In Vitro Evaluation of the Toxicity of Cobalt Ferrite Nanoparticles in Kidney Cell

Kobalt Ferrit Nanopartiküllerinin Böbrek Hücresi Üzerine Güvenliğinin İn Vitro Değerlendirmesi

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ABSTRACT

Objectives: The remarkable properties of hard magnetic cobalt ferrite nanoparticles (CoFe₂O₄-NPs) and their physicochemical stability lead to various applications in different industrial and medical fields. Although CoFe₂O₄-NPs have been reported to cause toxic effects, there is a serious lack of information concerning their effects on the kidneys. In this study, it was aimed to investigate the toxic effects of CoFe₂O₄-NPs on NRK-52E kidney cells.

Materials and Methods: The particle characterisation and cellular uptake were determined using transmission electron microscopy, dynamic light scattering and inductively coupled plasma-mass spectrometry. Then, the cytotoxicity was evaluated by MTT and neutral red uptake assays, the genotoxicity by comet assay, and the apoptotic potentials by Annexin V-FITC apoptosis detection assay with propidium iodide.

Results: After 24 h exposure to CoFe₂O₄-NPs (39±17 nm), it was observed they did not affect the cell viability at concentration ranging from 100 to 1000 μg/mL, but significantly induced DNA damage at concentration ≤100 μg/mL. No apoptotic or necrotic effect was observed in the exposed cells.

Conclusion: According to the results obtained, CoFe₂O₄-NPs are promising for safe use in various applications. However, further in vivo studies are needed to fully understand their mechanisms of action.

Key words: DNA damage, cell death, apoptosis, cobalt ferrite nanoparticle

ÖZ

Amaç: Sert manyetik kobalt ferrit nanopartiküllerin (CoFe₂O₄-NP) dikkate değer özellikleri ve fizikokimyasal kararlılıkları farklı endüstri ve tip alanlarında çeşitli uygulamalarla kullanımlarına yol açmaktadır. CoFe₂O₄-NPs'lerin bazı toksik etkilere neden olduğu bildirilmiş olsa da böbrek üzerindeki etkileri hakkında ciddi bilgi eksikliği vardır. Çalışmanın amacı, CoFe₂O₄-NPs'lerin NRK-52E böbrek hücreleri üzerine toksik etki potansiyellerinin araştırılmasıdır.

Gereç ve Yöntemler: Partikül karakterizasyonu ve hücre alınması elektron mikroskobu, dinamik ışık saçılma ve plazma-mass spektrometri ile gerçekleştirildi. Sonra, sitotoksisite MTT ve neutral kırmızı alım testi, genotoksisite comet testi, apoptotik potansiyel propidium iyodürlü Annexin V-FITC apoptosis tayini ile değerlendirildi.

Bulgular: CoFe₂O₄-NPs (39±17 nm) 100-1000 μg/mL arasında değişen konsantrasyonlarda 24 saat süre ile maruz bırakılan böbrek hücrelerinde hücre canlanması etkilemediler, ancak ≤100 μg/mL de önemli ölçüde DNA hasarı meydana geldiği gözlemlemiştir. Maruz kalan hücrelerde apoptotik veya nekrotik etki gözlemmedi.

Sonuç: Elde edilen sonuçlara göre, CoFe₂O₄-NP'ler çeşitli uygulamalarda güvenli kullanım vaat etmektedir. Bununla birlikte, etki mekanizmalarının tam olarak anlaşılması için in vivo çalışmalara ihtiyaç vardır.

Anahtar kelimeler: DNA hasarı, hücre ölümü, apoptoz, kobalt ferrit nanopartiküller

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INTRODUCTION

Today nanoparticles are important issue of concern with their widely application in industrial and medical sectors because of their special properties, which cause dramatic increases in intentional and inadvertent oral, dermal and inhalational human exposure. Also, nanoparticles can found as contaminant in water, air, and bulky materials as a result of the natural incident such as volcanic eruptions.\textsuperscript{1,2} Research database provides that nanoparticles could cause DNA damage, cell death, oxidative stress and change cell function and morphology \textit{in vitro}, damages and changes in liver, kidney, gastrointestinal and neuronal systems \textit{in vivo}.\textsuperscript{3,4}

