Uporaba elektropororacije v medicini: elektrokemoterapija in elektrogenska terapija

Maja Cemazar
Onkološki inštitut Ljubljana
Univerza na Primorskem Fakulteta za vede o zdravju
History

1937

The Regional Institute for Research and Treatment of Neoplasms was established by the decree issued by the authorities of Drava Province. On August 1st, 1938, first patients were admitted into the reconstructed buildings of the former Šempeter Barracks (presently Building A). In the first year of existence, the Institute, with a capacity of 28 beds, accommodated 818 patients.

Doc.dr. Cholewa
Institute of Oncology Ljubljana

- Department of Experimental Oncology – 340 m² located in building B basement
- Registered as a user organisation for experimentation on laboratory animals. Laboratories for the work with animals occupy 90 m²
- Registered as organisation with license to work with the GMO at safety level 2.
- Registered as an organisation with license to work with closed sources of ionising radiation (X-ray irradiation apparatus)
Main research areas – translational research

- Specific targeting of solid tumors
- Use of delivery systems
  - Electroporation
  - Nanothechnology – magnetic nanoparticles
- Therapeutic approaches:
  - Gene therapy: antivascular, immune response stimulation
  - Electrochemotherapy
  - Radiotherapy combined with electrochemotherapy and gene therapy
What is translational oncology?

- Translational oncology forms a bridge between basic science and clinical utilisation of this knowledge.
Transplantable tumor models and human tumor xenografts

Methods:
tumor implantation

Measurement of tumor diameter
Metastases count

Subcutaneous tumors
Induced lung metastases
Reversible electroporation - principle

- At electric field intensity higher than a critical threshold short lived primary pores are formed in the plasma membrane.
- Accumulation and expansion of pores.
- Resealing of plasma membrane.

Electric pulses (DC)

Rectangular

Exponential

Propidium iodide uptake
Reversible electroporation

- Universal
- Easily, rapidly and reproducibly obtained
- Non-thermal
- Limited to the plasma membrane

Introduction of hydrophilic and non-permeant molecules:
- Dyes
- Proteins
- Oligo and polinucleotides (siRNA, DNA)
- Drugs
Electro Chemo Therapy: ECT

Definition

• Potentiation of antitumor effectiveness of the non-permeant anticancer drugs by application of electric pulses at the tumor site.

• Electric pulses transiently permeabilize plasma membrane and thus increase drug delivery into the cells.
Electrochemotherapy: Increased cytotoxicity of chemotherapeutic drugs

- Effective for hydrophilic drugs with hampered transport through the plasma membrane
- Drugs that have clinical applicability:
  - Bleomycin (BLM)
  - Cisplatin (CDDP)
Preclinical data on electrochemotherapy

- **In vitro:**
  - potentiation of cytotoxicity
- **In vivo:**
  - antitumour effectiveness in different:
    - animal models: mice, rats, cats, dogs, horses
    - tumour models:
      - melanoma, carcinomas, sarcomas, glioma
      - spontaneous, transplanted
      - primary, metastatic
  - route of administration (intravenous, intratumoural, intramuscular)
  - optimization of drug dosage, sequencing and electric pulses amplitude
  - mechanisms of action
  - effect on metastases
Antitumor effectiveness on tumor models
Electroporation increases cytotoxicity of chemotherapeutic drugs on different tumour cell lines.
Protocol of electrochemotherapy

**Electric pulse generator**

Electric pulses: 8 x 0.1 ms; 1300 V/cm, 1 Hz

**Intravenous or intratumoral drug injection**

**Application of electric pulses**

**Injection of the drug**
**Electrochemotherapy: variable anti-tumor effect on different tumor models**

Gregor Serša a,*, Maja Čemažar a, Damijan Miklavčič b, Lluis M. Mir c

a Institute of Oncology, Department of Tumor Biology, Založka 2, 61105 Ljubljana, Slovenia
b University of Ljubljana, Faculty of Electrical and Computer Engineering, Tržaška 25, 61000 Ljubljana, Slovenia
c Department of Antitumor Pharmacology, URA 147 CNRS, Institut Gustave-Roussy, rue C. Desruelles, F 94805 Villejuif, France

Intrinsic Sensitivity of Tumor Cells to Bleomycin as an Indicator of Tumor Response to Electrochemotherapy

Maja Čemažar,1,3 Damijan Miklavčič2 and Gregor Serša1

Local tumor control of sarcoma and carcinomas after ECT with Bleomycin
Antitumor Effectiveness of Electrochemotherapy with 
cis-Diamminedichloroplatinum(II) in Mice

Gregor Serša, Maja Čemažar, and Damijan Miklavčič

SA-1 tumors

Control

EP: 8 pulses, 1300 V/cm, 1 Hz, 100 μs

Cisplatin 4 mg/kg i.v.

