PERGAMON

Computers in Biology and Medicine 28 (1998) 439–448

Computers in Biology and Medicine

Calculation of the electrical parameters in electrochemotherapy of solid tumours in mice

D. Šemrov *, D. Miklavčič

University of Ljubljana, Faculty of Electrical Engineering, Tržaška 25, SI-1000 Ljubljana, Slovenia

Received 8 December 1997

Abstract

Electrochemotherapy is a novel approach in chemotherapeutic drug delivery into tumours. Short intense direct current electric pulses are applied to tumour tissue causing electropermeabilisation thus enabling entrance of chemotherapeutic drugs into cells which otherwise do not easily penetrate. A three dimensional anatomically based finite element model of the mouse with injected subcutaneous solid tumour was built. The main goal of the study was to evaluate the influence of the electrode orientation on the distribution of electric field in the tumour and surrounding tissue during electrochemotherapy. Two electrode configurations, previously examined in experimental study, were modelled. Electric field distributions were calculated for each configuration. The main conclusion of our study is that changing electrode orientation strongly influences the distribution of the electric field inside the tumour in the electrochemotherapy of solid tumours in mice, which is in good agreement with the results of the experimental study. The efficacy of the electrochemotherapy depends on the magnitude of the electric field intensity inside tumour tissue.

Keywords: Electrochemotherapy; Electroporation; Murine tumour models; Electrode configuration; Numerical modelling; Finite element method

1. Introduction

Electric conductivity of the plasma membrane in normal physiological conditions is much lower than the conductivity of cytoplasm and extracellular medium. When a cell is exposed to an external electric field, the anode-facing side becomes hyper-polarized and the cathode-facing side becomes depolarized depending on the size and the shape of the cell [1]. If the externally induced transmembrane potential is high enough, then reversible permeabilization of plasma

* Corresponding author. Fax: +386-61-126-46-58; E-mail: dejan@svarun.fe.uni-lj.si.

0010-4825/98/$19.00
PII: S0010-4825(98)00017-1

membrane occurs which results in local increase of its permeability [2]. The phenomenon is called electropermeabilization and is characterized by electric field intensity, duration, number of pulses and their shape. Electric field intensity of the pulses of selected duration must reach a threshold. At the molecular level, the phenomenon is still a subject of the research [3, 4]. However, under appropriate conditions, these membrane changes are reversible and cell viability is maintained [5, 6].

Many chemotherapeutic drugs used in cancer therapy have poor access into the tumour cells, therefore membrane permeabilization offers an approach for enhanced drug delivery into the cells and better antitumour effectiveness. It was shown that in vitro cytotoxicity of some chemotherapeutic drugs can be potentiated several-fold by exposing cells to short intense electric pulses [7–9]. Cells in tissues can also be electropermeabilized and the antitumour effectiveness of chemotherapeutic drugs potentiated by increasing drug delivery into the cells [10]. This novel approach, termed electrochemotherapy, was introduced by Okino and Mir [11, 12]. Lower systemic doses of chemotherapeutic drugs are thus required for successful therapy and adverse side effects are reduced. For example, antitumour effectiveness of bleomycin can be greatly potentiated with electric pulses, inducing partial and complete responses of the tumours. Furthermore, the treatment requires such a low amount of bleomycin, that it is ineffective without electric pulses and does not induce side effects. Antitumour effectiveness has also been shown for the chemotherapeutic drug cisplatin [9].