The exceptional features of cobalt based nanoparticles motivate their uses in different technologies like sensors, catalysts, pigments, and magnetism and energy storage devices.\textsuperscript{5,6} Because of the high physicochemical stability of cobalt ferrite nanoparticles (CoFe\textsubscript{2}O\textsubscript{4}-NPs), researchers also focus on using as drug carriers, anticancer treatment, and as magnetic resonance imaging contrast enhancement.\textsuperscript{7,8} However, some researchers have shown that CoFe\textsubscript{2}O\textsubscript{4}-NPs could cause oxidative damage, cell death and inflammatory responses in exposed mice, guinea pigs, zebrafish and human cell lines.\textsuperscript{10-14} Therewith, both \textit{in vitro} and \textit{in vivo} studies should be gradually carried out to get comprehensive toxicity profiles of nanoparticles to predict their effects on human. There is no study evaluating the effects of CoFe\textsubscript{2}O\textsubscript{4}-NPs or any other cobalt based nanoparticle on kidney. Therefore, we aimed to evaluate the toxic effects of CoFe\textsubscript{2}O\textsubscript{4}-NPs on kidney (NRK-52E) cells by \textit{in vitro} assays.

MATERIALS AND METHODS

CoFe\textsubscript{2}O\textsubscript{4}-NPs (CAT. No: 773352), neutral red dye and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) were obtained from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Dulbecco’s modified eagle medium (DMEM F-12), fetal bovine serum (FBS), phosphate buffered saline (PBS) and antibiotic solutions from Multicell Wisent (Quebec, Canada); Annexin V-FITC apoptosis detection kit with propidium iodide (PI) from Biolegend (San Diego, CA, USA); the other chemicals from Merck (NJ, USA) were purchased.

To particle size and distribution characterization, CoFe\textsubscript{2}O\textsubscript{4}-NPs were suspended in Milli-Q water and cell culture medium with 10% FBS, and measured by transmission electron microscopy (TEM) (Jem-2100 HR, JEOL, USA).\textsuperscript{15-17} The average hydrodynamic size of CoFe\textsubscript{2}O\textsubscript{4}-NPs in cell culture medium was determined by dynamic light scattering (DLS) (ZetaSizer Nano-ZS, Malvern Instruments, Malvern, UK). One mg CoFe\textsubscript{2}O\textsubscript{4}-NPs was dispersed in cell culture medium, and then the suspension was sonicated at room temperature for 15 min at 40 W. Ten μL of the suspension were diluted with cell culture medium to reach final concentration 10 μg/mL, and sonicated for further 5 min. Then, DLS experiments performed.

NRK-52E rat kidney proximal tubular epithelial cells (CRL-1571) were obtained from American Type Culture Collection (Rockville, MD, USA). The cells were incubated in DMEM-12 medium supplemented with FBS (%10) and 100 U/mL antibiotic solution at 5% CO\textsubscript{2}, 90% humidity and 37°C for 24 h. The cell densities were from 1x10\textsuperscript{4} to 1x10\textsuperscript{6} cells/mL. CoFe\textsubscript{2}O\textsubscript{4}-NPs were freshly suspended at 1 mg/mL concentration in cell culture medium with 10% FBS and sonicated at room temperature for 15 min to avoid the aggregation/agglomeration of the nanoparticles before exposure.\textsuperscript{15,16} The exposure times to the particle suspensions were 24 h.

The cellular uptake of nanoparticle was evaluated with inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Elemental X series 2, USA). After exposure to 200 μg/mL of nanoparticles, the cells were washed several times with equal volumes of PBS and counted by Luna cell counter (Virginia, USA).\textsuperscript{15,16} The acid-digested samples were assayed for Co amount with ICP-MS. Also, Co content of the untreated cells for every cell line was measured.

The cytotoxic potentials of CoFe\textsubscript{2}O\textsubscript{4}-NPs were determined by MTT and neutral red uptake (NRU) assay based on different cellular mechanisms.\textsuperscript{15,16,18,19} The cell exposed final concentrations of 0-100 μg/mL. Optical density was read at 590 and 540 nm for MTT and NRU, respectively, using a microplate spectrophotometer system (Epoch, Germany). In every assay, the untreated cells were evaluated as negative control. It was calculated the inhibition of enzyme activity observed in cells compared with untreated (negative control) cells. Results were expressed as ratio of negative control.

