,13} found that quercetin and kaempferol could partially inhibit the toxicity of Ag NPs to Caco-2 cells through the inhibition of oxidative stress. However, we recently showed that a panel of flavones and flavonols could inhibit the cytotoxicity of ZnO NPs to Caco-2 cells in a manner that was not dependent on the anti-oxidative properties of the phenolic compounds.¹⁴ Rather, further analysis suggested that

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phytochemicals might alter the cytotoxicity of ZnO NPs to Caco-2 cells through the modulation of autophagy and/or the endoplasmic reticulum (ER) stress signaling pathway.^{15,16}

We recently found that myricetin (MY), a typical flavonol, could effectively inhibit the toxicity of ZnO NPs to both Caco-2 cells and endothelial cells.¹⁴ MY is a flavonoid with well-documented beneficial effects, such as anti-oxidative, anti-cancer and anti-inflammatory properties; therefore it is widely used in food and food related products.^{17,18} ZnO NPs have been used in commercial products due to their well-documented antibacterial activities, which could lead to the contamination of NPs in food.^{19–21} Meanwhile, oral exposure to ZnO NPs could also occur when they are used as biomedical materials.²² Thus, co-exposure to MY and ZnO NPs is possible in real life. In this study, we investigated the possible cytoprotective effects of MY against ZnO NPs in Caco-2 cells. To this end, we focused on two aspects. First, we investigated the changes of colloidal aspects of ZnO NPs and the internalization of ZnO NPs into Caco-2 cells due to the presence of MY, because it has been suggested that NP–cell interactions could be changed due to the fact that phytochemicals alter the colloidal stability of NPs.⁵ For comparison, we also used dihydromyricetin (DMY), the chemical analog of MY, so the possible influence of chemical structures of phytochemicals could be evaluated.¹⁰ Second, we investigated the changes of the apoptosis-ER stress signaling pathway after combined exposure to ZnO NPs and MY. ER stress is an adaptive response to the dysfunction of ER, and alleviation of ER stress has been shown to promote the survival of cells under harsh conditions, including NP exposure,²³ and we recently found that 3-hydroxyflavone enhanced the cytotoxicity of ZnO NPs through the modulation of the apoptosis-ER stress signaling pathway.¹⁵

Materials and methods

Cell culture

Caco-2 cells were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured as we described elsewhere.²⁴

ZnO NP preparation and exposure

ZnO NPs (code XFI06; 20 nm) were purchased from Nanjing XFNANO Materials Tech Co., Ltd and have been thoroughly characterized as we reported earlier. X-ray diffractograms (XRD) suggest the hexagonal phase of NPs, and the average XRD size was calculated as 22.3 nm. The specific surface area was measured as 19.07 m² g^{−1}.²⁵ To make the suspension of ZnO NPs, 1.28 mg mL^{−1} NPs in 2% fetal bovine serum (FBS) were first sonicated for a total of 16 min with continuous cooling on ice using an ultrasonic processor FS-250N (20% amplitude; Shanghai Shengxi, China) and then diluted in full cell culture medium for exposure. To indicate the changes of colloidal properties of ZnO NPs due to the presence of MY and DMY, the hydrodynamic size, zeta potential and polydispersity index (PDI) of 25 µg mL^{−1} ZnO NPs with or without the pres-

ence of 50 µM MY or DMY were measured by using a Zetasizer nano ZS90 (Malvern, UK). Each sample was measured three times, and mean ± SD was calculated.

UV-Vis spectra

The UV-Vis spectra of 25 µg mL^{−1} ZnO NPs, 50 µM MY or DMY, and XFI06 plus MY or DMY were recorded by using an Agilent Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA), with MilliQ water used as the blank.