In preclinical in vitro and in vivo experiments, several research groups proved potential applicability of cisplatin and bleomycin for electrochemotherapy [13, 14]. In one of previous studies, it was demonstrated that changing electrode orientation improves the efficacy of electrochemotherapy of solid tumours in mice [15]. In short, experimental protocol used was following. Ehrlich ascites tumour in CBA mice was used as a tumour model. Tumour cells were injected to the left flank of the mouse. When the tumours reached approximately 40 mm^3 in volume, they were treated with the combination of intravenously injected bleomycin (Mack, Germany) and trains of square-wave high voltage direct current pulses (amplitude 1040 V, pulse width 100 μs, repetition frequency 1 Hz). Electric pulses were delivered by two parallel stainless steel plate electrodes 8 mm apart (two stainless steel strips, 7 mm in width with rounded tips). Good contact between the electrodes and the skin was provided by means of conductive gel. It was shown that the electrode orientation strongly influences the efficiency of electrochemotherapy. Namely, in previous studies many tumours regrew in the areas where the intensity of the electric field was lower. In the last study tumours were treated either with 4, 8, or 4 + 4 pulses in train [15]. In the last group the second train of 4 pulses was delivered with electrodes oriented perpendicularly with respect to the first one with a time interval of 1 s between the two trains of pulses. This changing of the electrode orientation resulted in improved antitumour efficacy of the electrochemotherapy; prolonged tumour growth delay and higher percentage of short and long term complete responses of the tumours [15].

Numerical modelling was used as a tool for the explanation of the observed effects. In our study an anatomically based numerical model was built to determine electric field distributions inside the tumour for different electrode configurations, described in the previous paragraph. A parametric study on the model was performed to evaluate different electrode configurations and gain more precise knowledge on the levels of electric field intensity inside the tumour.

2. Materials and methods

The electric field in the biological tissue, resulting from application of constant direct electric current, can be considered quasi-stationary i.e. its time variations can be neglected. Its distribution is described by equations for the steady electric currents in the volume conductor. For the homogenous and isotropic volume conductor the electric potential distribution is governed by the Laplace equation which together with two types of boundary conditions describe electric field inside the conductor. The two types of boundary conditions are a Dirichlet boundary condition defined as a fixed scalar electric potential, i.e. applied voltage on the surface of the model; and a Neumann boundary condition defined as a first derivative of the scalar electric potential in the direction normal to the boundary surface of the model, i.e. current density flowing in/out of the model divided by the conductivity of the tissue.

2.1. Geometry and mesh generation

A three dimensional (3-D) anatomically based finite element (FE) model of the mouse with injected subcutaneous solid tumour was built using MSC/EMAS (electromagnetic analysis system) software package (trademark of MacNeal-Schwendler, USA) [16]. The geometry of the model was based on the 14 cross section scans of one typical animal with a subcutaneous tumour, obtained by magnetic resonance imaging (MRI). The distance between two neighboring cross sections in the longitudinal direction was 2.7 mm. There were eleven MRI scans in the abdominal and three in the thoracal part of the body. The geometry of the model was described with 1390 points which defined 3859 curves/lines. A total of 1379 3-D geometric bodies were defined using those curves. All data about geometrical entities, i.e. coordinates of points, definitions of curves and geometric bodies, were arranged into a database using dBase IV software package (trademark of Ashton Tate Corporation) in order to simplify further modifications of the geometry of the model.

Resulting three dimensional geometric structure was built of eleven different tissues (organs), i.e. skin, fat, skeletal and heart muscles, bone, connective tissue, intestine, kidney, liver, lung

Table 1
Electric conductivities of tissues (organs) used in the model

Tissue	Conductivity γ (S/m)
Skin	0.04
Fat	0.046
Muscle	$\gamma_{xx} = 0.225$ $\gamma_{yy} = 0.225$ $\gamma_{zz} = 0.9$
Bone	0.025
Connective tissue	0.025
Intestine	0.55
Kidneys	1.01
Liver	0.333
Lungs	0.07
Heart	$\gamma_{xx} = 0.2$ $\gamma_{yy} = 0.2$ $\gamma_{zz} = 0.44$
Tumour	0.125