The genotoxic potentials of CoFe\textsubscript{2}O\textsubscript{4}-NPs were determined by comet assay.\textsuperscript{15,16,20,21} The cell exposed final concentrations of 0.1-100 μg/mL. Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) (100 μM) and PBS were used as positive and negative controls, respectively. Briefly, the cells were layered on microscope slides coated with agarose gel. The slides were incubated for 1 h at 4°C in lysis solution (2.5 M NaCl, 100 mM EDTA, and 10 mM tris-HCl, pH 10), added with 10% DMSO and 1% Triton X-100. Then, DNA was unwound for 20 min in cold-fresh electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) at 4°C and electrophoresis was performed at 4°C for 20 min (20 V / 300 mA). After electrophoresis, slides were neutralized with 0.4 M tris-HCl buffer (pH 7.5) 3 times for 5 min. The number of DNA breaks were scored under a fluorescent microscope (Olympus BX53, Olympus, Tokyo, Japan) at 400 magnification using an automated image analysis system (Comet Assay IV, Perceptive Instruments, Suffolk, UK). DNA damage to individual cells was expressed as a percentage of DNA in the comet tail (tail intensity %).

Annexin V-FITC apoptosis detection kit with PI was used to evaluate the cellular apoptosis or necrosis.\textsuperscript{7,15} In every assay, negative controls and blank were evaluated. The cell exposed final concentrations of 0.1-100 μg/mL. The apoptotic or necrotic cells, distributed on the slides, were immediately counted at 400 magnification under a phase-contrast fluorescent microscope (Olympus BX53, Olympus, Tokyo, Japan). Results were expressed as percent of the total cell amount.

All experiments were done in triplicates and each assay as repeated four times. Data (n=12) was expressed as mean± standard deviation. The significance of differences between
the untreated and treated cells with the nanoparticles was
calculated by one-way ANOVA Dunnett t-test using SPSS
version 17.0 for Windows (SPSS Inc., Chicago, IL). p values
of less than 0.05 were selected as the levels of significance.

RESULTS

The aim of this study is to evaluate the toxicity profiles of
CoFe$_2$O$_4$-NPs in NRK-52E kidney cells that could simulate
specific target organ or system affected by occupational and
environmental exposure to nanoparticles.

According to TEM images, the average size of CoFe$_2$O$_4$-NPs
was 39±17 nm with narrow size distribution after dispersing in
water (Figure 1). The nanoparticles slightly agglomerated and/
or aggregated after dispersing in the culture medium, and their
average sizes (range) increased to 101.5 nm (32.6 to 157.1 nm).
The average hydrodynamic size of CoFe$_2$O$_4$-NPs was evaluated
by DLS technique. The nanoparticle size was 183.6 nm (ranging
from 5.6-342.1 nm), and 52% of the particles had a size lower
than 33.6 nm. In addition, the cellular uptake of CoFe$_2$O$_4$-
NPs was evaluated using ICP-MS. Results confirmed that
nanoparticles were taken into the cells. Cobalt concentration
was 8.3 μg/mL/10$^5$ cell compared to the negative control.

In the evaluation of their cytotoxic potential, it was shown that
CoFe$_2$O$_4$-NPs did not decrease the cell viability at concentration
≤1000 μg/mL (Figure 2). Annexin V-FTIC apoptosis detection
assay with PI was used to assess the cell death pathway. The
maximum levels of apoptotic and necrotic induction were 4.02
and 2.25 fold, respectively. The induction level was statistically
significant at 100 μg/mL. Our results showed that apoptosis
could be the main cell death pathway in kidney NRK-52E cells
exposed to CoFe$_2$O$_4$-NPs (Figure 3).

As to Comet assay results, CoFe$_2$O$_4$-NPs could be genotoxic
because it was observed an increase in tail intensity, and induced
DNA damage. The increase in DNA damage was significant in
the range of 10-100 μg/mL, and occurred in a concentration-
dependent manner (p<0.05). At the highest concentration of
CoFe$_2$O$_4$-NPs (100 μg/mL), the tail intensity was approximately
1.7-fold of the negative control. In the positive controls (100 μM
H$_2$O$_2$), the tail intensity was 16.9 (Figure 4).

DISCUSSION

CoFe$_2$O$_4$-NPs toxicity still controversial since the previous
studies have contrary estimations. Horev-Azaria et al.$^{13}$
investigated the in vitro toxicological effects of CoFe$_2$O$_4$-NPs
on lung (A549 and NCIH441), liver (HepG2), kidney (MDCK),
testin (Caco-2 TC7), and lymphoblast (TK6) cells in the
concentration range of 11.7-281.5 mg/mL. They reported that
CoFe$_2$O$_4$-NPs produced no toxic effects in all cell types at ≤46.9
mg/mL. In that study, a significant decrease in viability was

Figure 2. Effects of CoFe$_2$O$_4$-NPs on cell viability as assayed by MTT and NRU
All experiments were done in triplicates and each assay was repeated four
times, The results are expressed as mean
NRU: Neutral red uptake

