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ARAŞTIRMA / RESEARCH

In vitro effects of iron chelation of curcumin Fe (III) complex

Kurkumin demir (III) kompleksinin demir şelasyonunun in vitro etkileri

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Abstract

Purpose: The aim of this study was to investigate the cytotoxicity effect, iron chelator and antioxidant activities of iron (III) ions with curcumin ligand that may be used in the treatment of iron overload.

Materials and Methods: The cytotoxic activities of the ligand and the complex were evaluated by the MTT assay. The SOD activity of the complex of curcumin was determined by using its ability to inhibit the reduction of NBT. The catalytic activity studies of Fe(III) complex in DMSO towards the disproportionation of hydrogen peroxide were also performed.

Results: The IC50 values are found in 6.8 µM catalase activity was measured. Where at a concentration of 2.0 mM, the activity was equivalent to 183.30 U/L. The complex shows a catalase activity. The complex showed minimal toxicity. IC50 values found 5.3 mg/ml. The observed cytotoxicity could be pursued to obtain a potential drug. The iron chelator effects were determined by Ferrozine reagent. Curcumin, the most active extract interfered with the formation of ferrous and ferrozine complex. It demonstrated strong chelating activities. The result showed that the complexes possess considerable SOD activity. This finding indicates that the iron complex is capable of removing free radicals.

Conclusion: The study results revealed that the iron(III) complex of curcumin with an appropriate potential drug may act as a protector against oxidative stress. Therefore, all results suggest that curcumin may represent a new approach in the treatment of iron overload.

Keywords: Curcumin, iron overload, iron(III)

Öz

Amaç: Bu çalışmanın amacı, aşırı demir yüklenmesinin tedavisinde kullanılması muhtemel olan kurkumin ligandı ve demir(III) iyonlarının sitotoksik etkisini, demir şelatörünü ve antioksidan etkinliğini araştırmaktır.

Gereç ve Yöntem: Ligandın ve kompleksin sitotoksik etkileri MTT yöntemi kullanılarak değerlendirildi. Kurkumin kompleksinin SOD etkinliği, kompleksin NBT azaltımını inhibe etme kabiliyetine göre belirlendi. Buna ilaveten, demir(III) kompleksinin DMSO'daki hidrojen peroksitin disproporsiyonu reaksiyonuna yönelik katalitik etkinliği de çalışıldı.

Bulgular: IC50 değerleri, 6.8 µM katalaz etkinliğinde ölçüldü. Konsantrasyonun 2.0 mM, olduğu durumda etkinlik seviyesi 183.30 U/L olarak ölçüldü. Kompleksin katalaz etkinliği gösterdiği ve minimal seviyede toksisiteye sebep olduğu görüldü. IC50 değerlerinin, 5.3 mg/ml'ye denk geldiği görüldü. Gözlenen sitotoksisitenin takip edilmesiyle, potansiyel bir ilacın elde edilmesinin muhtemel olduğu görüldü. Demir şelatörün etkileri Ferrozin reaktif bileşiği ile belirlendi. Demir ve Ferrozine kompleksinin oluşmasına müdahale eden en aktif ekstraktın kurkumin olduğu görüldü. Ayrıca, kurkuminin güçlü şelasyon etkinliğine sahip olduğu görüldü. Elde edilen bulgular bu komplekslerin önemli derecede SOD etkinliğine sahip oldukları görüldü. Dolaysıyla bu bulgular demir kompleksinin serbest radikalleri yok etme gücüne sahip olduğuna işaret etmektedirler.

Sonuç: Bu çalışmada elde edilen bulgular, uygun bir potansiyel ilaç ile beraber kullanıldığında demir(III) kurkumin kompleksinin oksidatif strese karşı bir koruyucu olarak işlev görebileceğini göstermektedir. Dolayısıyla elde edilen bütün bulgular, kurkuminin aşırı demir yüklenmesinin tedavisinde yeni bir yaklaşım olabileceğine işaret etmektedirler.

Anahtar kelimeler: Kurkumin, demir yüklenmesi, demir(III)

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INTRODUCTION

Iron is an essential micronutrient incorporated into proteins responsible for cellular respiration, survival, and growth. At the systemic level, iron is required as a component of heme, including in hemoglobin and myoglobin, which are vital for the delivery and storage of oxygen. Iron is an essential element for cellular metabolism with a major role in redox cycling. Since it functions both as an electron donor and an acceptor, iron is also a co-factor in the active site of several key enzymes via critical biochemical pathways including ATP generation, oxygen transport, cell cycling, and DNA synthesis[1,2,3].