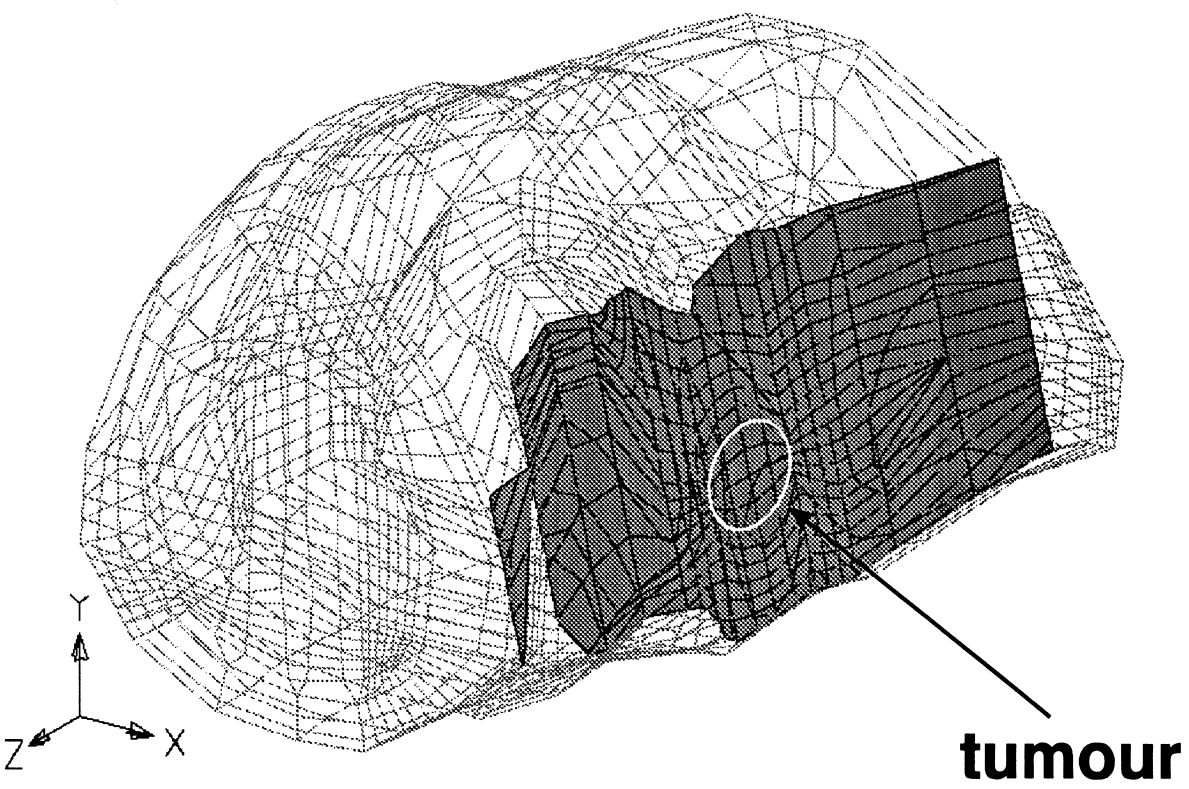

Fig. 1. Three dimensional anatomically based finite element model of the mouse with subcutaneous solid tumour. Shaded plane represents cross section through the middle of the tumour in which the results were observed.

and tumour. Each of these tissues (organs) formed a closed region and a mesh of 3-D finite elements was generated inside with appropriate material properties assigned to the elements in each region. Anisotropic characteristics were considered for skeletal and heart muscles, while all other tissues (organs) were modelled as isotropic. The values of the electric conductivities of tissues (organs) used in the model were collected from literature and used in one of previous studies where similar model was verified with the measurements of electric potential in the five points in the tumour and surrounding tissue [17]. All tissue conductivities are listed in Table 1. All data about mesh generation were also arranged into a database to simplify further model modifications and mesh generation procedure. Resulting 3-D model was made of 7089 3-D finite elements which were defined by 7578 grid points. It is shown in Fig. 1 where the cross section alongside tumour, in which the results were observed, is also drawn.

