CdTe QUANTUM DOTS MODIFIED BY GLUTATHIONE AS A LABEL FOR MONITORING OF DNA INTERACTIONS WITH PLATINUM-BASED CYTOSTATICS

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Abstract

Cancer, a plaque of 21st century, is a leading cause of a death in developed countries [1]. There are several treatment strategies including chemotherapy based on using of platinum cytostatics. Cisplatin, carboplatin and oxaliplatin represent three generations of Pt-based compounds applied successfully in clinical practice. As a consequence of the employment of Pt-based cytostatics in the cancer treatment, it became necessary to study the mechanism of their main activity - formation of Pt-DNA adducts. The mechanism of their formation is still unclear, but nanomaterials as progressively developing branch, can offer a tool for study the interactions of these drugs with DNA. The fluorescence intensity of QDs can be significantly quenched by the presence of metals [2] and therefore in this work, fluorescent CdTe nanocrystals - quantum dots (QDs, λ_{em} = 525 nm) were employed to study interactions of platinum cytostatics (cisplatin, carboplatin and oxaliplatin) with DNA fragment (500 bp, c = 25 μg/ml). First, fluorescent behavior of QDs in the presence of platinum cytostatics was monitored and major differences in the interaction of QDs with tested drugs were observed. It was found that the presence of carboplatin (c = 0.25 mg/ml) had no significant influence on QDs fluorescence (average decrease of 1%); however cisplatin and oxaliplatin at the same concentration quenched the fluorescence significantly (average decrease 20%). Subsequently, the amount of platinum incorporated in DNA was determined by QDs fluorescence quenching. Carboplatin-DNA adducts quenched the QDs fluorescence by 0.2% and cisplatin-DNA adducts by 0.6%. Best results were reached using oxaliplatin (9.4%). Linear trend (R^2 = 0.9811) was observed for DNA platinated by three different concentrations of oxaliplatin (0.250, 0.125 and 0.063 mg/ml). In average, 20% of Pt-drug was incorporated into DNA. Correlation with differential pulse voltammetric measurements provided linear trend (R^2 = 0.9511). As a conclusion, especially in the case of oxaliplatin-DNA adducts, the quenching was the most significant compared to cisplatin and non-quenching carboplatin. Considering the fact that the tested drugs have different way of action, the quenching of QDs fluorescence could be a new approach to study their influence on DNA.

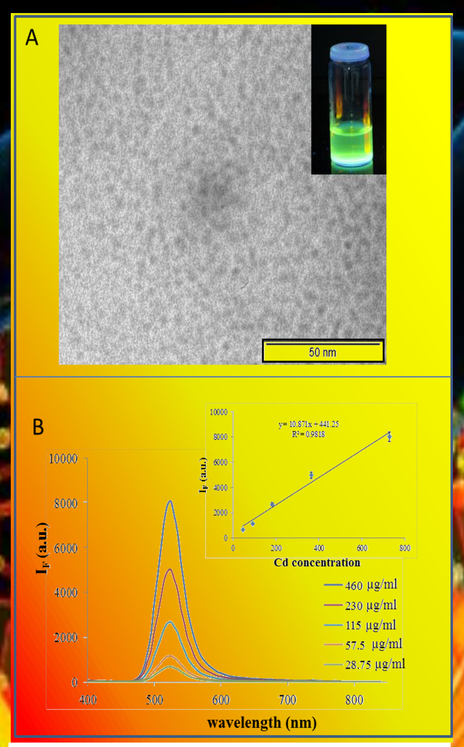

Figure 1:
Characterization of CdTe QDs. A) TEM micrograph of synthesized CdTe QDs (inset: photograph of CdTe QDs solution under UV light illumination); B) Emission spectra of CdTe QDs, excitation 480 nm (inset: calibration curve of CdTe QDs)

Experimental

Synthesis of QDs

330 μl of the $CdCl_2$ solution (c = 0.04 mol/L^{-1}) was diluted with 2.5 ml of water During constant stirring, 8 mg of sodium citrate, 330 μl of Na_2TeO_3 solution (c = 0.01 mol/L^{-1}), 15 mg of GSH and 3.3 mg of $NaBH_4$ were added into water-cadmium(II) solution. The mixture was kept at 95°C under the reflux cooling for 2.5 hours.

Formation of DNA-cytostatic adducts

The solution of 500 bp DNA fragment (25 mg/ml) was mixed with the cytostatic drug -cisplatin, carboplatin and oxaliplatin of various concentrations (0.5, 0.25 and 0.125 mg/ml) in ratio 1:1 (v/v). The mixture was incubated for 24 hours at 37°C. Subsequently the mixture underwent the dialysis using centricons Amicon Ultra 50K (Millipore, MA, USA) to remove the excess of cytostatics.

