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Short communication

SHORT COMMUNICATION

Application of UV–Vis spectrophotometric and chemiluminescent methods for the evaluation of the antioxidant action of curcumin

STANCHO STANCHEV[1]*, IVANKA PENCHEVA[2], SPIRO KONSTANTINOV[3], DANKA OBRESHKOVA[4] and VERA HADJIMITOVA[5]

[1]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, 2 Flemingovo namesti, CZ-16610 Prague 6, Czech Republic, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, 2 Dunav St. BG-1000 Sofia,Bulgaria, [3]Department of Pharmacology and Toxicology, Faculty Pharmacy, Medical University ,2 Dunav St., BG-1000 Sofia, Bulgaria, [4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, 2 Dunav St. BG-1000 Sofia and [5]Department of Physics and Biophysics, Faculty of Medicine, Medical University, 2 Zdrave St. BG-1431 Sofia, Bulgaria

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Abstract: Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5--dione) is a natural biologically active substance with antioxidant activity. The ability of curcumin to inhibit free radical reactions can be used in the prevention of diseases such as cancer and coronary heart disease. UV–Vis spectrophotometric and chemiluminescent dynamic methods for determination of the antioxidant activity of curcumin were developed. The spectrophotometric method includes investigation of the interaction between DNA, isolated from HL-60 cells, and curcumin. The decreasing absorption of curcumin in the presence of HL-60 DNA against the blank sample can be a measurement for some complex formation between curcumin and DNA. The chemiluminescent method involves three tests for the detection of luminol- dependent chemiluminescence based on model systems that generate superoxide, hydroxide and hypochlorite radicals. The strongest decay of chemilunimescence was registered at the highest concentration of curcumin (100 μmol L^{-1}).

Keywords: curcumin; antioxidant; UV–Vis spectrophotometry; DNA complexation; chemiluminescence.

INTRODUCTION

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the principal curcuminoid of the popular spice *Curcuma longa* L. (turmeric),

* Corresponding author. E-mail: stancho_stanchev@yahoo.com
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which is a member of the ginger family (Zingiberaceae). The curcuminoids are polyphenols and are responsible for the yellow color. Curcumin (Fig. 1) can exist in at least two tautomeric forms - keto and enol. The enol form is more energetically stable in the solid phase and in solution.[1]

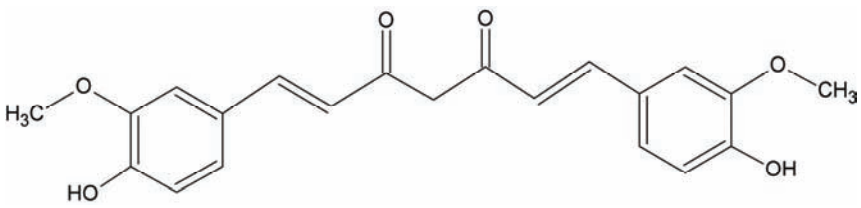

Fig. 1. Chemical structure of curcumin.

In vitro and animal studies suggested a wide range of potential therapeutic or preventive effects associated with curcumin on numerous diseases, including multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, Alzheimer's disease, gastric ulcer and dermal wound healing and liver protection.[2–9] Curcumin has been tested in the clinical stage for anticancer activity – phase 1 and it showed very low toxicity (even at a dosage of 12 g day^{-1}).[10] The major problem is its very low bioavailability dues to poor absorption and rapid metabolism and elimination. Many methods were used, in order to increase its bioavailability, such as combination with piperine, which interferes with glucoronidation, and application as liposomes and nanoparticles.[11]

The antioxidant properties of curcumin were evaluated by different methods (mainly spectrophotometric and spectrofluorimetric), including the determination of thiobarbituric acid reactive substances (TBARS), as a result of lipid peroxidation caused by AAPH (2,2'-azobis (2-amidinopropane) hydrochloride),[12] the decrease in the reduction of Tetrazolnitro Blue to formazane caused by superoxide radicals, generated in the xantine/xantine oxidase system, and the scavenging of the DPPH radical, based on the decrease in the absorption of the stable radical at 517 nm.[13–14] The other methods are based on electronic paramagnetic resonance.[15–16]