2.2. Boundary conditions–electrode configurations

Different electrode orientations were obtained by applying appropriate boundary conditions in the grid points corresponding to each of the two electrodes. Increased area with the same electric potential under each electrode resulting from the use of conductive gel was also

considered. Fixed values of scalar electric potential, i.e. Dirichlet boundary conditions, were assigned to grid points in the regions where electrodes were placed. Two electrode configurations were modelled according to the position of the electrodes with respect to the tumour, e.g. cranial/caudal and dorsal/ventral. Potentials of 0 V and 1040 V were assigned to groups of appropriate grid points of the FE mesh corresponding to each of the electrodes thus modelling the conditions in the experimental study. The width of both electrodes was 7 mm and their thickness was 0.9 mm. In the cranial/caudal electrode configuration, the positive electrode was placed on the left side of the tumour in the direction towards the head of the mouse. The distance between the electrode and the edge of the tumour was 0.9 mm. The negative electrode was placed on the other (right) side of the tumour in the direction towards the tail of the mouse and the distance between the electrode and the edge of the tumour was again 0.9 mm. In the dorsal/ventral electrode configuration the positive electrode was placed above the tumour in the direction towards the back of the mouse. The distance between the electrode and the edge of the tumour was 0.9 mm. The negative electrode was placed on the other side below the tumour and the distance between the electrode and the edge of the tumour was again 0.9 mm.

On the remaining outer surfaces of the model, a Neumann boundary condition was applied. This boundary was considered as the interface between a conducting medium and air (assimilated to an ideal dielectric). Since the conductor (skin layer) was linear and isotropic, the usual Neumann condition was applied, i.e. the normal derivative of the electric potential on the interface between the model and surrounding air was zero.

3. Results

Distributions of scalar electric potential were calculated for cranial/caudal and dorsal/ventral electrode configurations. Distributions of electric field intensity were then calculated from the values of the scalar electric potential in the grid points of the model. The results were observed in the cross section plane through the middle of the tumour alongside it, since we were most interested in the electrical phenomena inside the tumour tissue.

In Fig. 2 the results for the cranial/caudal electrode configuration are shown. The region of the maximal electric field intensity is near the electrodes where its magnitude reaches 4400 V/cm. The magnitude of electric field intensity falls rapidly towards edges of the tumour in proximity of the electrodes where its value is 520 and 490 V/cm on the opposite sides of the tumour. In the middle of the tumour, the magnitude of the electric field intensity is lower (300 V/cm) and it falls towards edges of the tumour in the direction away from the line connecting electrodes to the value of 250 V/cm.

In Fig. 3 the results for the dorsal/ventral electrode configuration are shown. The region of the maximal electric field intensity is again near the electrodes where its magnitude reaches 4800 V/cm. The magnitude of electric field intensity falls rapidly towards edges of the tumour near electrodes where its value is 530 and 500 V/cm on the opposite sides of the tumour. In the middle of the tumour, the magnitude of the electric field intensity is lower (390 V/cm) and it falls towards edges of the tumour in the direction away from the line connecting electrodes to the value of 280 and 300 V/cm.