Cell counting kit-8 (CCK-8) assay

The cytotoxicity of ZnO NPs under different conditions was evaluated by the CCK-8 assay by using a commercial kit purchased from Beyotime (Nantong, China). The CCK-8 assay could reflect the activities of mitochondria in living cells. Briefly, Caco-2 cells were seeded at a density of 4 × 10⁴ per well on 24-well plates and exposed to 0, 6.25, 12.5, 25, 50 and 100 µg mL^{−1} ZnO NPs with or without the presence of 50 µM MY or DMY. After 24 h exposure, the CCK-8 assay was performed according to the manufacturer's instructions.

Transmission electron microscopy (TEM)

For ultrastructural observations, Caco-2 cells were seeded at a density of 5 × 10⁵ on 60 mm diameter cell culture Petri dishes and grown for 2 days before exposure to 25 µg mL^{−1} ZnO NPs, 25 µg mL^{−1} ZnO NPs plus 50 µM MY, and 25 µg mL^{−1} ZnO NPs plus 50 µM DMY. After 3 h exposure, the cells were observed under a TEM (JEM-1230, JEOL Ltd, Tokyo, Japan) as we previously described.²⁶

Atomic absorption spectroscopy (AAS)

AAS was used to determine the solubility of ZnO NPs in different suspensions. Briefly, 25 µg mL^{−1} ZnO NPs, 25 µg mL^{−1} ZnO NPs plus 50 µM MY, and 25 µg mL^{−1} ZnO NPs plus 50 µM DMY were aged for 24 h at 37 °C in a CO₂ incubator prior to centrifugation at 16 000g for 30 min. After centrifugation, the concentrations of total soluble Zn in the supernatants were measured by using an AA7000 AAS (Shimadzu CO., LTD, Japan) equipped with a Zn hollow cathode lamp as described.²⁷ The experiment was performed once with *n* = 4, and mean ± SD was calculated.

AAS was also used to measure the total cellular Zn elements in ZnO NP-exposed cells. Briefly, Caco-2 cells were seeded at a density of 2 × 10⁵ per well on 6-well plates and exposed to 100 µg mL^{−1} ZnO NPs with or without the presence of 50 µM MY or DMY. After 3 h, the cells were rinsed and dissolved in HCl. The total cellular Zn elements were then measured by using AAS.

Intracellular Zn ions

Caco-2 cells were seeded at a density of 1 × 10⁴ per well on 96-well black plates. The accumulation of intracellular Zn ions in Caco-2 cells after 3 h exposure to various concentrations of ZnO NPs with or without the presence of 50 µM MY or DMY was measured by using a fluorescent probe Zinquin ethyl ester (Sigma-Aldrich, USA) as we described elsewhere.²⁸

Real time RT-PCR

The mRNA levels of apoptosis genes (*CASP3* and *BCL2*), ER stress genes (*DDIT3*, *XBP-1s*, *ERN1*), and internal control *GAPDH* were determined by quantitative real time RT-PCR. Briefly, 2×10^5 per well Caco-2 cells were seeded on 6-well plates and grown for 2 days before exposure to $0 \mu\text{g mL}^{-1}$ (control), $25 \mu\text{g mL}^{-1}$ ZnO NPs or $25 \mu\text{g mL}^{-1}$ ZnO NPs plus $50 \mu\text{M}$ for 24 h. After exposure, total mRNA extraction (using TRI Reagent®, Sigma-Aldrich, USA), cDNA synthesis (using HiFiScript cDNA Synthesis Kit, Cwbiotech, Beijing, China) and quantitative real-time RT-PCR (using UltraSYBR Mixture, Cwbiotech, Beijing, China on PikoReal™ qPCR system, Thermo-Fisher, USA) were performed as we previously described.²⁶ The primers for each gene are summarized in Table 1. The mRNA levels were expressed as the ratio between the mRNA level of the target genes and the internal control gene.