A mononuclear (1:1) copper complex of curcumin was synthesized and examined for its superoxide dismutase (SOD) activity. The complex was found to be regenerated completely, indicating catalytic activity in neutralizing superoxide radicals. The SOD mimicking activity of the complex was determined by the xanthine/xanthine oxidase assay, from which it was found that 5 μg of the complex is equivalent to 1 unit of SOD. The complex inhibited radiation-induced lipid peroxidation and showed radical-scavenging ability.[14]

Curcumin is using as a supplement and food additive because of its broad antioxidant activity for the prevention of different diseases caused by the overproduction of free radicals. As a food additive, its E number is E100. The aim of

this study was to develop a spectrophotometric and chemiluminescent method for the evaluation of its antioxidant activity in different model systems, which could find application in the quality control of drugs and food additives containing curcumin.

EXPERIMENTAL

Collection of cells from an HL-60 cell culture

5×10^6 cells with the ordinary number of chromosomes were centrifuged for 5 min at 300 rpm in 1.5 mL centrifuge test tubes. The supernatant was removed carefully in order to keep the cell pellets intact.

Liberation of DNA from the collected cells

Collected cell pellets are suspended in phosphate buffer up to a final volume of 200 μL. 20 μL of QIAGEN-protease (protease K) and 200 μL buffer AL were added to the suspension. The mixture was vortexed for 15 s in order to obtain homogenous suspension, followed by incubation at 56 °C for 10 min. The suspension was centrifuged shortly in order to remove the drops from the inner side of the lid. Ethanol (200 μL, 96–100 %) was added to the sample and the latter was vortexed for 15 s and then centrifuged shortly, in order to remove the drops from the lid.

Purification of DNA by QIAmp ion-exchange column

A QIAmp DNA Blood Mini Kit (QIAGEN, UK), consisting of proteolytic enzymes (lyophilized QIAGEN-protease) and a series of buffer solutions (buffer AL, buffer AW1, buffer AW2), was used for the step-by-step purification of DNA from leukemic HL-60 cells using an ion-exchange column QIAmp (QIAGEN).

The mixture, obtained in the previous step was loaded onto a QIAamp-rotating column (with a 2 mL collection tube), without wetting of the edge of the column. The whole system was centrifuged at 6000 rpm for 1 min. The collection test-tube was changed with a clean one.

The column was eluted in the same manner subsequently with 500 μL of buffer AW1 and 500 μL of buffer AW2. The centrifugation of the buffer AW2 was performed at 20000 rpm for 3 min. The final centrifugation was performed at full rate for 1 min., in order to remove the residual quantities of buffer AW2 in a new test tube. The final elution was performed with 200 μL of distilled water, followed by incubation for 1 min and centrifugation at 6000 rpm for 1 min. The yield of the isolated DNA was around 6 μg.

Analysis of the isolated DNA by UV–VIS spectrophotometry

Two samples of DNA were prepared as aqueous solutions, 5 and 20 μL mL^{-1}. These solutions were analyzed on Hewlet-Packard UV–Vis spectrophotometer (Hewlet-Packard, USA, with a diode array matrix with a 1 cm cell). This spectrophotometer was connected with PC and the data were collected and processed by integrated software.

The absorptions (A_1 and A_2) were measured at the wavelengths 248 and 280 nm and the factor of resolution was calculated as the ratio between A_1 and A_2 at λ_1 and λ_2.

UV–Vis spectrophotometric assay of the interaction between DNA and curcumin

Two samples of curcumin reference substance (Fluka, Switzerland) were prepared in Eppendorf tubes: sample 1 – containing 100 μL DNA, 400 μL distilled water and 5 μL 10000 M solution of curcumin, sample 2 – containing 495 μL distilled water and 5 μL 10000 M solution of curcumin (blank sample).

Samples 1 and 2 were incubated 24 h at room temperature. Sample 1 was filtered through paper filter and subsequently diluted to 3 mL with distilled water. The spectrophotometric analysis of this sample was made before and after filtration and dilution at λ = 422 nm. The absorption of sample 2 (blank sample) was measured at 422 nm after dilution to 3 mL with distilled water.