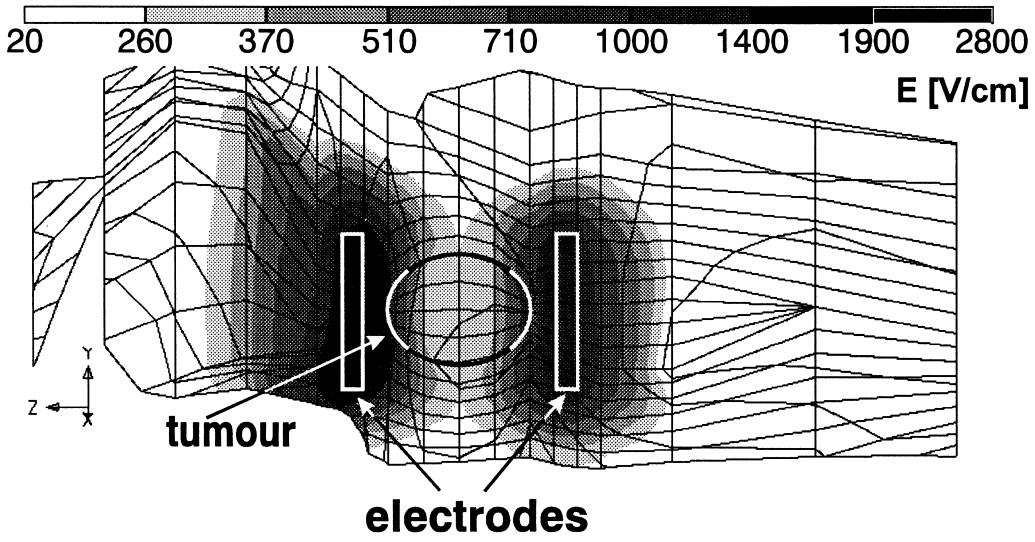

Fig. 2. Electric field distribution for the cranial/caudal electrode configuration. The location of the tumour and electrodes is schematically shown.

The distributions of the electric field were more precisely studied for 48 elements representing subcutaneous tumour, since we were most interested in the electrical phenomena inside tumour tissue. The mean magnitude of the electric field intensity inside tumour for the cranial/caudal electrode configuration is 360 V/cm. The magnitudes of the electric field intensity for the dorsal/ventral electrode configuration are higher with the mean of 400 V/cm.

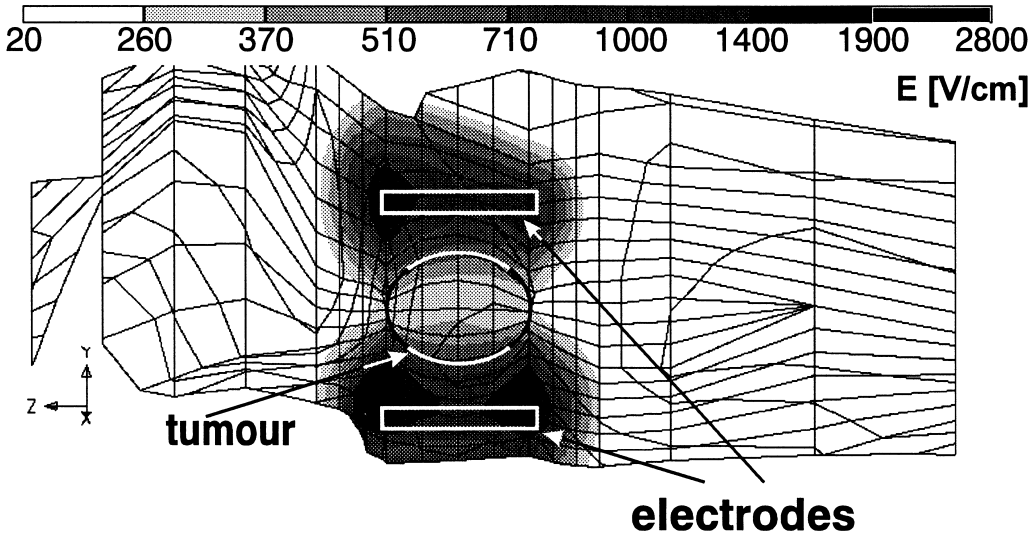

Fig. 3. Electric field distribution for the dorsal/ventral electrode configuration. The location of the tumour and electrodes is schematically shown.