Figure 3. Evaluation of the apoptosis- and necrosis-inducing potentials
of CoFe$_2$O$_4$-NPs as assayed by Annexin V-FTIC apoptosis detection assay
with propidium iodide, Results are presented as percentage of the total
cell amount, All experiments were done in triplicates and each assay was
repeated four times, The results are presented as meanstandard deviation.
*p≤0.05 were selected as the levels of significance by one-way ANOVA
Dunnett t-test
observed in NCIH441, HepG2, MDCK, and Caco-2 TC7 cells after 72 h, while there was no cytotoxic effect on A549 and TK6 cells even after 24 h of exposure.

Marmorato et al. reported CoFe$_{2}$O$_{4}$-NPs caused interference with lipid metabolism in Balb/3T3 cells depending on concentration. In another study, CoFe$_{2}$O$_{4}$-NPs were observed to have a weakly embryotoxic effect with an IC$_{50}$ value of 243.91 and 20.05 mg/mL in mouse 3T3 fibroblast and D3 embryonic stem cell lines, respectively. Human glioblastoma-astrocytoma (U87MG) cells were observed to have peculiar features including a white corona around the nucleus and other morphological changes after exposure to CoFe$_{2}$O$_{4}$-NPs at 58 and 235 mg/mL for 24 h. They suggested CoFe$_{2}$O$_{4}$-NPs caused cellular stress, and indicated the vesicles appeared to be lipid droplet organelles.

The genotoxicity of CoFe$_{2}$O$_{4}$-NPs was evaluated by studying the interaction with Salmon sperm DNA. It was reported the interaction between CoFe$_{2}$O$_{4}$-NPs and nucleic acid occurred, and the linkage was based on a coordination interaction of the phosphate groups and the oxygen atoms on the heterocyclic bases of DNA on the particle surface. Also, Ahmad et al. pointed out the genotoxicity of CoFe$_{2}$O$_{4}$-NPs. Similarly, Colognato et al. reported the induction of genotoxicity in human peripheral lymphocytes exposed those CoFe$_{2}$O$_{4}$-NPs.

CONCLUSION

In conclusion, CoFe$_{2}$O$_{4}$-NPs did not show cytotoxic potentials on the kidney cells, whereas only their highest concentration induced DNA damage. The intensity of toxicological effects of nanoparticles could be varied among different cell lines. In light of the results and previous researches, low but effective concentrations of CoFe$_{2}$O$_{4}$-NPs could be evaluated to be used safely in biomedicine, electronic, magneto-optic, sensor, data storage, catalysis and microwave applications. However, in vivo studies should be carried out to fully understand the mechanism of CoFe$_{2}$O$_{4}$-NPs toxicity.

