Comparison of *in vivo* electropermeabilization of normal and malignant tissue using the ^{51}CR-EDTA uptake test

D. Batiuškaitė[1*], D. Cukjati[2], L. M. Mir[3]

[1] *Department of Biology, Vytautas Magnus University, Vileikos 8, LT-3000 Kaunas, Lithuania*
[2] *Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia*
[3] *UMR 8121 CNRS, Institute Gustave-Roussy, Villejuif Cedex, France*

The aim of our study was to use the ^{51}Cr-EDTA uptake test to compare the electropermeabilization of a malignant and a normal tissue, and to analyze their specific electropermeabilization thresholds. After exposing the tissues to the voltage ranging from 150 to 1600 V/cm, we found that the threshold for reversible electropermeabilization for rat skeletal muscle was about 550 V/cm and for LPB fibrosarcoma murine tumor was about 750 V/cm. The threshold for irreversible electropermeabilization for rat skeletal muscle was about 800 V/cm, while it could not be determined for the LPB fibrosarcoma murine tumors. The two tissues have thus shown very different responses to the electric pulses applied.

Key words: electropermeabilization, electroporation, *in vivo*, ^{51}Cr-EDTA, cell membrane, tumor, muscle

INTRODUCTION

Electropermeabilization (EPN), also termed electroporation, is a process that results in cell membrane permeability changes under the influence of strong and short electric pulses [1]. This phenomenon has wide biomedical applications, among which the most important are electrogenetherapy and electrochemotherapy [1]. For the optimal application of these approaches it is necessary to understand the kinetics of uptake of various molecules and the effects that the electric fields have on the cells *in vitro* as well as *in vivo* [2]. *In vitro* cell EPN has been well described [3, 4]. *In vivo* investigations are more difficult to perform. Some specific indicators as ^{57}Co-bleomycin, ^{111}In-bleomycin and ^{99m}Tc-DTPA have already been used *in vivo* [5–7]. However ^{57}Co-Bleomycin has a slow renal clearance and requires considerable radiation safety measures, and the use of ^{111}In-bleomycin or ^{99m}Tc-DTPA is restricted by the requirement of sophisticated gamma-camera equipment.

Recently, a new quantitative method of investigation of *in vivo* EPN has been developed [8, 9]. *In vivo* EPN of mice muscle tissue, which results from membrane perturbations caused by the electric field, was determined by assaying the uptake of ^{51}Cr-EDTA, a small complex used in clinical scintigraphic analysis [10] and as an EPN indicator [11]. Moreover, this method allowed to detect two phases in the uptake of ^{51}Cr-EDTA: the first indicates increasing EPN, and the second shows the development of irreversible membrane damage.

The aim of this study was to investigate the EPN of a malignant and a normal tissue and their specific EPN thresholds using the ^{51}Cr-EDTA uptake test.

MATERIALS AND METHODS

Animals. Female Wistar rats and female C57Bl/6 mice (Janvier, France) were used for experiments. They were maintained at 22 °C with a natural day/night light cycle in a conventional animal colony, fed and watered *ad libitum*. Subcutaneous tumors were implanted by subcutaneously injecting viable syngeneic LPB fibrosarcoma cells in the flanks of 8–12 week old mice. The tumors were pulsed 10 to 12 days later, when they reached at least 5.2 mm in diameter. Animals were anaesthetized before experiments by the intraperitoneal administration of the anesthetics Ketamine (100 mg/kg; Ketalar, Panpharma, France) and Xylazine (10 mg/kg; Rompun, Bayer, France).

* Corresponding author: E-mail: a8daba@vaidila.vdu.lt

Delivery of electric pulses. In all experiments, 8 square-wave pulses of 100 μs duration, delivered at a frequency of 1 Hz, were generated by a PS 15 electropulsator (Jouan, St. Herblain, France). Plate electrodes consisting of two opposing metal plates separated by 5.2 or 8.2 mm for tumor treatment and by 5.7 mm for rat's skeletal muscles (the *musculus triceps brachii* of the hind limb and the *musculus gastrocnemius medialis* of the forelimb) were used. Good contact between electrodes and the underlying skin was assured by shaving hairs at the treatment site and by the use of a conductive gel (Parker Laboratories, New York, USA).

^{51}Cr-EDTA uptake. Mice were given 100 μl and rats 200 μl of ^{51}Cr-EDTA (Amersham, UK) with a specific activity of 3.7 MBq/ml, by an intravenous injection into the retroorbitary sinus, 5 min before the delivery of the electric pulses. The animals were sacrificed in a CO_2 camera 24 hours after ^{51}Cr-EDTA injection and tissues exposed to electric pulses were taken out, weighed and gamma-counted (Cobra 5002 gammacounter, Packard Instrument, Meridien, CT, USA). The net uptake as a result of EPN was calculated as the measured activity per gram of the tissue exposed to the electric pulses. The measured activity then was converted to the corresponding nanomoles of ^{51}Cr-EDTA internalized per gram of tumor tissue.

Statistics. For each point at least four independent experiments were performed. Data are presented by the arithmetical mean and the standard error of the mean (SE) of the values of each experimental group; the t test was used to analyze the differences between independent groups of data.

RESULTS

The net uptake of ^{51}Cr-EDTA as a function of electric field strength (the ratio of applied voltage to the electrode distance) is shown in Figure.