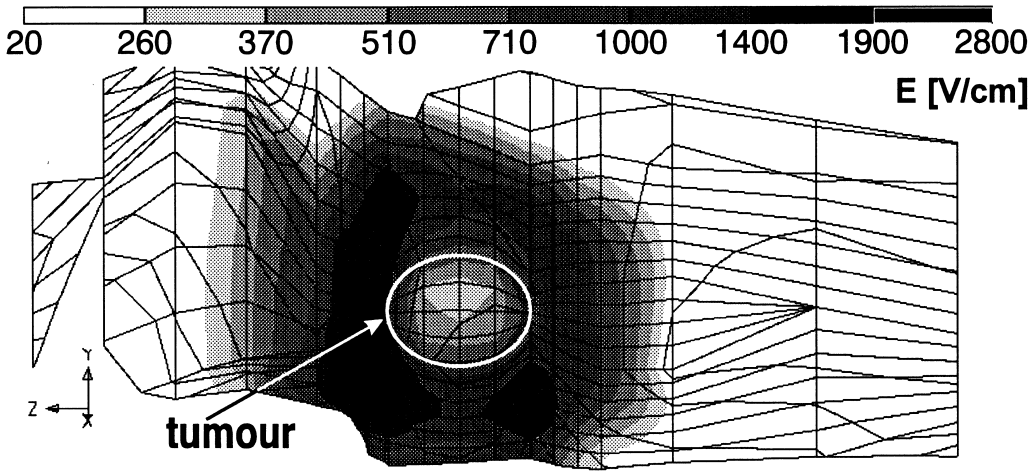

Fig. 4. Electric field distribution for the 4 + 4 electrode configuration.

Electric field distribution for the electrode configuration 4 + 4, where for the last four pulses the electrodes were oriented perpendicularly with respect to the position of the electrodes for the first four pulses, was determined as a combination of the results for the cranial/caudal and dorsal/ventral electrode configurations. Since electropermeabilization is a threshold phenomenon, it can be assumed that in the 4 + 4 electrode configuration the effective magnitude of electric field intensity in each finite element of the model is the higher of the magnitudes for cranial/caudal and dorsal/ventral electrode configurations in that particular element. Based on this assumption we determined the values of the electric field intensity for all 7089 finite elements and the resulting distribution in the cut surface is shown in Fig. 4. The mean magnitude of electric field intensity for the 48 elements representing tumour is 430 V/cm, i.e. higher than in the cranial/caudal or dorsal/ventral electrode configurations.

4. Discussion

In the present study, the three dimensional, anatomically based model of the mouse with subcutaneous tumour during electrochemotherapy was built by means of the finite element method. Different electrode configurations, i.e. orientations of the plate electrodes, were modelled by applying appropriate boundary conditions. Distributions of electric field intensity were determined in the cut surface through the middle of the tumour.

As it was previously shown, changing electrode orientation during the treatment improves the efficacy of electrochemotherapy of solid tumours in mice [15]. Electric field intensity in all electrode configurations has its maximum near the electrodes outside the tumour. The summary data for the magnitude of the electric field intensity in selected points inside tumour tissue are listed in Table 2. The maximum magnitudes of the electric field intensity inside tumour tissue for the cranial/caudal and the dorsal/ventral electrode configurations are near electrodes. In the middle of the tumour, these magnitudes are lower and they fall even more

Table 2
Magnitudes of electric field intensity (V/cm) in the selected points inside tumour

Electrode configuration	Center of the tumour	Left edge of the tumour	Right edge of the tumour	Upper edge of the tumour	Lower edge of the tumour
Cranial/caudal	300	520	490	250	250
Dorsal/ventral	390	280	300	500	530

rapidly towards the edges in the direction away from the line connecting electrodes. This pattern, which can also be noted in Figs. 2 and 3, is in good agreement with the experimental results. Namely, in the group where electrode orientation was not changed many of the tumours regrew after successful treatment in the margins where there was no contact with the electrodes [15].

The magnitudes of electric field intensity inside the tumour for the dorsal/ventral electrode configuration are higher than for cranial/caudal electrode configuration. This is a result of the higher electric conductivity of the muscle tissue in the longitudinal direction. Namely, in the cranial/caudal electrode configuration, this direction coincides with the direction of the electrical current for that particular electrode position. As a result of this, more current was flowing through the muscle tissue deeper in the model than through tumour.