Chemiluminescent assay

For registration of active oxygen species, a luminol-dependent chemiluminescence was detected using a chemiluminometer LKB 1251 (Bioorbit, Turku, Finland). The chemiluminometer was connected with IBM computer system (IBM, USA) and the obtained data were collected and analyzed with the MultiUse program version 1.08 (Bioorbit). All tests were made at a fixed temperature of 37 °C. The chemiluminescence responses of all the systems described below were measured. The ratio in percentage between the magnitude of the chemiluminescence in the presence and the absence of curcumin was termed chemiluminescence-scavenging index (*CL-SI*).

Xanthine/xanthine oxidase system. This test for the determination of the luminol-dependent chemiluminescence is based on the model system in which superoxide is generated from xanthine/xanthine oxidase: To 1 mL PBS (0.1 mmol L^{-1} luminol and 1 mmol L^{-1} xanthine), curcumin in different concentrations in DMSO was added. In parallel, blank samples were prepared without curcumin. After 10 min incubation at 37 °C to 20 μL xanthine oxidase (100 IU L) were added to the samples and the chemiluminescence was measured against reference sample for 5 min.

Fe–ethylenediaminetetraacetic acid(EDTA)–H_2O_2 system. This test for the determination of the luminol-dependent chemiluminescence is based on the model system in which hydroxide radicals are generated from Fe-EDTA, ascorbic acid and peroxide. Luminol (100 μmol L^{-1}), Fe^{3+} (100 μmol L^{-1} and EDTA, 100 μmol L^{-1}), ascorbic acid (100 μmol L^{-1}) and peroxide (1 mmol L^{-1}) were dissolved in 1 mL phosphate buffer of pH 7.4 (50 mM K_2HPO_4/ /KH_2PO_4). Curcumin in the three different concentrations as a DMSO solution was added. The chemiluminescence was measured against the reference sample for 1 min.

Hypochlorite system. This test for the determination of luminol-dependent chemiluminescence is based on the model system in which hypochlorite ions are generated. To 1 mL phosphate buffer of pH 7.4 (50 mM K_2HPO_4/KH_2PO_4), luminol (100 μmol L^{-1}), sodium hypochlorite (30 μmol L^{-1}) and curcumin in the investigated concentrations were added. The chemiluminescence was measured against the reference sample for 1 min.

RESULTS AND DISCUSSION

UV–Vis spectrophotometric evaluation of the interaction between DNA and curcumin

Purity of DNA, isolated from HL-60 cells by the above-described procedure was evaluated by UV–Vis spectrophotometry. Two samples, containing DNA with concentration respectively 5 μL DNA/mL water and 20 μL DNA/mL water were analyzed. The absorbances (A_1 and A_2) were measured at the wavelengths 248 and 280 nm and the factor of resolution was calculated. Factor of resolution was measured as the ratio between A_1 and A_2 at 248 and 280 nm respectively, which is an indicator for purity of DNA and normally must be between 1.7 and

1.9. The measured value in our case is 1.86, which corresponds to the requirement of purity.

The absorption maxima of the complex DNA–curcumin were at 248, 250, 252, 260 and 422 nm. The range of the minimal overlapping between DNA and curcumin is the visible area and for this reason, all the measurements were made at 422 nm. The absorption of the complex DNA–curcumin decreased by 24.5 % and after filtration by 66 %, compared to the blank sample 1. The decrease of the absorbance (resp. concentration) of curcumin in the sample containing DNA and curcumin, compared to the blank sample, containing only curcumin, can serve as proof for an interaction between DNA and curcumin. This complex was probably formed by hydrogen bonds and/or van der Waals interactions between phenolic hydroxyl groups and the keto-groups of curcumin and the keto-, amino- and imino-groups of the nucleotide bases.