Electrochemotherapy
Time dependence of electrochemotherapy with cisplatin injected intratumorally

The best antitumour effect was obtained when electroporation was performed immediately after the drug injection.

*SA-1 tumours*

*Cemazar et al; Anticancer Drugs 1998; 9: 525-530.*
Mechanisms associated with antitumour effectiveness of electrochemotherapy

- Electroporation
- Drug entrapment by tumour blood flow modification
- Anti-vascular effect
- Immune system response
Antitumor effectiveness of electrochemotherapy is not due only to direct cytotoxic effect to tumor cells, therefore other mechanisms must be involved

In tumors treated with electrochemotherapy, cell kill was increased by a factor of 20, compared with treatment with cisplatin.

log cell kill (CDDP): 0.017
log cell kill (EP): 0.04
log cell kill (ECT): 0.77

**PF (IC\textsubscript{50}) = 10**
Electroporation increases the amount of platinum bound to DNA after electrochemotherapy with cisplatin.

Vascularization of the tumours and effect of applied electric pulses
Application of electric pulses to the tumours induces transient reduction in tumour blood flow

Electroporation of tumours induces drug entrapment by vascular lock in the tumours. 

Platinum content in the tumors after electrochemotherapy and cisplatin injected intravenously. Prolonged accumulation of platinum in the tumors exposed to electric pulses.

Anti-vascular effect of electrochemotherapy with bleomycin and cisplatin

Cytoskeletal F-actin changes in EP and ECT-treated HMEC-1 monolayers

The differential effect between EP and ECT induced changes are detectable already at 10 min post-treatment.

8 EP, 100 μs, 1 Hz d_{ele}: 7.3 mm
Bleomycin 300 nM

Meulenber C JW et al PlosOne 2012
Cytoskeletal $\beta$-tubulin changes in EP and ECT-treated HMEC-1 monolayers

The differential effect between EP and ECT induced changes are detectable already at 10 min post-treatment.

*Meulenberg CJW et al PlosOne 2012*
Disruption of F-actin and VE-cadherin junctions in endothelial cells after EP

Kanthou et al. Mol Cancer Ther 2006
HMEC-1 monolayer permeability changes occur quicker and to higher degree after ECT compared to EP.

Meulenberg CJW et al PlosOne 2012
Proposed mechanism of EP-induced changes in tumor blood vessels – vascular lock and increased permeability of tumor blood vessels

Jarm et al. Expert Rev. Anticancer Ther. 2010
Vascular disrupting effect of electrochemotherapy at chemotherapeutic drug doses that cause tumor destruction

QUESTION: Are the same mechanisms also involved in vivo?
Intravital microscopy

- Direct visual access to the blood vessels and surrounding tissue
- Repetitive observations of the same animal
- In combination with modern microscopy techniques it enables high spatial and time resolution of imaging

Tumor grown in dorsal window chamber

Masks of blood vessels: calculation of fluorescence intensity inside the vessels and in extravascular space

Koehl GE. et al, Clin Ex Metastasis, 2009
Application of EP to tumor causes increased permeability of tumor blood vessels

**in vivo**

**Control**

**EP**

*In vivo* conformation of data obtained in *in vitro* HUVEC monolayers

Application of EP to tumor causes „vascular lock“ that lasts ~10 mins, followed by partial restoration of tumor blood flow.

Proposed mechanism of EP-induced changes in tumor blood vessels — vascular lock and increased permeability of tumor blood vessels

**NORMAL BLOOD FLOW** (before EP)

**DECREASED BLOOD FLOW** (~0-2 h after EP)

- endothelial cells rounding up;
  - increased resistance to flow

- increased IFP, tissue oedema;
  - passive vasoconstriction

**GRADUAL RECOVERY OF BLOOD FLOW** (~8-12 h after EP)

- gradual recovery of endothelial lining

**NORMAL BLOOD FLOW** (~24 h after EP)

Diffusion constant of blood vessel wall (Dwall) of normal blood vessels

<table>
<thead>
<tr>
<th>Diffusion Constant</th>
<th>Dwall (AM±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 kDa</td>
<td>0,0126±0,0037 μm²/s</td>
</tr>
<tr>
<td>2000 kDa</td>
<td>0,0081±0,0008 μm²/s</td>
</tr>
</tbody>
</table>