Iron overload could be defined as an increase of storage iron. Iron overload disorders represent a heterogenous group of conditions resulting from inherited and acquired causes. Iron can accumulate in human in a variety of conditions, including congenital, systemic iron-loading conditions (hereditary hemochromatosis), conditions associated with systemic macrophage iron accumulation (hemolytic conditions, transfusions, etc), in some hepatitidies (hepatitis C), and liver-specific iron accumulation of uncertain pathogenesis in cirrhosis[4,5]. Excess iron forms insoluble complexes that are deposited in, and cause damage to, internal organs[6].

Although iron is an essential component of life, an excessive amount may become extremely toxic to the human body both in its ability to generate reactive oxygen species such as the hydroxyl radical[7]. The highly reactive hydroxyl radical is able to induce cell death through initiating a series of chemical reactions, resulting in DNA oxidation, mitochondrial damage and the peroxidation of membrane lipids[8,9].

Iron overload can be largely prevented by the use of iron specific chelation ligands [10,11]. Currently, three iron chelators are licensed for clinical use. Of these, Desferrioxamine B, a naturally occurring trihydroxamic acid derived from cultures of Streptomyces pilosus, is the only drug currently available for clinical use as an iron chelator Desferoxamine is highly expensive and poorly absorbed from the gastrointestinal tract, and these disadvantages limit its regular use in the clinic[3,12].

Over the past twenty years there has been a growing interest in the orally active iron chelators, deferiprone and exjade, both having been extensively studied. Deferiprone has been the first orally active iron chelator used in clinical practice. Deferiprone, a typical 3-hydroxypyridine-4-one (HPO), has emerged as a prominent therapeutic, able to remove accumulated excess iron from the heart. Although the oral administration route is associated with better patient compliance deferiprone therapy may be associated with several adverse effects. These drugs are extremely useful in the treatment of iron overload, but they too present some disadvantages. Accordingly, a new avenue is required to provide more effective treatment with lesser side effects to patients with iron overload[13-16].

Curcumin is the main constituent of the Curcuma longa. It is a natural phenolic compound which has antioxidant, antibacterial and anti-inflammatory properties. Curcumin can chelate numerous metal ions and form metal curcumin complexes[17-21]. It is shown that the coordination of metal ions, and so forth, with bioactive ligands can actually improve the pharmaceutical activity of drugs. Unfortunately, the chelation of iron(III) in the iron overload of curcumin action has been very less investigated[22-24].

Today, some drugs are extremely useful in the treatment of iron overload. These drugs are extremely useful in the treatment of iron overload, but they too present some disadvantages, which make urgent the need for new chelation agents more suitable from a clinical point of view [25]. The aim of this study was to investigate the cytotoxicity effect, iron chelators and antioxidant activities of iron (III) ions with curcumin ligand that may be used in the treatment of iron overload.

MATERIALS AND METHODS

Iron chelating activity

The chelation of ferrous ions by sample was estimated by method of Dinis et al. It was measured as described previously, by adding 0.1 mM $FeSO_4$ (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of curcumin. After incubating at room temperature for 10 min, absorbance of the solution was there after measured at 562 nm.

Chelating activity was calculated using the following formula: Iron chelating activity = $(A_{control} - A_{sample})/ A_{control} \times 100$. Where A control is the absorbance of control reaction (without curcumin), and A sample is the absorbance in the presence of a curcumin.

Superoxide dismutase (SOD) activity

The superoxide activity of the complex was determined by using its ability to inhibit the reduction of chromophoric nitrotetrazolium blue choride (NBT). Briefly, superoxide radicals were generated *in situ* by the xanthine/ xanthine oxidase system, and they subsequently react with the NBT. They were followed spectrophotometrically by measuring the absorbance at 560 nm. Complex with SOD activity inhibit reaction by abstracting the superoxide reactant. Therefore SOD performance of a complex can be given as IC50 value. This value is concentration of the complex where inhibition of the reaction NBT → NBT-diformazan is 50%.