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REFERENCES

1. Dhawan A, Sharma V. Toxicity assessment of nanomaterials: methods and challenges. Anal Bioanal Chem. 2010;398:589-605.
2. Kim YJ, Yu M, Park HO, Yang SI. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by silica nanomaterials in human neuronal cell line. Mol Cell Toxicol. 2010;6:337-344.
3. Arora S, Rajwade JM, Paknikar KM. Nanotoxicology and in vitro studies: the need of the hour. Toxicol Appl Pharmacol. 2012;258:151-165.
4. Brooking J, Davis SS, Ilium L. Transport of nanoparticles across the rat nasal mucosa. J Drug Target. 2011;19:267-279.
5. Alarifi S, Ali D, Verma A, Alakhtani S, Ali BA. Cytotoxicity and genotoxicity of copper oxide nanoparticles in human skin keratinocytes cells. Int J Toxicol. 2013;32:296-307.
6. Alinovi R, Goldoni M, Pinelli S, Campanini M, Aliatis I, Bersani D, Lottici PP, Iavicoli S, Petyx M, Mozzoni P, Mutti A. Oxidative and pro-inflammatory effects of cobalt and titanium oxide nanoparticles on aortic and venous endothelial cells. Toxicol In Vitro. 2015;29:426-437.
7. Maaz K, Mumtaz A, Hasanaei SK, Ceylan A. Synthesis and magnetic properties of cobalt ferrite (CoFe2O4) nanoparticles prepared by wet chemical route. J Magn Magn Mater. 2007;308:289-295.
8. Di Guglielmo C, López DR, De Lapuente J, Mallafre JM, Suarez MB. Embryotoxicity of cobalt ferrite and gold nanoparticles: a first in vitro approach. Reprod Toxicol. 2010;30:271-276.
9. Amiri S, Shokrollahi H. The role of cobalt ferrite magnetic nanoparticles in medical science. Mater Sci Eng C Mater Biol Appl. 2013;33:1-8.
10. Ahmad F, Yao H, Zhou Y, Liu X. Toxicity of cobalt ferrite (CoFe2O4) nanobeads in Chlorella vulgaris: interaction, adaptation and oxidative stress. Chemosphere. 2015;139:479-485.
11. Gianoncelli A, Marmorato P, Ponti J, Pascolini L, Kaulich B, Uboldi C, Rossì F, Makovec D, Kiskinova M, Ceccone G. Interaction of magnetic nanoparticles with U87MG cells studied by synchrotron radiation X-ray fluorescence techniques. X-Ray Spectrom. 2013;42:316-320.
12. Matsuda S, Nakanishi T, Kaneko K, Osaka T. Synthesis of cobalt ferrite nanoparticles using spermine and their effect on death in human breast cancer cells under an alternating magnetic field. Electrochim Acta. 2015;183:153-159.
13. Horev-Azaria L, Baldi G, Beno D, Bonacchi D, Golla-Schindler U, Kirkpatrick JC, Kolle S, Landsiedel R, Maimon O, Marche PN, Ponti J, Romano R, Rossi F, Sommer D, Ubolfi C, Unger RE, Villiers C, Korenstein R. Predictive toxicology of cobalt ferrite nanoparticles: comparative in vitro study of different cellular models using methods of knowledge discovery from data. Part Fibre Toxicol. 2013;10:32.
14. Hwang DW, Lee DS, Kim S. Gene expression profiles for genotoxic effects of silica-free and silica-coated cobalt ferrite nanoparticles. J Nucl Med. 2012;53:106-112.
15. Abudayyak M, Alincekic T, Özhan G. In vitro toxicological assessment of cobalt ferrite nanoparticles in several mammalian cell types. Biol Trace Elem Res. 2017;175:458-465.

Figure 4. Evaluation of DNA damage potentials of CoFe$_{2}$O$_{4}$-NPs as assayed by comet assay

All experiments were done in triplicates and each assay was repeated four times. The results are presented as mean tail intensity (%) with ±standard deviation, NC and PC mean negative and positive controls, respectively. *p≤0.05 were selected as the levels of significance by one-way ANOVA.
16. Uzar NK, Abudayyak M, Akcay N, Algun G, Özhan G. Zinc oxide nanoparticles induced cyto- and genotoxicity in kidney epithelial cells. Toxicol Mech Methods. 2015;25:334-339.

17. Chattopadhyay S, Dash SK, Tripathy S, Das B, Mandal D, Pramanik P, Roy S. Toxicity of cobalt oxide nanoparticles to normal cells: an in vitro and in vivo study. Chem Biol Interact. 2015;226:58-71.

18. Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat Protoc. 2008;3:1125-1131.

19. Van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. Methods Mol Biol. 2011;731:237-245.

20. Collins AR. The comet assay for DNA damage and repair principles, applications, and limitations. Mol Biotechnol. 2004;26:249-261.

21. Speit G, Hartmann A. The comet assay (single-cell gel test): a sensitive genotoxicity test for the detection of DNA damage and repair. Methods Mol Biol. 1999;113:203-212.

22. Marmorato P, Ceccone G, Gianoncelli A, Pascolo L, Ponti J, Rossi F, Salomé M, Kaulich B, Kiskinova M. Cellular distribution and degradation of cobalt ferrite nanoparticles in Balb/3T3 mouse fibroblasts. Toxicol Lett. 2011;207:128-136.

23. Mariani V, Ponti J, Giudetti G, Broggi F, Marmorato P, Gioria S, Franchini F, Rauscher H, Rossi F. Online monitoring of cell metabolism to assess the toxicity of nanoparticles: the case of cobalt ferrite. Nanotoxicology. 2012;6:272-287.

24. Pershina AG, Sazonov AE, Novikov DV, Knyazev AS, Izaak TI, Itin VI, Naiden EP, Magaeva AA, Terechova OG. Study of DNA interaction with cobalt ferrite nanoparticles. J Nanosci Nanotechnol. 2012;11:2673-2677.

25. Colognato R, Bonelli A, Bonacchi D, Baldi G, Migliore L. Analysis of cobalt ferrite nanoparticles induced genotoxicity on human peripheral lymphocytes: comparison of size and organic grafting-dependent effects. Nanotoxicology. 2009;1:301-308.