Finite element model
ECT induce vascular lock and completely abrogates tumor blood flow; increased permeability of blood vessels

8 EP, 780 V, 100 μs, 1 Hz; d_{el}: 6mm
Bleomycin 100 μg/mouse
FITC dextran 70KDa

Time in relation to EP

- Control
- Bleomycin
- EP+1 min
- ECT+1 min
- EP+60 min
- ECT+60 min

Before EP

EP

EP+4 h

EP+8 h

EP+24 h

EP+72 h
EP and ECT do not affect normal blood vessels surrounding tumor
Limb sparing treatment of bleeding melanoma recurrence by electrochemotherapy

Marko Snoj, Maja Cemazar, Tinkara Srnovrsnik, Snezna Paulin Kosir, and Gregor Sersa
Vascular disrupting effect of electrochemotherapy

Quicker and more pronounced decrease in blood flow—occurring already at 10 min after ECT: changes in cytoskeletal proteins and proteins involved in cell to cell junctions in endothelial cells also due to the drug action?
Clinical application of electrochemotherapy in human medicine
Electrochemotherapy - procedure

Marty and Sersa et al. EJC Suppl 2006
Fifth framework research programme
Quality of Life and Management
of Living Ressources Programme

QLK3-2002-02003
EU Project Officer:
Dr. Beatrice Lucaroni
Coordinator: L.M.Mir

Institute Gustave-Roussy
Dr. L.M. Mir, Pr. M. Marty

Institute of Oncology Ljubljana
Pr. G. Sersa

IGEA s.r.l.
Dr. R. Cadossi

Herlev Hospital
Dr. J. Gehl

Cork Cancer Research Center
Pr. G. O’Sullivan
Electric pulse generator

- Cliniporator™, CE marked, produced by IGEA
- Appropriate pulse configurations set for each electrode type
- Two options for frequency (1Hz, 5kHz)
- Visual confirmation of the quality of the delivered pulses
- Stores voltage and current data
Possible ways of performing ECT

• ECT with BLM
  – BLM given intravenously
  – BLM given intratumorally

• ECT with CDDP
  – CDDP given intratumorally
Electrochemotherapy proved to be effective in treatment of various cutaneous tumor nodules:

- Melanoma
- Basal cell carcinoma
- Kaposi sarcoma
- Cervix leiomyosarcoma
- Head and neck cancer
- Hypernephroma
- Squamous cell carcinoma of the skin
- Breast cancer

Predominant use:

- **Melanoma metastases**
  - Palliative intent
  - When other treatment modalities have failed or proved insufficient
  - Cytoreductive treatment before surgical excision
  - Management of bleeding metastases
Electrochemotherapy in treatment of tumours

G. Sersa a, D. Miklavcic b, M. Cemazar a, Z. Rudolf a, G. Pucihar b, M. Snoj a, *

Electrochemotherapy with bleomycin.
The first clinical experience in malignant melanoma patients

Zvonimir Rudolf,1 Borut Štabuc,1 Maja Čemažar,1 Damijan Miklavčič,2 Lojze Vodovnik2 and Gregor Serša1

1 Institute of Oncology, Ljubljana, 2 Faculty of Electrical and Computer Engineering, University of Ljubljana, Slovenia
Malignant melanoma
ECT with CDDP i.t., plate electrodes, single application

• CR for 16 years

Long lasting complete response in melanoma treated by electrochemotherapy

Marko Snoj, Zvonimir Rudolf, Snezna M. Paulin-Kosir, Maja Cemazar, Rudolf Snoj, Gregor Sersa*
Malignant melanoma:
ECT with BLM i.v., hexagonal electrodes, single application

The state-of-the-art of electrochemotherapy before the ESOPE study; advantages and clinical uses

Before treatment

After 2 weeks

After 4 weeks

Fig. 1 - Antitumour effectiveness of electrochemotherapy with bleomycin given intravenously. Electric pulses to melanoma nodules on the leg were applied by hexagonal centred electrodes. The melanoma nodules were located in surgically pre-treated area. Complete and partial responses of the treated nodules are visible after single electrochemotherapy session, with good cosmetic effect, without scaring of the treated area.
Anal malignant melanoma

ECT-CDDP i.t.