Western blot

2×10^5 per well Caco-2 cells were seeded on 6-well plates and grown for 2 days before exposure to $0 \mu\text{g mL}^{-1}$ (control), $25 \mu\text{g mL}^{-1}$ ZnO NPs or $25 \mu\text{g mL}^{-1}$ ZnO NPs plus $50 \mu\text{M}$ for 24 h. After exposure, the protein level of caspase-3 (including pro-caspase-3 and cleaved caspase-3), BCL-2, chop, p-chop and IRE1 was determined by western blot as we described elsewhere.²⁹

Statistics

All the data were expressed as means $\pm$ SD of means of 3–5 independent experiments. Two-way ANOVA followed by the Tukey HSD test was performed using R 3.2.2. A p value < 0.05 was considered as statistically different.

Results

The changes of cytotoxicity

Fig. 1 shows the dose–response curve of ZnO NPs in different suspensions. The value of EC₅₀ of ZnO NPs is $25.12 \mu\text{g mL}^{-1}$, and was increased to $31.61 \mu\text{g mL}^{-1}$ in the presence of MY. In contrast, the presence of DMY only slightly increased the EC₅₀ value to $26.73 \mu\text{g mL}^{-1}$.

The interactions between ZnO NPs and MY/DMY

As shown in Fig. 2A, yellow color development was observed when ZnO NPs were mixed with MY. Fig. 2B shows the UV-Vis spectra of ZnO NPs in different suspensions. The suspensions of ZnO NPs and MY all have a peak of absorbance at approxi-

Fig. 1 The dose–response curves. Caco-2 cells were exposed to various concentrations of ZnO NPs (code XF106) for 24 h, and CCK-8 assay was performed to indicate cytotoxicity. The EC₅₀ values were calculated by using a four-parameter logistic model.

mately 370 nm, and the height of the peak was increased when ZnO NPs were mixed with MY. The suspension of DMY has a peak of absorbance at 290 nm, which was shifted to about 320 nm in the presence of ZnO NPs.

The result from AAS measurement as shown in Fig. 3 showed that ZnO NPs were partially soluble both in water and cell culture medium. The solubility of ZnO NPs was not obviously changed by MY or DMY.

Fig. 4 shows the size distribution of ZnO NPs in different suspensions, and Table 2 summarizes the changes of the hydrodynamic size, zeta potential and PDI of ZnO NPs under different conditions. The hydrodynamic size of ZnO NPs was increased due to MY but decreased due to DMY in water, but this difference was not obvious when ZnO NPs were suspended in medium. The absolute value of zeta potential of ZnO NPs in water was larger than 20 mV and was not obviously changed by the presence of MY or DMY. When suspended in medium, the absolute values of zeta potential of ZnO NPs with or without the presence of MY or DMY were decreased to be about 5 mV. The PDI of ZnO NPs in water was increased by both MY and DMY, with MY being more potent to increase the PDI of the ZnO NP suspension.

The internalization of XF106

TEM was used to visualize the internalization of ZnO NPs. As shown in Fig. 5, NPs were observed both in the nuclei and

Table 1 The forward (F-) and reverse (R-) primers used in this study

Gene names	F-primer	R-primer	Product length
<i>GAPDH</i>	ACAGCCTCAAGATCATCAGC	GGTCATGAGTCCTTCCACGAT	104 bp
<i>DDIT3</i>	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTTCATTCTC	116 bp
<i>XBP-1s</i>	CCGCAGCAGGTGCAGG	GAGTCAATACCGCCAGAATCCA	70 bp
<i>ERN1</i>	GCCTCCAACCACTCGCTCT	TCAAACATGCCCCGGTACACA	182 bp
<i>CASP3</i>	TTTGAGCCTGAGCAGAGACA	GGCAGCATCATCCACACATA	118 bp
<i>BCL2</i>	GGTGGGGTCATGTGTGTGG	CGGTTCAAGGTACTCAGTCATCC	89 bp

Fig. 2 A The color of suspensions of $25 \mu\text{g mL}^{-1}$ ZnO NPs (code XF106), $50 \mu\text{M}$ MY, $50 \mu\text{M}$ DMY, ZnO NPs plus MY and ZnO NPs plus DMY. B The UV-Vis spectra of $25 \mu\text{g mL}^{-1}$ code XF106, $50 \mu\text{M}$ MY, $50 \mu\text{M}$ DMY, XF106 plus MY and XF106 plus DMY.