Fluorimetric analysis

Platinum cytostatics (concentration range 0.5 - 0.004 mg/ml) were mixed with GSH-QDs in ratio 1:1 (v/v). 350 nm was used as an excitation wavelength and the fluorescence scan in the range from 400 to 850 nm was measured with 5 nm steps. Each intensity value is an average of 5 measurements.

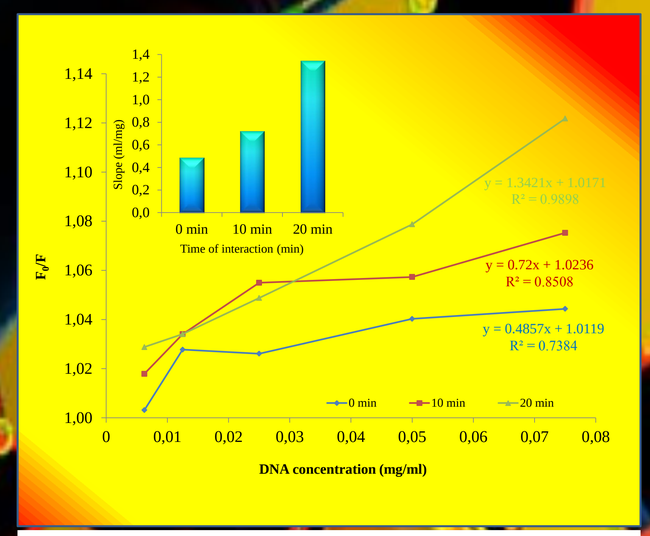

Figure 3:
Stern-Volmer plot of CdTe QDs fluorescence quenching effect caused by DNA fragment (500 bp) and its dependence on time

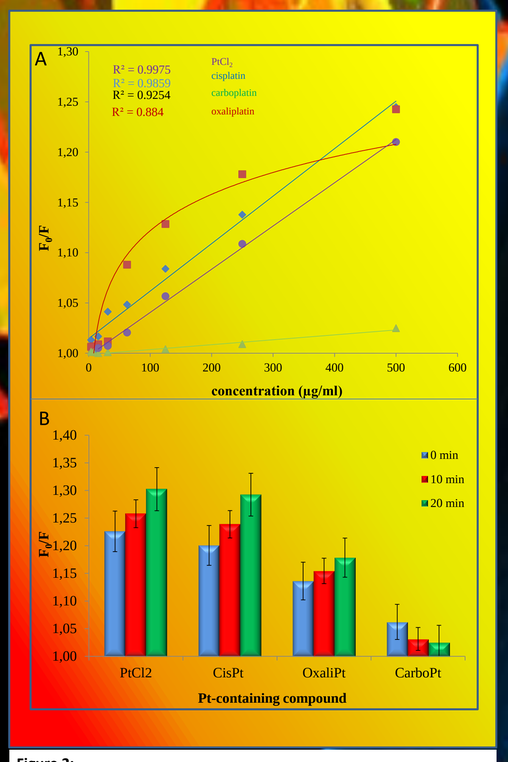

Figure 2:
A) Ster-Volmer plots of CdTe QDs fluorescence quenching effect caused by platinum containing compounds, B) Time dependence of the fluorescence quenching effect caused by platinum containing compounds.

Gel electrophoresis

Agarose gel (1%) was prepared by boiling of 1× TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM ethylenediaminetetraacetic acid, Bio-Rad, USA) for 2 minutes in microwave. Then the gel was cooled to 60°C and ethidium bromide was added (5 μl per 100 ml of the gel). The gel was transferred into electrophoretic bath containing TAE buffer. Samples prepared with 5% (*v*/*v*) bromophenol blue and 3% (*v*/*v*) glycerol were loaded into a gel in 10 μl aliquots. DNA ladder in the size range 0.5-1.5 kb was used to monitor the size of analyzed fragment.

Electrochemical determination of Platinum

Platinum concentration was determined by differential pulse voltammetry. Platinum detection was performed using mercury drop electrode (HMDE). Method parameters were chosen as follows: modulationtime 0.057 s, time interval 0.2 s, potential step of 1.95 mV, scan rate 10 mV/s, modulation amplitude 49.95 mV, deposition potential of -0.7 V, deposition time of 20 s. Platinum was measured by adsorptive stripping voltammetry in 1,98 ml of ACS reagent containing 17.822 mol/l sulfuric acid, 0.24 ml of 0.015 mol/l hydrazine and 0.01 ml of 2 mol/l formaldehyde. Pt-formazan complex were created in measuring container during the 2 min purging time and was accumulated for 15 second on the HMDE. The scan was from -0.5 to -1.2 V with scan rate 10 mV/s. Characteristic peak for platinum was recorded at potential -0.9 V.