Immediately after treatment

Day 46
19 days after retreatment
2 days before operation

Day 27, Retreatment

14 months after the beginning of treatment

Snoj et al. Anticancer Drugs 2005
Breast cancer

ECT with BLM i.v., plate electrodes, multiple applications

Antitumour Treatment

Electrochemotherapy of chest wall breast cancer recurrence

Gregor Sersa a,e, Tanja Cufer b, Snezna Marija Paulin a,c, Maja Cemazar a,d, Marko Snoj a,c

Before treatment

After 2 weeks

After 4 weeks

After 8 weeks

After 3 month

After 5 month
Response rate of the patients treated before and in ESOPE study

Electrochemotherapy with BLM
- 202 pts were included
- 655 tumor nodules treated

Electrochemotherapy with CDDP
- 45 pts were included
- 354 tumor nodules treated

<table>
<thead>
<tr>
<th>Electrochemotherapy</th>
<th>Patients</th>
<th>Nodules</th>
<th>Response %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PD  NC  PR  CR  OR</td>
</tr>
<tr>
<td>Before ESOPE study</td>
<td>247</td>
<td>1009</td>
<td>6  11  19  64  83</td>
</tr>
<tr>
<td>ESOPE study</td>
<td>41</td>
<td>171</td>
<td>5  10  11  74  85</td>
</tr>
</tbody>
</table>

ESOPE project:
- 41 pts were included
- 171 tumor nodules treated

Toxicity evaluation

Memory of the procedure
The majority of the patients (N=57, 93%) were willing to undergo electrochemotherapy again.
Perspectives

ECT included in guidelines

Malignant melanoma in Slovenia

Surgery and radiotherapy in the treatment of cutaneous melanoma

A. Testori¹*, P. Rutkowski², J. Marsden³, L. Bastholt⁴, V. Chiarion-Sileni⁵, A. Hauschild⁶ & A. M. M. Eggermont⁷

Treatment of apparent in-transit metastases. Treatment of ITM of the limb depends on their number, site and size [57]. Resectable ITM should be treated surgically with narrow but clear margins. Amputation is not indicated and does not improve survival. With multiple dermal ITM, carbon dioxide laser ablation can be used, but the recurrence rate is very high and this technique is limited to lesions <1 cm in diameter. Other local modalities including radiotherapy, cryotherapy, intralesional injections and electrochemotherapy may be used in specific situations. Regional chemotherapy with ILP or isolated limb infusion (ILI) is the preferred method of treating multiple and frequently recurrent ITM. It treats the whole limb below the point of tourniquet isolation, can achieve 20–50 times higher concentrations of melphalan compared with systemic therapy, and can be performed with minimal locoregional toxicity and minimal systemic leakage [58]. ILP with melphalan can be used in combination with tumour necrosis factor (TNF)-α [59, 60], especially in the case of bulky lesions [60, 61] or after failure of a prior ILP or ILI using melphalan alone [62, 63]. Iliac ILP has the advantage of treating the whole limb up to the groin; ILI only treats to the upper third of the thigh. ILI is probably slightly less effective than ILP, but is less invasive and easier to repeat.

Electrochemotherapy can be indicated for palliation of superficial metastatic lesions when ILP or ILI is not indicated for the general conditions of the patient; a 90% response on the superficial metastases has been reported [64, 65].
Electrochemotherapy: A New Technological Approach in Treatment of Metastases in the Liver

Figure 3: A: Overlay of the computational geometry and patient's anatomy. The red lines represent the direction of insertion of the electrodes, while line represents the cross-section of Figure 2. B: Photograph of the surgical setup with electrodes penetrating into the tumor is clearly seen (cables: neglected).

Figure 5: Resection of Sg 1 with common trunk and MHV exposed. Necrotic metastasis (M) is visible in Sg 1, close to the MHV and IVC.
## ECT centres in Europe - 2012

<table>
<thead>
<tr>
<th>Country</th>
<th>Centres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>32</td>
</tr>
<tr>
<td>Germany</td>
<td>31</td>
</tr>
<tr>
<td>Austria</td>
<td>1</td>
</tr>
<tr>
<td>Portugal</td>
<td>1</td>
</tr>
<tr>
<td>Spain</td>
<td>5</td>
</tr>
<tr>
<td>Sweden</td>
<td>4</td>
</tr>
<tr>
<td>Denmark</td>
<td>1</td>
</tr>
<tr>
<td>Greece</td>
<td>5</td>
</tr>
<tr>
<td>Great Britain/Ireland</td>
<td>6</td>
</tr>
<tr>
<td>Hungary</td>
<td>1</td>
</tr>
<tr>
<td>Poland</td>
<td>1</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>1</td>
</tr>
<tr>
<td>Lithuania</td>
<td>1</td>
</tr>
<tr>
<td>France</td>
<td>6</td>
</tr>
<tr>
<td>Slovenia</td>
<td>1</td>
</tr>
<tr>
<td>Belgium</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
</tr>
</tbody>
</table>
Clinical application of electrochemotherapy in veterinary medicine
Electrochemotherapy Compared to Surgery for treatment of Canine Mast Cell Tumours

VERONIKA KODRE¹, MAJA CEMAZAR², JANI PECAR³, GREGOR SERSA², ANDREJ CŐR⁴ and NATASA TOZON³

Figure 2. Kaplan Meier survival curves for tumour regression for patients treated with surgery and ECT with cisplatin. Circles represent censored data. At the end of the study 7 dogs were still in CR of ECT treated group and 8 dogs treated by surgery.