Catalase activity

Catalase activity was measured using Biodiagnostic Kit which is based on the spectrophotometric method described by Aebi. The catalases-like activities of different concentrations of complex was done by reacting with known quantity of H2O2 catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-Dichloro-2- hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm.

Cytotoxicity assay (Cell culture and MTT assay)

Cytotoxic effects of the compounds on the HUVEC cell lines were evaluated by the MTT assay. The cells were cultured in a humidified atmosphere with 5% CO2 at 37 °C. After 24 h of the incubation of the cells, the medium was replaced with 100 μL medium containing various doses of ligand and complex (50, 10, 5, 1, 0.1, 0.01 mg/ml). After 72 h of the treatment, cell viability was determined by the MTT assay. The absorbance was measured at 570 nm on a microplate reader (Elisa 2100C). Cell proliferation was calculated as the ratio of absorbance in the treated group divided by the absorbance in the control group, multiplied by 100 to give a percentage proliferation. IC50 is defined as the concentration of an agent inhibiting cell survival by 50% compared with the treated control. The IC50 values were calculated from the dose curves by a computer program (CalcuSyn).

Statistical analysis

Statistical analyses were performed by SPSS statistical software. Data were analyzed using one way ANOVA and expressed as mean ± SD. The data are expressed as mean ± standard deviation in triplicate for each experimental point. The percentages of cell growth were used to obtain the full dose response curves and to determine the IC50 values (concentration inhibiting of 50% the cell growth compared with control). The IC50 values were calculated from the dose curves.

RESULTS

Due to its ability to undergo cyclic oxidation and reduction, iron generates reactive oxygen species (ROS). In the presence of molecular oxygen, the "loosely-bound" iron is able to undergo redox cycling between its two most stable oxidation states, namely iron (II) and iron (III), thereby generating oxygen-derived free radicals such as hydroxyl radicals. Hydroxyl radicals are highly reactive and capable of interacting with most types of biological molecules [5,7]. We tested my curcumin in a iron chelating assay. Tested in the concentration range of 1 to 10 mg/mL. Curcumin, the most active extract interfered with the formation of ferrous and ferrozine complex. It demonstrated strong chelating activities. In this study We reported for the first the strong iron chelating activity of curcumin (IC50= 0.46, 95% CI (Confidence Interval)= 0.39-0.43 mg/ml).

Firstly, the cytotoxic potential of ligand and complex was investigated in HUVEC endothelial cells by the colorimetric MTT assay. The cells were exposed to different concentrations of complex for 72 h at 37°C. The data were calculeted % cytotoxicity formula and expressed as mean ± S.D. against HUVEC cell line is summarized in Table 1. It showed minimal toxicity. IC50 values found 5.3 mg/ml.

The SOD activity of iron(III) complex of curcumin was determined by using its ability to inhibit the reduction of NBT. The SOD activity of the complex prepared in this thesis were measured and IC50 and Kcat values. In this colorimetric based assay, superoxide is generated by xhantine-xhantine oxidase enzyme The superoxide then converts NBT to formazan, a colored product that absorbs light at 560 nm. The result showed that the complexes possess considerable SOD activity with IC50 value 6.8 μM.

The catalytic activity studies of Fe(III) complex in DMSO towards the disproportionation of hydrogen peroxide were also performed. The studies showed that complex is catalytically active. Where at a concentration of 2.0 mM, the activity was equivalent to 183.30 U/L. The complex shows an catalase activity It may constitute a new and interesting basis for the future search of new potential drugs.

Table 1. Cytotoxicity results for HUVEC cell lines (mean ± standard deviation of n=8 experiments).

Cell line	50 mg/ml	10 mg/ml	5 mg/ml	1 mg/ml	0.1 mg/ml	0,01 mg/ml
HUVEC	56.30± 9.55	39.16± 3.85	18.35± 15.62	9.43±12.75	-12.10±6.25	-7.45± 6.98
Control (DMSO)	7.25 ± 11.30	2.25± 3.12	-2.56± 5.15	-4.85± 6.42	2.55± 3.52	1.95± 3.45

DISCUSSION

Iron overload is a serious clinical condition which can be largely prevented by the use of iron specific chelating agents. The failure to find the ideal iron chelator can be ascribed to inherent difficulties deriving from the biological and clinical restraints. Curcuminoids are organic compounds existing in turmeric, a popular spice. Curcumin is (bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) a yellow spice and pigment from Curcuma longa. While curcumin has been well-recognized as a compound and extensively studied in experimental cancer models, e.g. in the therapeutic models of Alzheimer, the chelation of iron(III) in the biological activity of curcumin has been studied in a limited number of studies.