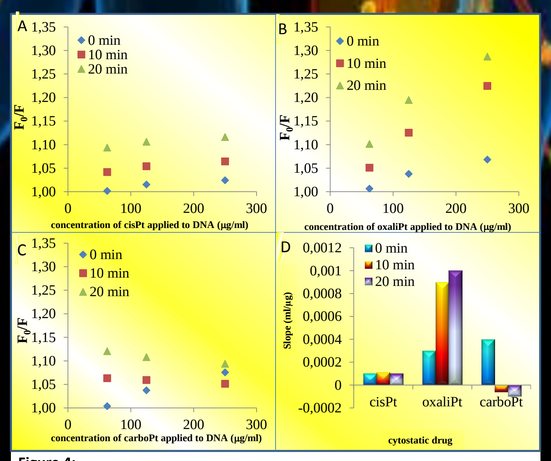

Figure 4:
CdTe QDs fluorescence quenching caused by platinated DNA fragment. A) Stern-Volmer plot of CdTe QDs fluorescence quenching effect caused by cisplatin and its dependence on time; B) Stern-Volmer plot of CdTe QDs fluorescence quenching effect caused by oxaliplatin and its dependence on time; C) Stern-Volmer plot of CdTe QDs fluorescence quenching effect caused by cisplatin and its dependence on time; D) Comparison of slopes of the Stern-Volmer curves for each platinum based cytostatics in different times of interaction.

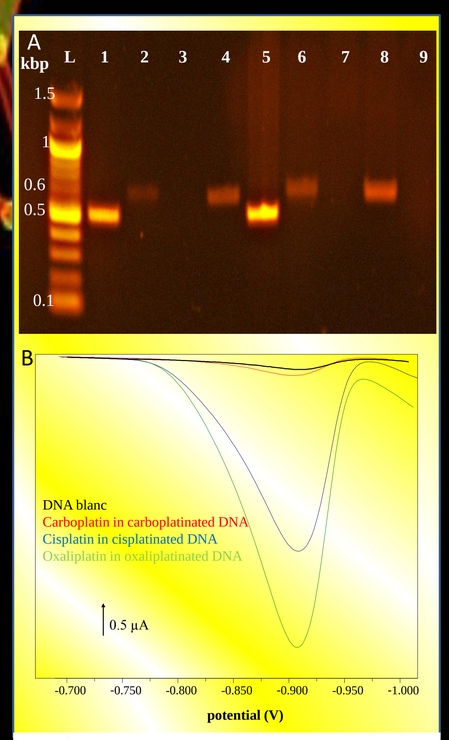

Figure 5:
A) Agarose gel analysis of DNA and DNA-cytostatic drug adducts (L – DNA ladder, 1 – DNA after incubation at 37°C for 24 hours without paltinum cytostatic drugs, 2 – DNA incubated with cisplatin, 3 – DNA after incubation with oxaliplatin, 4 – DNA after incubation with carboplatin, 5-8 – samples in lanes 1-4 with addition of GSH-QDs, 9 – GSH-QDs); B) Determination of platinum-based cytostatic drugs in DNA-drug adducts

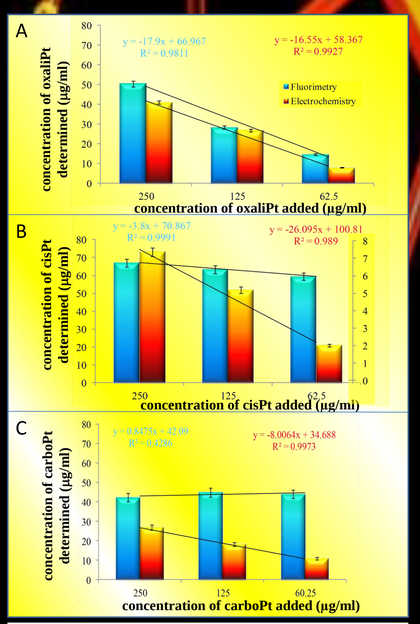

Figure 6:
Correlation between amount of platinum-based cytostatic drug incubated with DNA and amount determined fluorimetrically and electrochemically. A) oxaliplatin, B) cispaltin, C) carboplatin

Conclusion

It can be concluded that especially in the case of oxaliplatin-DNA adducts, the quenching was the most significant compared to cisplatin and non-quenching carboplatin. Considering the fact that the tested drugs have different mechanism of action, the quenching of QDs intensity could be a new how to study their action with DNA.

Acknowledgement

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References

[1] Jemal, A., Siegel, R., Ward, E., Hao, Y. P., *et al.*, *CA-Cancer J. Clin.* 2009, *59*, 225-249.
[2] Zhang, L. J., Xu, C. L., Li, B. X., *Microchim. Acta* 2009, *166*, 61-68.

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