Figure 3. Patient No. 18 with tumour nodule in the hind leg before and 4 and 8 weeks after ECT treatment. After treatment in some cases superficial scab is formed, that fell off within 8 week. The tumour completely regressed and the dog is free of disease for more than 3.5 years.

Table II. Tumour volumes, histology and response to treatment in dogs
Electrochemotherapy with cisplatin of squamous cell carcinoma in cat

Before therapy

3 months after
Cat SCC: Queeny

- 14 y; F
- SCC
- Number of tumors: 1
- Nose
- T3
- TH: ECT bleomycin IV
- Number of sessions: 1
- Response: CR

Before therapy

4 weeks after ECT

8 weeks after ECT

12 week after ECT
Electrochemotherapy with cisplatin of sarcoid tumors in horses

Before treatment

After 6 weeks
Sarcoid on the head treated with ECT with cisplatin. CR was obtained.
Gene electrotransfer

- optimisation studies using reporter genes
- therapeutic efficiency
# Strategies of cancer gene therapy

<table>
<thead>
<tr>
<th>GENE THERAPY STRATEGY</th>
<th>GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic replacement or correction therapy</td>
<td><em>p53, CTS1, MDA7, Bcl-2-, BclXL-, survivin-antisense...</em></td>
</tr>
<tr>
<td>Suicide gene therapy (gene chemotherapy)</td>
<td>Endogenous precursors</td>
</tr>
<tr>
<td></td>
<td>Exogenous precursors</td>
</tr>
<tr>
<td>Gene based immunotherapy</td>
<td></td>
</tr>
<tr>
<td>Vascular-targeted gene therapy</td>
<td></td>
</tr>
</tbody>
</table>

*CTS1, Chimeric Tumor Suppressor 1(synthetic variant of wild-type p53); HSV-tk, Herpes Simplex Virus thymidine kinase; CD, Cytosine Deaminase; HRP, Horseradish Peroxidase; IAA, Indol-3-Acetic Acid; iNOS, inducible Nitric Oxide Synthase; TNF-α, Tumor Necrosis Factor-α; IL-12, Interleukin-12; PSA, Prostate Specific Antigen; VEGF, Vascular Endothelial Growth Factor.*
Gene electrotransfer: principle

DNA injection

Permeabilization and electrophoresis

Insertion  Translocation  Expression

Pulse  After pulse

Tumor

Electric pulses generator

Plasmid DNA injection

Expression of GFP

tumor  muscle
Optimization of gene electrotransfer

- Comparison to other methods
- Time dependence of transfection
- Different electrical parameters
- Timing of the procedure
- Tumor histological properties
Effective gene transfer to solid tumors using different nonviral gene delivery techniques: Electroporation, liposomes, and integrin-targeted vector

Maja Cemazar,¹,² Gregor Sersa,² John Wilson,¹ Gillian M Tozer,¹ Stephen L Hart,³ Alenka Grosel,² and Gabi U Dachs¹

- DNA only
- EP1+DNA
- EP2+DNA
- Liposomes+DNA-LD
- LDEP
- Lipos. + Peptides+ DNA-LPD
- LPDEP
Sequence and Time Dependence of Transfection Efficiency of Electrically-Assisted Gene Delivery to Tumors in Mice

Maja Cemazar¹,*, Darja Pavlin², Simona Kranjc¹, Alenka Grosel¹, Suzana Mesojednik¹ and Gregor Sersa¹
**Correlation between transfection efficiency and histological properties at 5 min interval**

**Tumors with bigger cells and less extracellular matrix are easier to transfec**t.
Skeletal muscle is an attractive target tissue for delivery of therapeutic genes, since it is well vascularized, easily accessible, and has a high capacity for protein synthesis. For efficient transfection in skeletal muscle, several protocols have been described, including delivery of low voltage electric pulses and a combination of high and low voltage electric

---

**Gene Electrotransfer into Murine Skeletal Muscle:**
**A Systematic Analysis of Parameters for Long-term Gene Expression**