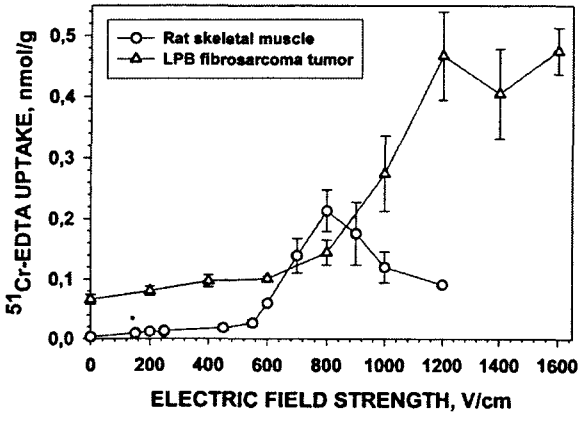

Figure. Net uptake of ^{51}Cr-EDTA 24 hours after delivery of electric pulses

^{51}Cr-EDTA uptake in the skeletal muscles. At 0 V/cm net uptake was very close to 0 nmol/g, indicating that after 24 hours ^{51}Cr-EDTA was almost washed out from the unpulsed muscles and that it was possible to detect even very low levels of radioactivity entrapped by the electropermeabilized muscles. Between 150–450 V/cm, the values of net uptake insignificantly ($p > 0.05$) increased in comparison with the control value. From 450 V/cm to 800 V/cm, a progressive large increase was observed. The values of 450 V/cm and 800 V/cm were statistically different ($p < 0.01$). At 900 V/cm a decrease of the net uptake was detected, which was still more prominent at larger voltages. The uptake values at 800 V/cm and 1200 V/cm were statistically different ($p < 0.05$).

^{51}Cr-EDTA uptake in the LPB fibrosarcoma tumors. Contrary to the muscles, at 0 V/cm the net uptake was not null. A value as high as 0.0661 nmol/g was detected. Between 200–600 V/cm, the values of net uptake insignificantly ($p > 0.05$) increased in comparison with the control value. When the pulse strength exceeded 600 V/cm, a progressive large increase of ^{51}Cr-EDTA uptake (up to 1200 V/cm) was observed. The ^{51}Cr-EDTA uptake values at 600 V/cm and 1200 V/cm were statistically different ($p < 0.01$). Two points at a higher pulse strength (1400 and 1600 V/cm) indicated that the uptake reached the plateau values: contrary to muscles, no significant decrease of ^{51}Cr-EDTA uptake was detected.

DISCUSSION

Analyzing the dynamics of the net uptake threshold for the reversible EPN of skeletal muscle in rats (field strength corresponding to the intersection of the line describing the progressive increasing part of the uptake curve and the line corresponding to the basal level uptake) was about 550 V/cm, while that of the LPB fibrosarcoma tumors was about 750 V/cm. The difference between the two thresholds could be due to the difference in the size of the tumor and the muscle cells: it is known that, *in vitro*, the larger the cell, the lower the value of the external field strength necessary to obtain the EPN of the cell [12]. Our data *in vivo* are thus consistent with the known previous data obtained *in vitro*.

However, while in the skeletal muscle it was possible to easily identify the threshold for irreversible EPN, no consistent value could be deduced from the data obtained in tumors. The reason for obtaining a plateau at very high field strengths is unclear. Further experiments are required to understand the particular behavior of the tumor tissue.

Received 14 October 2002

References

1. Mir LM. Bioelectrochemistry 2000; 53: 1–10.
2. Mir LM, Orlowski S. Adv Drug Delivery Rev. 1999; 35: 107–18.
3. Neumann E, Kakorin S, Toensing K. Bioelectrochem Bioenerg 1999; 48: 3–16.
4. Rols MP, Teissie J. Biophys J 1990; 58: 1089–98.
5. Belehradek JJr, Orlowski S, Ramirez LH et al. Biochim Biophys Acta 1994; 1190: 155–63.
6. Engstrom P, Persson BR, Salford LG. Bioelectrochem Bioenerg 1998; 46: 241–8.
7. Engstrom P, Persson BR, Salford LG. Biochim Biphys Acta 1999; 1473: 321–8.
8. Gehl J, Sorensen TH, Nielsen K et al. Biochim Biophys Acta 1999; 1428: 233–40.
9. Gehl J, Mir LM. Biochem Biophys Res Comm 1999; 261: 377–80.
10. Rubin K, Sjoquist M, Gustafsson AM et al. Int J Cancer 2000; 86: 636–43.
11. Satkauskas S, Bureau MF, Puc M et al. Mol Ther 2002; 5: 133–40.
12. Teissie J, Rols MP. Biophys J 1993; 65: 409–13.

D. Batiuškaitė, D. Cukjati, L. M. Mir

NORMALIŲ IR PIKTYBINIŲ AUDINIŲ *IN VIVO* ELEKTROPERMEABILIZACIJOS PALYGINIMAS NAUDOJANT ^{51}CR-EDTA TESTĄ

Santrauka

Elektrochemoterapija ir elektrogenoterapija yra vienos perspektyviausių elektropermeabilizacijos biomedicininio pritaikymo sričių. Norint optimizuoti šiuos terapinius metodus, būtina žinoti elektros lauko, lemiančio grįžtamąją ir negrįžtamąją elektropermeabilizaciją, slenkstines vertes. Šiame darbe buvo tirti normalių ir piktybinių audinių elektropermeabilizacijos ypatumai *in vivo* naudojant ^{51}Cr-EDTA testą. Nustatyta, kad grįžtamosios elektropermeabilizacijos slenkstis pelių skeleto raumenyse buvo apie 550 V/cm, o pelių LPB fibrosarkomos augliuose – apie 750 V/cm. Negrįžtamosios elektropermeabilizacijos slenkstis pelių skeleto raumenyse buvo apie 800 V/cm, o pelių LPB fibrosarkomos augliuose jis nenustatytas dėl specifinės šio navikinio audinio reakcijos į stiprų elektros lauką.