Fig. 3 The solubility of ZnO NPs (code XF106) in different suspensions. $25 \mu\text{g mL}^{-1}$ XF106 was aged in water or medium with or without the presence of $50 \mu\text{M}$ MY or DMY for 24 h. After that, the concentrations of soluble Zn were determined by AAS.

cytoplasm of Caco-2 cells exposed to ZnO NPs (Fig. 5A), ZnO NPs plus MY (Fig. 5B) or ZnO NPs plus DMY (Fig. 5C). To quantitatively measure the internalization of ZnO NPs under different conditions, intracellular Zn ions and cellular Zn elements were determined. As shown in Fig. 6A, exposure to

Fig. 4 The size distribution of ZnO NPs (code XF106) with or without the presence of $50 \mu\text{M}$ MY or DMY.

all concentrations of ZnO NPs with or without the presence of MY or DMY significantly increased intracellular Zn ions compared with the control ($p < 0.01$), and ANOVA indicates no effect of MY or DMY ($p > 0.05$). As shown in Fig. 6B, the presence of MY or DMY did not significantly influence cellular Zn elements in ZnO NP-exposed Caco-2 cells ($p > 0.05$).

The activation of the apoptosis pathway

The expression of *CASP3* (Fig. 7A) was significantly increased by ZnO NPs with or without the presence of MY ($p < 0.01$), but exposure to ZnO NPs plus MY led to higher levels of *CASP3* expression compared with the exposure to ZnO NPs ($p < 0.05$). The expression of *BCL-2* (Fig. 7B) was only significantly decreased by ZnO NPs ($p < 0.05$) but not ZnO NPs plus MY ($p > 0.05$). The ratio of cleaved caspase-3/pro-caspase-3 (Fig. 7D) was significantly increased after exposure to ZnO NPs ($p < 0.01$), whereas exposure to ZnO NPs plus MY led to a significantly lower ratio of cleaved caspase-3 compared with the exposure to ZnO NPs ($p < 0.01$), although it was still higher compared with the control ($p < 0.05$). The protein levels of *BCL-2* (Fig. 7E) were significantly down-regulated by both ZnO NPs ($p < 0.05$) and ZnO NPs plus MY ($p < 0.01$), but exposure to ZnO NPs plus MY led to a significantly lower level of *BCL-2* compared with XF106 ($p < 0.05$).

ER stress pathway

The expression of *DDIT3* (Fig. 8A) and *XBP-1s* (Fig. 8B) was not significantly affected by XF106 ($p > 0.05$), whereas *ERN1* mRNA was significantly decreased by ZnO NPs ($p < 0.01$; Fig. 8C). The protein levels of chop (Fig. 8E), p-chop (Fig. 8F) and IRE1 (Fig. 8G) were all significantly decreased by ZnO NPs ($p < 0.01$). Exposure to ZnO NPs plus MY significantly down-regulated the mRNA levels of *DDIT3* ($p < 0.05$) and *ERN1* ($p < 0.01$) as well as protein levels of chop, p-chop and IRE1 ($p < 0.01$), and ZnO NPs plus MY led to significantly lower levels of *DDIT3* ($p < 0.05$), *ERN1*, chop and p-chop ($p < 0.01$) compared with

Table 2 The average hydrodynamic size, zeta potential and PDI of ZnO NPs (code: XF106) in different suspensions