Curcumin can chelate various metal ions and form metal curcumin complexes. Curcumin is also promising because it can bind iron. Unfortunately, the chelation of iron(III) in the iron overload of curcumin action has been very less investigated[25-30].

We investigated that coordination of iron(III) ions with curcumin ligand that may be used in the treatment of iron overload. Curcumin reacted with iron in high concentrations at physiological pH at room temperature. Subsequently, a brown-red complex was formed. Data regarding magnetic susceptibility showed that the complexes with a 1:2 (metal/ligand) mole ratio had octahedral geometry. The complex showed higher anti-oxidant effect towards the cell line ECV304 at IC50 values of 4.83 compared to curcumin. Electrochemistry studies showed that Fe^{3+}/Fe^{2+} couple redox process occurred at low potentials. This value was within the range of compounds that are expected to show superoxide dismutase activity. This finding indicates that the iron complex is capable of removing free radicals. The observed cytotoxicity could be pursued to obtain a potential drug[3].

In this study, the cytotoxic activities of the ligand and the complex were evaluated by the MTT assay. The SOD activity of the complex of curcumin was determined by using its ability to inhibit the reduction of NBT. The catalytic activity studies of Fe(III) complex in DMSO towards the disproportionation of hydrogen peroxide were also performed. The cytotoxic activities of the ligand and the iron(III) complex were studied on the HUVEC cells using the MTT viability test. It showed minimal toxicity. An IC50 value was found 5.3 mg/ml. The observed cytotoxicity could be pursued to obtain a potential drug. The catalytic activity studies of iron (III) complex in DMSO towards the disproportionation of hydrogen peroxide were also performed. Where at a concentration of 2.0 mM, the activity was equivalent to 183.30 U/L. The complex shows a catalase activity It may constitute a new and interesting basis for the future search for new and more potent drugs. When the coordinate iron(III) ion is present in the structure, the catalytic reactivity greatly enhances. The SOD activity of iron(III) complex of curcumin was determined by using its ability to inhibit the reduction of NBT. The IC50 values are found in 6.8 μM. The iron(III) complex of curcumin shows a significant increase in the SOD activity was found. The chelation of ferrous ions by the sample was estimated by the method of Dinis et al. curcumin demonstrated strong chelating activities. In this study, we reported for the first the strong iron chelating activity of curcumin.

In conclusion, our study confirms previous reports on the therapeutic potential of curcumin. My results confirm the hypothesis that curcumin acts as an iron chelator. We in-vitro results demonstrated that curcumin has the potential to exhibit a positive effect on iron overload. Therefore, our results suggest that

curcumin may represent a new approach in the treatment of iron overload.