Gregor Tevz, B.Sc.¹
Darja Pavlin, D.V.M.²
Urska Kamensek¹
Simona Kranjc, Ph.D.¹
Suzana Mesojednik¹
Andrej Coer, Ph.D.³
Gregor Sersa, Ph.D.¹
Maja Cemazar, Ph.D.¹,²

---

**Technology in Cancer Research and Treatment**
ISSN 1533-0346
Volume 7, Number 2, April 2008
©Adenine Press (2008)
Gene electrotransfer for treatment of cancer

• **local approach** → gene electrotransfer into tumor tissue
  – local production of proteins – direct effect on tumor cells
  – The protein can also enter blood stream – effect on metastases

• **systemic approach** → gene electrotransfer into muscle tissue (or skin – vaccination)
  – easily accessible tissue
  – high capacity for protein synthesis
  – efficient transfection
  – long-term expression of transgene (~ 1 year)

• therapeutic effects
  • suppression of growth of primary and metastatic tumors
  • prevention of metastatic spread
  • induction of long-term antitumor memory
  • prolongation of survival time
**Translational gene therapy studies**

- **Concept**
  - Gene electro transfer of therapeutic genes
  - Plasmids coding for therapeutic proteins or si RNAs
  - Tumors: melanoma; sarcoma, carcinoma

- **Therapeutic approaches**
  - Immunogene therapy – interleukin 12 (IL-12)
  - Antiangiogenic gene therapy
    - AMEP
    - siRNA against Endoglin
    - siRNA against MCAM

- **Preclinical testing**
  - Antitumor and anti-angiogenic effects
    - Direct antitumor effect by intratumoral transfection
    - Systemic effect through intramuscular transfection
    - Combined with tumor irradiation and electrochemotherapy

- **Clinical testing**
  - IL-12 gene therapy entered clinical testing
  - AMEP entered clinical testing
Interleukin-12 antitumor effect
Concentration of mIL-12 and mIFN-γ in serum of mice is dependent on the amount of plasmid DNA and number of intramuscular transfections.
Controlled systemic release of interleukin-12 after gene electrotransfer to muscle for cancer gene therapy alone or in combination with ionizing radiation in murine sarcomas.
$mIL-12$ gene intramuscular electrotransfer has anti-metastatic effect on induced lung metastases

Control

EP

pORF-mIL12

-24h GET

+24h GET

+72h GET

+120h GET

4×GET

Percent of metastases (%)

0

20

40

60

80

100

120

140

* **

SA-1

LPB

injection of SA-1 cells into tail vein
Local and systemic antitumor effect of intratumoral and peritumoral IL-12 electrogene therapy on murine sarcoma

Darja Pavlin, Maja Cemazar, Urska Kamensek, Natasa Tozon, Azra Pogacnik and Gregor Sersa

Intratumoral EGT resulted in high level of complete responses (18/20 tumors) with significant inhibition of tumor growth in the remaining 2 tumors.

Peritumoral EGT resulted in lower complete response rate (3/19 tumors), with remaining 16/19 showing significant delay in tumor growth.
Gene electrotransfer induces local and systemic release of IL-12 and IFN-γ and has antitumor effect on distant untreated tumors.

**SERUM**

- IL-12
- IFN-γ

**TUMOR**

- IL-12
- IFN-γ

**Growth curves of distant untreated tumors**

*Pavlin et al. Cancer Biol Ther 2009; 8:2112-2120*
IL-12 gene therapy combined with tumor irradiation - radiosensitization

- Systemic mIL-12 release (electrotransfection of muscle) and local tumor irradiation
- Local mIL-12 release (electrotransfection of tumor) and local tumor irradiation
**Intratumoral mIL-12 gene electrotransfer improves therapeutic index of radiotherapy in murine sarcoma**

**Radiation dose-modifying factor = 2.17**

44% higher probability of local tumor control in combined treatment with no significant change to skin reaction.

Dry desquamation < 20% of irradiated area

*Sedlar et al. BMC Cancer 2013*
Intramuscular mIL-12 gene electrotransfer has radiosensitizing effect on solid subcutaneous tumors

mIL-12 i.m. gene electrotransfer has radiosensitizing effect on induced lung metastases
Endoglin (CD105)

- Transforming growth factor-β (TGF-β) co-receptor
- Function: activation of signaling pathway that mediates endothelial cell proliferation, migration and adhesion
- Elevated expression in proliferating vascular endothelial cells in the tumors
Multiple Delivery of siRNA against Endoglin into Murine Mammary Adenocarcinoma Prevents Angiogenesis and Delays Tumor Growth