	Suspensions	Hydrodynamic size (nm)	Zeta potential (mV)	PDI
ZnO NPs	Water	268.27 ± 0.67	−25.67 ± 0.25	0.123 ± 0.048
	Medium	111.80 ± 13.75	−6.38 ± 0.56	0.803 ± 0.153
ZnO NPs + MY	Water	432.50 ± 16.66	−23.77 ± 0.35	0.473 ± 0.081
	Medium	96.78 ± 8.06	−4.52 ± 0.74	0.864 ± 0.142
ZnO NPs + DMY	Water	179.53 ± 2.67	−26.83 ± 0.39	0.290 ± 0.023
	Medium	118.40 ± 8.13	−4.23 ± 0.31	0.727 ± 0.140

Fig. 5 The TEM images of Caco-2 cells exposed to ZnO NPs (code XF106) under different conditions. Caco-2 cells were exposed to 25 $\mu\text{g mL}^{-1}$ ZnO NPs with (A) or without the presence of 50 μM MY (B) or DMY (C) for 3 h. After exposure, TEM was used to visualize the internalized ZnO NPs (white arrows in the images). **Fig. 6** A The intracellular Zn ions. Caco-2 cells were exposed to various concentrations of ZnO NPs (code XF106) with or without the presence of 50 μM MY or DMY for 3 h. After exposure, the intracellular Zn ions were measured by using a fluorescent probe. *, $p < 0.01$, compared with the control. B The cellular Zn elements. Caco-2 cells were exposed to 100 $\mu\text{g mL}^{-1}$ ZnO NPs with or without the presence of 50 μM MY or DMY for 3 h. After exposure, the cellular Zn elements were measured by AAS.

the exposure to ZnO NPs alone. But for IRE1 protein, ZnO NPs plus MY also led to a relatively higher level of IRE1 protein compared with the exposure to ZnO NPs ($p < 0.01$).

Discussion

In this study, we investigated the interactions between MY and ZnO NPs and the possible mechanism associated with the cytoprotective effects of MY against ZnO NP exposure. It was shown that MY and its chemical analog DMY only had minimal impact on the changes of colloidal stability of ZnO NPs. Meanwhile, the internalization of ZnO NPs was not sig-

nificantly changed by MY or DMY. ZnO NPs activated the biomarkers of apoptosis, although ER stress biomarkers were not significantly elevated by ZnO NPs, whereas co-exposure to ZnO NPs and MY partially decreased the ratio of cleaved caspase-3/pro-caspase-3, accompanying a decrease of ER stress biomarkers. Thus, it is possible that the cytoprotective effects of MY against ZnO NPs are due to the changes of the ER stress-apoptosis signaling pathway rather than the changes of colloidal aspects of ZnO NPs or NP-Caco-2 cell interactions.

We first investigated the changes of colloidal properties of ZnO NPs due to the presence of MY or DMY by the measurement of the UV-Vis spectra, solubility, hydrodynamic size, zeta potential and PDI. The measurement of UV-Vis spectra is a

Fig. 7 The activation of the apoptosis pathway. Caco-2 cells were exposed to $25 \mu\text{g mL}^{-1}$ ZnO NPs (code XF106) with or without the presence of $50 \mu\text{M}$ MY for 24 h. After exposure, the expression of *CASP3* (A) and *BCL-2* (B) was determined by real time RT-PCR. The protein levels of cleaved caspase-3, pro-caspase-3 (D) and *BCL-2* (E) were determined by western blot. C shows the bands of each protein. *, $p < 0.05$, compared with the control; #, $p < 0.05$, compared with the cells exposed to XF106 only.