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REFERENCES

1. Sun, CC. Vaja V, Babitt JL, Lin HY. Targeting the hepcidin–ferroportin axis to develop new treatment stra-tegies for anemia of chronic disease and anemia of inflammation. Am J Hematol. 2012;87:392-400.
2. Kim A, Nemeth E. New insights into iron regulation and erythropoiesis. Curr Opin Hematol. 2015;22:199–205.
3. Özbolat G, Yegani AA, Tuli A. Synthesis, characterization and electrochemistry studies of iron(III) complex with curcumin ligand. Clin Exp Pharmacol Physiol. 2018;45:1221-6.
4. Siah CW, Ombiga J, Adams LA, Trinder D, Olynyk JK. Normal iron metabolism and the pathophysiology of iron overload disorders. Clin Biochem Rev. 2006;27: 5–16.
5. Batt KP. Iron overload syndromes and the liver. Modern Pathology. 2007;20:S31–9.
6. Lindsey WT, Bernie PD, harm RD. Deferasirox for transfusion-related iron overload: a clinical review. Clin Ther. 2007;29:2154-66.
7. Özbolat G, Tuli A. Iron chelating ligand for iron overload diseases. Bratisl Med J. 2018;119:308–11.
8. Shander A, Cappellini MD, Goodnough L. Iron overload and toxicity: the hidden risk of multiple blood transfusions. Vox Sang. 2009;97:185–97.
9. Ehteram H, Bavarsad MS, Mokhtari M. Prooxidant-antioxidant balance and hs-CRP in patients with beta-thalassemia major. Clin Lab. 2014;60:207-15.
10. Jordan LB, Vekeman F. Sengupta A. Persistence and compliance of deferoxamine versus deferasirox in edicaid patients with sickle-cell disease. J Clin Pharm Ther. 2012;37:173-181.
11. Tam T.F, Leung-Toung R, Li W, Wang Y, Karimian K, Spino M. Iron chelator research: past, present and future. Cur Med Chem. 2003;10:983.
12. Nisbet-Brown E, Olivieri NF, Giardina PJ, Grady RW, Neufeld EJ et al. Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomized, double-blind, placebocontrolled, dose-escalation trial. Lancet. 2003;361:1597-602.
13. Jordan LB, Vekeman F. Sengupta A, Corral M, Guo A, Duh MS.. Persistence and compliance of deferoxamine versus deferasirox in Medicaid patients with sickle-cell disease. J Clin Pharm Ther. 2012;37:173-81.
14. Kalinowski DS, Richardson DR. The evolution of iron chelators for the treatment of iron overload disease and cancer. Pharmacol Rev. 2005;57:547-83.
15. Zhang J, Hou X, Ahmad H, Zhang H, Zhang L, Wang T. Assessment of free radicals scavenging activity of seven natural pigments and protective effects in AAPH-challenged chicken erythrocytes. Food Chem. 2014;145:57–65.
16. Crisponi G, Nurchi VM, Zoroddu MA. Iron chelating agents for iron overload diseases. Thalasemia Reports. 2014;4(2).
17. Aggarwal BB. Sundaram C. Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv Exp Med Biol. 2007;595:1-75.
18. Mei X. Luo X. Xu S, Xu D, Zheng Y, Xu S et al. Gastroprotective effects of a new zinc(II)-curcumin complex against pylorus-ligature-induced gastric ulcer in rats. Chem Biol Interact. 2009;181:316–21.
19. Jiao Y. Wilkinson J. Pietsch EC, Buss JL, Wang W, Planalp R et al. Iron chelation in the biological activity of curcumin. Free Radic Bio Med. 2006;40:1152–60.
20. Banerjee R. Inhibitory effect of curcumin-cu(II) and curcumin-Zn(II) complexes on amyloid-beta peptide fibrillation. Bioinorg Chem Appl. 2014;2014:325873.
21. Kocaadam B. Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Crit Rev Food Sci Nutr. 2017;57:2889-95.
22. Messne DJ, Sivam G, Kowdley KV. Curcumin reduces the toxic effects of iron loading in rat liver. Liver Int. 2009;29:63–72.
23. Oberley LW. Spitz DR. Greenwald RA. Handbook for Methods for Oxygen Radicals Research. Boca Ratón, FL, CRC Press, 1986.
24. Khalil M.I., Al-Zahem A., Al-Qunaibi MH. Synthesis, characterization, mössbauer parameters, and antitumor activity of fe(III) curcumin complex. Bioinorg Chem Appl. 2013;2013:982423..
25. Zhoua T, Hider RC, Liu ZD, Neubert H. Iron(III)-selective dendritic chelators. Tetrahedron Lett. 2004;45:9393-6.
26. Lebda M.A. Acute iron overload and potential chemotherapeutic effect of turmeric in rats. Int J Pure App Biosci. 2014;2:86-94.
27. Barik A, Mishra B, Kunwar A. Comparative study of copper(II)-curcumin complexes as superoxide dismutase mimics and free radical scavengers. Eur J Med Chem. 2007;42:431-9.
28. Messne DJ, Sivam G, Kowdley KV. Curcumin reduces the toxic effects of iron loading in rat liver.

Liver Int. 2009;29:63–72.
29. Gupta SC, Patchva S, Aggarwal BB. Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J. 2013;15:195–218.
30. EL-Maraghy SA. Rizk SM. El-Sawalhi MM. Hepatoprotective potential of crocin and curcumin against iron overload-induced biochemical alterations in rat. Afr J Biochem Res. 2003;5:215-21.