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Monitoring of DNA-doxorubicin interactions by electrochemical methods

Hana Kynclova, Dalibor Huska, Jaromir Hubalek, Petr Babula, Tomas Eckschlager, Marie Stiborova, Rene Kizek

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Abstract

Cancer is presently one of the most studied lifestyle disease. Its treatment is among others based on chemotherapy. One of the drugs used in chemotherapy is doxorubicin, which interacts with DNA and inhibits division of tumour cells. For better usage of this compound as drug, it is necessary to know its interactions with DNA. In this present study interactions between doxorubicin and DNA were monitored using electrochemical methods. In addition, intercalation of doxorubicin into DNA isolated from resistant as well as sensitive tumour cells was monitored and mutual correlation was searched.

Keywords: Cancer, Doxorubcin, DNA, Electrochemical

Hana Kynclova, Jaromir Hubalek

Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Udolni 53, CZ-602 00 Brno, Czech Republic

Rene Kizek*

Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

Dalibor Huska

Department of Plant Biology Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

Petr Babula

Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, CZ-612 42 Brno, Czech Republic

Tomas Eckschlager

Department of Paediatric Hematology 2nd Faculty of Medicine, Charles University, V Uvalu 84, CZ-150 06 Prague 5, Czech Republic

Marie Stiborova

Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, CZ-128 40 Prague 2, Czech Republic

*Tel: +420 545 133 350, Fax: +420 545 212 044
E-mail: kizek@sci.muni.cz

Introduction

Recently, interest in development of biosensor methods, which would improve and facilitate investigation and analysis of properties of deoxyribonucleic acid (DNA) and its interactions with other biomolecules, are significantly increasing. It is well known that electrochemical methods are very efficient for these purposes. In addition, these methods can be used for study of cell processes connected with presumptive drug resistance.

Chemotherapy belongs to the group of basic therapeutic strategies for treatment of tumour diseases. Doxorubicin is one of the most used compounds in tumour therapy. Doxorubicin is included in group of anthracycline antibiotics (Huska et al. 2009; Drummond et al. 2003). Its mechanism of action is still unclear, despite of its utilization in therapy. Primary target of anthracyclines is DNA One of the possibilities of action is intercalation, eventually formation of covalent adducts of doxorubicin with DNA nucleotides, which lead to inhibition of processes of replication, eventually transcription.

For better utilization of this drug, it is necessary to study and know its interaction with DNA. In this work, this interaction is studied by the help electrochemical methods. By the use of electrochemical methods, covalent adducts of doxorubicin with DNA isolated from resistant and sensitive cells were studied. Mutual correlation was also searched (Huska et al. 2009; Drummond et al. 2003).

Materials and methods

Electrochemical determination was carried out by the help of apparatus AUTOLAB analyzer (EcoChemie, Netherlands) in connection with VA-Stand 663 (Metrohm, Switzerland). Cell lines were obtained by isolation and selection from neuroblastoma tissue cell cultures. For measurement, DNA isolated from cells sensitive and resistant to doxorubicin was used.

Cell characteristics was measured using sensitive and resistant UKF-NB-4 lines to the presence of cisplatin. DNA was isolated using Wizard Genomic DNA Purification Kit (Promega, Madison, WI USA). The principle is cell lysis, enzymatic digestion of RNA, deproteination and precipitation of genomic DNA by isopropanol. DNA concentration was determined spectrometrically (Hermo spectronic, Orchester NY, USA) at 260 nm. Moreover, the absorbance was measured at 280 nm due to the presence of impurities in the form of proteins (Frederick et al. 1990).

Results and discussion

The statistical analysis was done for comparing the differences between signals of DNA isolated from sensitive and resistant tumour cells. For analysis itself, cyclic voltammetry with evaluation of CA signal was used. From dependencies of signals heights on rates of polarization (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 V/s), we determined angular coefficients, which were compared with angular coefficient of control sample (without doxorubicin addition) In Fig 1, value of angular coefficient in dependence on concentration of doxorubicin added into cultivation medium is demonstrated. Differences between angular coefficients of sensitive and resistant tumour cell lines are statistically significant (significance level α=0.05) and average difference of values of angular coefficients of sensitive and resistant cells is Δk= 0,167 ± 0,083. As it follows from experimental data, selection of biochemical processes leading to resistance origination can be expected.

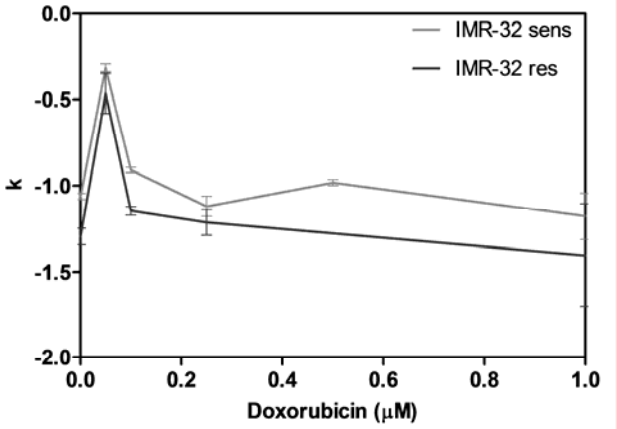

Figure 1: Dependence of values of angular coefficients on doxorubicin concentration

Conclusion

In our experimental work, differences between sensitive and resistant neuroblastoma cell lines were monitored by electrochemical techniques. Doxorubicin proved intercalation, but also covalent bond into DNA.

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