Chemiluminescent assay

The most significant decay of the luminal-dependent chemiluminescence in the three tested systems: xanthine–xanthine oxidase, generating superoxide radicals, Fe–EDTA/H_2O_2, generating hydroxyl radicals, and NaOCl, generating ClO^-, was at the highest concentration (100 μmol L^{-1}), referred to DMSO (Fig. 2). There was no significant decrease in the chemiluminescence between the lowest

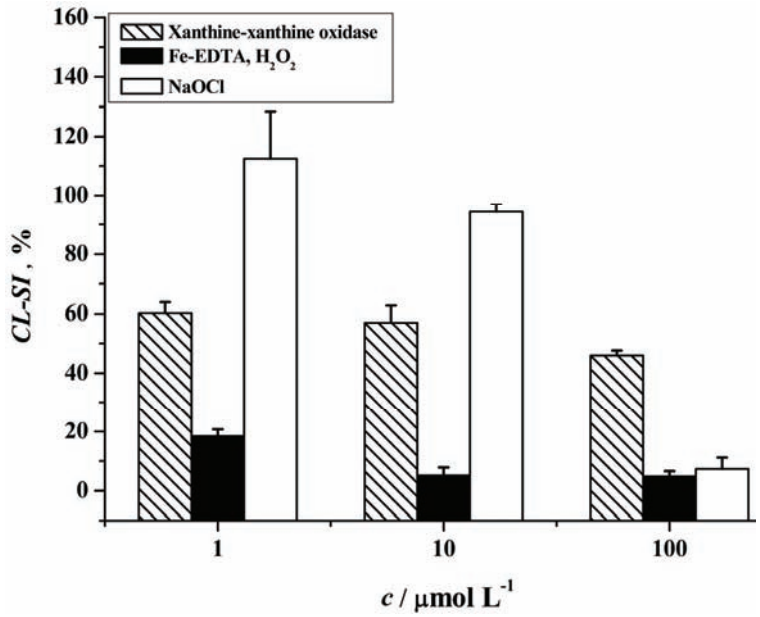

Fig. 2. Chemiluminescent scavenging index (*CL-SI*) as a marker of the antioxidant effect of curcumin in the three model systems: xanthine–xanthine oxidase, Fe–EDTA–H_2O_2 and NaOCl at the three concentrations *c* in μmol L^{-1}.

(1 μmol L^{-1}) and the middle concentration (10 μmol L^{-1}) for the systems xanthine–xanthine oxidase and NaOCl. In the Fe–EDTA/H_2O_2 model system, the decay of chemiluminescence at the highest (100 μmol L^{-1}) and the middle concentration (10 μmol L^{-1}) were relatively similar.

The most important functional groups from a structural point of view are conjugated double bonds, phenol and methoxy groups. Double bonds are probably oxidized and form epoxides and/or diols, especially under the influence of superoxide and hydroxyl radicals. The ClO^- can probably cause the formation of chlorohydrins at the double bonds of curcumin. Phenols have expressed redox properties, which can explain the neutralization of free radicals, singlet oxygen species and peroxides. Methoxy groups also have a free radical scavenging activity.

CONCLUSIONS

Three different model systems generating free radicals, *i.e.*, xanthine–xanthine oxidase, Fe–EDTA/H_2O_2 and NaOCl, were used in the investigation of the antioxidant properties of curcumin, based on the decay of luminol-dependent chemiluminescence. The decay was most expressed at the highest concentration, 100 μmol L^{-1}, of curcumin.

The developed methods could be very useful in the qualitative and quantitative control of the natural antioxidant curcumin in medicine and in the drug and food industries.

The interaction between DNA and curcumin was also investigated by UV–Vis spectroscopy. The obtained results evidenced some interaction between curcumin and DNA, probably based on hydrogen bonding and van der Waals interactions. This "complex" deserves further investigation, because it can be a part of the mechanism of protection of DNA against free radical damage.