Fig. 8 The changes of the ER stress signaling pathway. Caco-2 cells were exposed to $25 \mu\text{g mL}^{-1}$ ZnO NPs (code XF106) with or without the presence of $50 \mu\text{M}$ MY for 24 h. After exposure, the expression of *DDIT3* (A), *XBP-1s* (B) and *ERN1* (C) was determined by real time RT-PCR. The protein levels of chop (E), p-chop (F) and IRE1 (G) were determined by western blot. D shows the bands of each protein. *, $p < 0.05$, compared with the control; #, $p < 0.05$, compared with the cells exposed to ZnO NPs only.

relatively simple method to indicate the interactions between NPs and biological molecules, and we and others showed before that biological molecules altered the UV-Vis spectra of ZnO NPs.^{25,27,30} Here, we observed that ZnO NPs, ZnO NPs plus MY and ZnO NPs plus DMY appeared to have different UV-Vis spectra, and ZnO NPs plus MY showed color development (Fig. 2). These results might indicate that the MY and DMY have a different interaction with ZnO NPs. However, other methods such as surface enhanced Raman scattering are needed to further verify the interactions between ZnO NPs and phytochemicals.³¹ In our preliminary experiments we attempted the measurement of fluorescence to indicate NP-MY/DMY interactions. But this was not successful due to the low levels of fluorescence from ZnO NPs and MY/DMY.

The results from this study suggested that MY/DMY might influence the colloidal stability of ZnO NPs but the effects were only minimal. For example, the solubility of ZnO NPs in water or medium was not obviously changed by the presence of MY or DMY (Fig. 3). The zeta potential of ZnO NPs was not obviously changed by MY or DMY either, whereas the hydrodynamic size and PDI of ZnO NPs were only increased by the presence of MY in water but not medium (Table 2). In our previous study, we already found that phytochemicals could influence the colloidal stability of ZnO NPs in a manner that is dependent on the chemical structures of phytochemicals.¹⁴ Previous studies from us and others also indicated that small molecules with different chemical structures in food could affect the colloidal stability of NPs differently.^{32–34} The

changes of colloidal properties of NPs in medium might significantly alter NP–cell interactions.³⁵ Here the results from this study showed that ZnO NPs were internalized into Caco-2 cells accompanying the increase of intracellular Zn ions, but all of these endpoints were not significantly affected by the presence of MY or DMY (Fig. 5 and 6). This may be due to the fact that MY and DMY only have minimal impact on colloidal properties of ZnO NPs. Since we found that MY, as well as DMY to much less extent, partially protected Caco-2 cells from ZnO NP exposure (Fig. 1), we suggested that MY might elicit cytoprotective effects by other mechanisms rather than the changes of NP–cell interactions.

We then focused on the apoptosis-ER stress pathway. We observed that co-exposure to MY and ZnO NPs led to a significantly reduced ratio of cleaved caspase-3/pro-caspase-3 compared with the exposure to ZnO NPs (Fig. 7). Meanwhile, all of the biomarkers of ER stress were significantly down-regulated after co-exposure to ZnO NPs and MY (Fig. 8). Previous studies showed that a panel of NPs could induce ER stress leading to activation of caspase-3 as the mechanism for NP-induced toxicity.^{36–38} Therefore, it is possible that the reduced ER stress levels after co-exposure to ZnO NPs and MY could contribute to the cytoprotective effects of MY. Recently we also found that cyanidin, although showing minimal impact on ZnO NP internalization, protected Caco-2 cells from ZnO NPs through the modulation of autophagy.¹⁶ Therefore, it is possible that phytochemicals might alter the toxicity of NPs through the modulation of the molecular signaling pathway rather than the changes of NP–cell interactions. It should be noticed that in this study we sonicated ZnO NPs in the presence of FBS to facilitate the dispersion of ZnO NPs, and it is expected that serum proteins could also affect the apoptosis-ER stress pathway as we reported before.³⁹ This could also explain why the cytoprotective effects of MY appeared to be less effective as we reported before.¹⁴

In summary, in this study we found that MY could protect Caco-2 cells from ZnO NP exposure not by changing the colloidal stability of NPs or NP–cell interactions but rather by modulating the apoptosis-ER stress pathway. These data highlighted a need to further investigate the changes of the molecular signaling pathway after co-exposure to NPs and phytochemicals.

Conflicts of interest

There are no conflicts to declare.

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