In vivo

Delayed tumor growth and reduced endoglin mRNA

In vitro

Reduced endothelial cell proliferation and tube formation
Blood vessels were stained with anti CD31 antibody
Construction of plasmid DNA encoding shRNA against endoglin from the sequence of the most effective siRNA

Tumor growth was significantly delayed

Prevention of blood vessel growth into the tumors – dorsal window chamber model

Day 0
Day 6
Day 6

Control
shRNA against endoglin

Tumor were treated before the angiogenic switch

Red - blood vessels
Green - tumor
Clinical research - EGT with IL-12 in dogs

1. i.m. delivery of plasmid, encoding hIL-12
2. i.t. delivery of plasmid, encoding hIL-12
3. peri.t. delivery of plasmid, encoding hIL-12

Use of human IL-12 in dogs:
> 90% homology with canine IL-12 based on aminoacid sequence biologically active on canine perif. mononuclear cells *in vitro*
<table>
<thead>
<tr>
<th>Therapy</th>
<th>Follow up (avg mo)</th>
<th>CR</th>
<th>SD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECT</td>
<td>19</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>EGT i.t.</td>
<td>18</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>EGT i.m.</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ECT+EGT p.t.</td>
<td>14</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Addition of systemic EGT to local ECT offers an advantage over single therapies adding a systemic component to the local electrochemotherapy.
Mast cell tumor on the front leg of a dog, treated with a combination of ECT with cisplatin and IL-12 EGT as part of an ongoing clinical study at our institution. **A:** a large ulcerated tumor nodule before therapy, **B:** one week after the procedure, massive necrosis of the treated area can be seen; **C:** one month after the procedure, a cytologically confirmed complete response was achieved.
Safety of gene therapy – preliminary results

- RT-PCR detection of plasmid DNA in skin swabs of treated region at different post-treatment intervals
- Assessment of normal bacterial flora on dogs’ skin prior to therapy
- Assessment of presence of plasmid DNA in isolated bacteria
Preliminary results – post-therapy evaluation of presence of plasmid DNA and horizontal transfer

- Immediately after EGT: max value 40 ng of plasmid per ml of sample
- 1 week post-treatment: 0.13 ng/ml
- 2 weeks post-treatment: 0.06 ng/ml
- 4 weeks post-treatment: 0.01 ng/ml
- In most patients the plasmid pIL-12 was not detectable after 4 weeks
- Plasmid IL-12 was not detected in any of the residential bacteria.
- In vitro transformation of plasmid IL-12 into isolated strains was negative.

Performed by Mija Rak and Nataša Tešić, Ana Krhać and Jerneja Ambrožič Avguštin
Electrogene therapy in treatment of cancer patients

- ESOPE: Phase I clinical study on electrogene therapy using reporter gene for β-gal
Gene electrotransfer to melanoma skin nodule

Few positively staining cells were found, estimated less than 1/1000. The positive cells were characterized based on morphology, and were tumor cells, macrophages and fibroblast like cells.

- DNA: β-gal 100 µg DNA
- EP: 1 HV 1000 V/cm + 8 LV 140 V/cm
- Samples of tissue were excised 24 h after gene electrotransfer

There was consistent staining in gene electrotransfer samples DNA injection only as well as controls were negative. The number of positively staining cells was very low, but different cell types were stained.
Gene electrotransfer clinical trial with therapeutic plasmid AMEP

Angioskin EU FP6 project

CLINICAL TRIAL PROTOCOL

SAFETY AND EFFICACY OF INTRATUMOURAL ELECTROTRANSFER OF PLASMID AMEP 2 mg IN PATIENTS SUFFERING FROM ADVANCED OR METASTATIC MELANOMA: AN OPEN PHASE I CLINICAL TRIAL
Patient 001-003; Treatment: IT Plasmid AMEP™ 2mg

Exposure to treatment:
- All 5 patients received the two IT plasmid AMEP doses (D1 and D8) without delay.

Preliminary efficacy results:
- Based on evaluation of the local response in the treated and control lesion at D29
- Stabilization of treated lesion in 3 patients and a partial response in 1 patient (according to adapted RECIST 1.1 evaluation).
- In parallel, all cutaneous control lesions have shown an objective progression at D29

22.03.2011 Before treatment

29.03.2011 Before 2nd treatment
Tumour Necrosis

Nine weeks after treatment
Partial Tumour Response
Electrotransfer of therapeutic molecules into tissues
Maja Cemazar* & Gregor Sersa

Transfection efficiency

Therapeutic outcome

Electrode design
Electric pulse parameters

Electric field distribution
Plasmid construction and administration

Tissue properties

EMA, FDA – safety concerns – genes for antibiotic resistance

Blood flow modification
Tissue damage

Inflammation; Immune response

Intracellular DNA sensors

• different tumour types
• dose of plasmid DNA
• frequency of treatment
• required serum conc.