The electric field distribution for the 4 + 4 electrode configuration, shown in Fig. 4, clearly shows the rise of the magnitudes of electric field intensity inside the whole tumour compared to each of previous electrode configurations. The mean value of the electric field intensity for the 4 + 4 electrode configuration is also higher than in the case of the cranial/caudal or the dorsal/ventral electrode configurations.

The results obtained clearly demonstrate that the improvement in the tumour treatment for the experimental group where electrode configuration was changed [15], is due to higher electric field intensities reached inside the tumour.

5. Summary

Electrochemotherapy has proven to be an effective treatment of tumours, employing locally applied high voltage direct current electric pulses in combination with chemotherapeutic drugs. Plasma membrane hinders some antitumour drugs from entering the cells, thus reducing their efficacy. Biological consequences of electromagnetic fields are well described in the case of use of short intense direct current electric pulses, which result in electropermeabilization of the cells, i.e. transient and reversible changes in the plasma membrane. It becomes permeable to a number of hydrophilic molecules that are otherwise unable to diffuse through it, e.g. some chemotherapeutic drugs.

In one of the previous studies, it was shown that changing the electrode orientation improves the efficacy of electrochemotherapy of solid tumours in mice. Ehrlich ascites tumour in CBA mice was used as a tumour model. Tumours were treated with the combination of intravenously injected bleomycin and trains of square-wave high voltage direct current electric

pulses which were delivered via two parallel stainless steel plate electrodes. Changing of the electrode orientation during the treatment resulted in improved antitumour efficacy of the electrochemotherapy, prolonged tumour growth delay and higher percentage of short and long term complete responses of the tumours.

In our study, a three dimensional anatomically based finite element model of the mouse with injected subcutaneous solid tumour was built. The model consisted of the following eleven different tissues (organs): skin, fat, skeletal and heart muscles, bone, connective tissue, intestine, kidney, liver, lung and tumour. Their conductivities were collected from literature. Anisotropic characteristics were considered for skeletal and heart muscles. For the conductivity of the tumour tissue, we used a value determined in one of our previous studies. The main goal of the study was to evaluate the influence of the electrode orientation on the distribution of electric field in the tumour and surrounding tissue during electrochemotherapy. Two electrode configurations, previously examined in the experimental study, were modelled: cranial/caudal and dorsal/ventral, where electrodes were oriented perpendicularly with respect to each other.

Electric field distributions were calculated for each electrode configuration and magnitudes of the electric field intensity were analyzed throughout the model and more precisely inside the tumour tissue. Electric field distribution for the $4 + 4$ electrode configuration, where the orientation of the electrodes was changed during electrochemotherapy, was determined as the combination of the results of the cranial/caudal and the dorsal/ventral electrode configurations. Highest magnitudes of electric field intensity inside the tumour were obtained for the $4 + 4$ electrode configuration. Furthermore, the magnitudes of electric field intensity inside the tumour were higher for the dorsal/ventral electrode configuration than for the cranial/caudal electrode configuration. This is a consequence of the higher electric conductivity of the deeper lying muscle tissue in the longitudinal direction which coincides with the direction of the electric current for that particular electrode position.

The main conclusion of our study is that changing electrode orientation strongly influences the distribution of the electric field inside the tumour in the electrochemotherapy of solid tumours in mice, which is in good agreement with the results of the experimental study. The efficacy of the electrochemotherapy depends on the magnitude of the electric field intensity inside tumour tissue.

Acknowledgements

We appreciate the help of Franci Demšar, DSc. from Institute J. Stefan, Ljubljana, who provided us with the MRI scans. This study was supported by the Ministry of Science and Technology of the Republic of Slovenia.

References

[1] L.F. Jaffe, R. Nuccitelli, Electrical controls of development, Ann. Rev. Biophys. Bioeng. 6 (1977) 445.
[2] U. Zimmermann, Electrical Breakdown, electropermeabilization and electrofusion, Rev. Physiol. Biochem. Pharmacol. 105 (1986) 175.