ИЗВОД

ПРИМЕНА UV–Vis СПЕКТРОФОТОМЕТРИЈСКЕ И ХЕМОЛУМИНИСЦЕНТНЕ МЕТОДЕ У ЕВАЛУАЦИЈИ АНТИОКСИДАНТНОГ ДЕЈСТВА КУРКУМИНА

STANCHO STANCHEV[1], IVANKA PENCHEVA[2], SPIRO KONSTANTINOV[3], DANKA OBRESHKOVA[4] и VERA HADJIMITOVA[5]

[1]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, 2 Flemingovo namesti, CZ-16610 Prague 6, Czech Republic, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, 2 Dunav St. BG-1000 Sofia, Bulgaria, [3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Medical University ,2 Dunav St., BG-1000 Sofia, Bulgaria, [4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, 2 Dunav St. BG-1000 Sofia Bulgaria и [5]Department of Physics and Biophysics, Faculty of Medicine, Medical University, 2 Zdrave St. BG-1431 Sofia, Bulgaria

Куркумин (1,7-бис(4-хидрокси-3-метоксифенил)-1,6хептадиен-3,5-дион) је природна, биолошки активна супстанца са антиоксидантним дејством. Својство куркумина да инхибира механизме формирања слободних радикала користи се у превенцији

канцера и срчаних оболења. Развијене су УВ–ВИС спектрофотометријска и хемолуминисцентна динамичка метода за одређивње антиоксидантне активности куркумина. Спектрофотометријска метода укључује испитивање интеракција између ДНК, изоловане из ХЛ-60 ћелија, и куркумина. Мери се смањење апсорбанције формираних комплекса ХЛ-60 ДНК и куркумина у односу на слепу пробу. Хемолуминисцентна метода укључује три теста за детекцију луминола, на основу модел система који генерише супероксид, хидроксид и хипохлорит радикале. Највеће опадање интензитета хемолуминисценције је регистровано на концентрационом нивоу од 100 μmol L^{-1} куркумина.

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REFERENCES

1. T. Kolev, E. Valcheva, B. Stamboliiska, M. Spiteller, *Int. J. Quantum Chem.* **102** (2005) 1069
2. H. Hatcher, R. Planalp, J. Cho, F. Torti, S. Torti, *Cell. Mol. Life Sci*. **65** (2008) 1631
3. S. Swarnakar, K. Ganguly, P. Kundu, A. Banerjee, P. Maiti, A. Sharma, *J. Biol. Chem.* **280** (2005) 9409
4. M. Panchatcharam, S. Miriyala, V. Gayathri, L. Suguna, *Mol. Cell Biochem.* **290** (2006) 87
5. F. Marotta, Y. Shield, T. Bamba, Y. Naito, E. Minelli, E. Yoshioka, *Chin. J. Digestive Diseases* **4** (2003) 122
6. K. Maiti, K. Mukherjee, A. Gantait, B. Saha, P. Mukherjee, *Int. J. Pharm.* **330** (2007) 155
7. B. Aggarwal, S. Shishodia, *Biochem. Pharmacol*. **71** (2006) 1397
8. H. Choi, Y. Kim, S. Kim, M. Park, J. Wan, *Mol. Pharmacol*. **70** (2006) 1664
9. J. M. Matés, J. A. Segura, F. J. Alonso, J. Márquez, *Curr. Med. Chem*. **18** (2011) 2315
10. G. Sa, T. Das, S. Banerjee, J. Chakraborty, *Al Ameen J. Med. Sci*. **3** (2010) 21
11. F. Yen, T. Wu, C. Tzeng, L. Lin, C. Lin, *J. Agric. Food Chem.* **58** (2010) 7376
12. A. Banerjee, A. Kunwar, B. Mishra, K. Priydarsini, *Chem. Biol. Interact.* **174** (2008) 134
13. F. Bonte, M. Noel-Hudson, J. Wepierre, A. Maybeck, *Planta Med.* **63** (1997) 265
14. A. Barik, B. Mishra, L. Shen, H. Mohan, R. M. Kadam, S. Dutta, H. Y. Zhang, K. I. Priyadarsini, *Free Radic. Biol. Med*. **39** (2005) 811
15. H. Ahsan, N. Parveen, N. Khan, S. Hadi, *Chem. Biol. Interact.* **12** (1999) 161
16. K. Das, C. Das, *Biochem. Biophys. Res. Commun.* **295** (2002) 62.