Electroporation

Current Pharmaceutical Design, 2006, 12, 3817-3825

Electrically-Assisted Nucleic Acids Delivery to Tissues In Vivo: Where Do We Stand?

M. Cemazar¹,², M. Golzio¹, G. Sersa², MP. Rols¹ and J. Teissier¹,⁶
Perspectives

• Preparation and evaluation of plasmids with tissue specific and inducible promoters
• Preparation and evaluation of plasmids devoid of antibiotic resistance gene
Transfection efficacy of reporter plasmid DNA with promoter specific for skin cells (fibroblasts)

**pCOL1A2-GFP**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>% of cells expressing GFP</th>
<th>Normalized fluorescence intensity (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVEC4-10</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>HUVEC</td>
<td>40</td>
<td>2</td>
</tr>
<tr>
<td>L929</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>LPB</td>
<td>80</td>
<td>6</td>
</tr>
</tbody>
</table>

**pEGFP-N1**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>% of cells expressing GFP</th>
<th>Normalized fluorescence intensity (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVEC4-10</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>HUVEC</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>L929</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td>LPB</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

**Cell lines:**

Murine endothelial cells **SVEC4-10**
Human umbilical vein endothelial cells **HUVEC**
Murine fibroblasts **L929**
Murine fibrosarcoma cells **LPB**

COL1A2= eukaryotic promoter for alpha-2 type I collagen
Tumor irradiation combined with IL-12 gene therapy controlled by p21 radio-inducible promoter

Promoters of radiation inducible genes, that are activated by clinically relevant doses of irradiation, can be exploited to control transgenes expression spatially and temporally within the irradiated tumor tissue.
Radio-inducible expression of IL-12 resulted in equal antitumor effectiveness compared to constitutively expressed IL-12
Applications of electroporation-based technologies

**DRUG DELIVERY**
Electro-Chemo Therapy
ECT

**NUCLEIC ACIDS DELIVERY**
Gene Electro Transfer
GET

**HUMAN ONCOLOGY – PALLIATIVE TREATMENT**
- skin metastases
- liver tumors
- brain metastases
- colorectal tumors
- sarcomas

**VETERINARY ONCOLOGY: dogs, cats, horses**
- primary tumors & metastases

**COMBINED TREATMENT**
- ECT + GET
- ECT + irradiation
- GET + irradiation

Local or systemic
Acknowledgement

• Institute of Oncology Ljubljana
  – Gregor Sersa
  – Bostjan Markelc
  – Simona Kranjc
  – Vesna Todorovic
  – Tanja Dolinšek
  – Lara Prosen
  – Urška Kamenšek
  – Tanja Blagus
  – Ales Sedlar
  – Alenka Grošel
  – Suzana Vidic
  – Gregor Tevž
  – Mira Lavric
  – Marko Snoj
  – Eldar Gadžijev
  – Ibrahim Edhemović
  – Erik Brecelj
  – Tjaša Pečnik

• University of Ljubljana, Faculty of Electrical Engineering,
  – Damijan Miklavcic
  – Tomaž Jarm
  – Tadej Kotnik

• CNRS, Institut de Pharmacologie et de Biologie Structurelle, Toulouse
  – Justin Teissie
  – Muriel Golzio
  – Elizabeth Bellard

• Institute Jozef Stefan, Ljubljana
  – Radmila Milacic
  – Janez Scancar

• University of Ljubljana, Veterinary Faculty
  – Nataša Tozon
  – Darja Pavlin

• University of Ljubljana, Biotechnical Faculty
  – Jerneja Ambrožič Avguštin

• University of Ljubljana, Faculty of Medicine
  – Damjan Glavač
  – Mojca Kržan

• University of Primorska, Faculty of Health Science
  – Andrej Coer
  – Cecil J.W. Meulenberg
  – Nataša Tešić

• University of Sheffield, Sheffield
  – Ian Wilson
  – Chryso Kanthou
  – Gill Tozer

• Faculty of Chemistry, University of Bielefeld
  – Eberhard Neumann
• Institute of Oncology Ljubljana
• University of Primorska, Faculty of Health Sciences
• ARRS
• EBAM European Associated Laboratory (LEA)
• COST TD 1104
• Proteus project SI-FR
• Eu projects: Cliniporator, Esope, Angioskin