[3] J.C. Weaver, Molecular basis for cell membrane electroporation, Ann. NY Acad. Sci. 720 (1994) 141.
[4] S. Kakorin, S.P. Stoylov, E. Neumann, Electro-optics of membrane electroporation in diphenylhexatriene-doped lipid bilayer vesicles, Biophys. Chem. 58 (1996) 109.
[5] E. Neumann, M. Schaefer-Ridder, Y. Wang, P.H. Hofschneider, Gene transfer into mouse lyoma cells by electroporation in high electric fields, Eur. Mol. Biol. Organ. J. 1 (1982) 841.
[6] M.P. Rols, J. Teissie, Electropermeabilization of mammalian cells: quantitative analysis of the phenomenon, Biophys. J. 58 (1990) 1089.
[7] S. Orlowski, J. Belehradek Jr., C. Paoletti, L.M. Mir, Transient electropermeabilization of cells in culture. Increase in cytotoxicity of anticancer drugs, Biochem. Pharmacol. 37 (1988) 4727.
[8] A.K. Nutt, A. Mansouri, K.L. Henle, Response of cisplatin resistant tumour cells to electroporation and cisplatin, Proc. Annu. Meet. Am. Asoc. Cancer Res. 32 (1991) A2229.
[9] G. Serša, M. Čemažar, D. Miklavčič, Antitumour effectiveness of electrochemotherapy with cis-diamminedichloroplatinum (II) in mice, Cancer Res. 55 (1995) 3450.
[10] J. Belehradek Jr., S. Orlowski, L.H. Ramirez, G. Pron, B. Poddevin, L.M. Mir, Electropermeabilization of cells in tissues assessed by the qualitative and quantitative electroloading by bleomycin, Biochim. Biophys. Acta 1190 (1994) 155.
[11] M. Okino, H. Mohri, Effects of a high-voltage electrical impulse and an anticancer drug on in vivo growing tumours, Jpn. J. Cancer Res. 78 (1987) 1319.
[12] L.M. Mir, S. Orlowski, J. Belehradek Jr., C. Paoletti, Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses, Eur. J. Cancer 27 (1991) 68.
[13] G. Serša, M. Čemažar, D. Miklavčič, L.M. Mir, Electrochemotherapy: variable antitumour effect on different tumour models, Bioelectrochem. Bioenerg. 35 (1994) 23.
[14] L.M. Mir, S. Orlowski, J. Belehradek Jr., J. Teissié, M.P. Rols, G. Serša, D. Miklavčič, R. Gilbert, R. Heller, Biomedical applications of electric pulses with special emphasis on antitumour electrochemotherapy, Bioelectrochem. Bioenerg. 38 (1995) 203.
[15] G. Serša, M. Čemažar, D. Šemrov, D. Miklavčič, Changing electrode orientation improves the efficacy of electrochemotherapy of solid tumours in mice, Bioelectrochem. Bioenerg. 39 (1996) 61.
[16] J.R. Brauer, B.E. MacNeal, MSC/EMAS User's Manual Version 2.5, The MacNeal–Schwendler Corporation, Los Angeles, 1991.
[17] D. Šemrov, V. Valenčič, D. Miklavčič, L. Vodovnik, Electrical field distributions in the model of the mouse during DC electrotherapy of subcutaneous tumour, in: Abstract Book 17th Annual Meeting BEMS, Boston, 1995, p. 12.

Dejan Šemrov was born in 1967 in Šempeter pri Gorici, Slovenia. He received a BSc. in 1991 and a MSc. in 1994, both in electrical engineering from the Faculty of Electrical Engineering, University of Ljubljana. He is currently working towards his Ph.D. degree. His prime interests are in the field of mathematical modelling of biological systems.

Damijan Miklavčič was born in 1963 in Ljubljana, Slovenia. He received a BSc. in 1987, a MSc. in 1991 and a DSc. in 1993, all in electrical engineering from the Faculty of Electrical Engineering, University of Ljubljana. His prime interests are in the field of biomedical engineering and interactions between electromagnetic fields and biological systems.