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Research article:

Treatment of brain tumours with Electroporation in an *in vivo* rat model

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Abstract. The purpose of the study was to examine the possibilities of prolonging the life of rats with brain tumours using electroporation only while conducting impedance scans to evaluate the rate of electroporation. During the experiments, the treated rats in the first batch had tumors grow for 14 days and were then given electroporation with 8 + 8 exponential pulses at 800 v/cm, and 15 μ F. Three of the animals given electroporation and bleomycin and one of the animals given electroporation alone died within three days of treatment. The reason for this may be oedema and increased intracranial pressure due to the large amount of energy delivered to the brain. In the second batch of rats given electroporation with 8 + 8 exponential pulses at 800 V/cm but with the lower capacitance setting of 4.5 μ F, none of the treated rats died within a week after treatment. Among these rats, the tumors had also grown for only 10 days, thus further decreasing the risk of critical increases of the intracranial pressure directly after treatment.

Interpretation of the impedance scans made before and after is made difficult due to factors such as possible short-circuiting of electrodes due to bleeding in the operating area. Not counting the rats in the first batch that died within three days of treatment, the rats treated with electroporation had a significantly prolonged life compared to controls. These results combined with results of a prior study of treatment of brain tumors in rats with electroporation in combination with Bleomycin suggest that this form of treatment may prolong the life of patients with brain tumours.

Keywords: electroporation, impedance

1 Introduction

1.1 Purpose of the study

The purpose of the study is to examine the possibilities of prolonging the life of rats with brain tumours using electroporation while conducting impedance scans to evaluate the rate of electroporation.

1.2 What is electroporation?

In 1974, Zimmerman et al published observations that electric pulses bring about the breakdown of cell membranes [1]. They observed that red blood cells started losing haemoglobin when exposed to pulsed electric fields with a field strength in excess of a certain threshold value.

Immediately after electroporation, molecules and other substances outside cells can pass through the cell membrane for a limited time. This state is temporary and the cell membrane normally regains its normal structure within minutes. Electroporation is used widely in a variety of in vitro biochemical procedures to get molecules such as antibodies, nucleic acids, restriction nucleases and gene constructs into cells. One advantage of electroporation in these circumstances is to be independent of carrier molecules.

In 1987 Okino and Mohri showed that electroporation can be used in vivo to increase the concentration of cytotoxic drugs in solid tumours [2]. A single high voltage pulse was given to subcutaneously implanted tumours in rats after intramuscular injection of the chemotherapeutic agent bleomycin. None of these animals were cured, but it was discovered that the combination of an electric pulse with bleomycin had a strong antitumor effect while neither the electric pulse nor the bleomycin alone had any effect on tumour growth.

The combination of electroporation and cytotoxic drugs has mostly been used for the treatment of subcutaneous and coetaneous malignancies, but treatment of other tumours, such as tumors of the brain [3], the liver [4] and the pancreas [5] has also taken place in experimental animal models.

Clinical trials have also been performed, the first by Mir and colleagues in France 1991 on tumors of the head and neck [6]. Other clinical trials have followed such as those conducted by Heller and colleagues on cutaneous and subcutaneous tumours [7]. The results have been extremely promising. Bleomycin is the chemotherapeutic agent which has had the greatest effect, but other cytotoxic drugs, such as Cisplatin have also been shown to be effective [8].

Studies have shown that some other effects of electroporation are the stimulation of the host's immune system to react against the tumor after treatment [9], and that electroporation has effects on the blood vessels of tissues and tumours, mainly in an obstructive way [10]. It is still not exactly clear how electric pulses open channels in the cell membrane. Different theories are local destabilization of the phospholipid's head-groups, electromechanical forces acting on the membrane [11,12], and permeabilization due to lipid per-oxidation [13,14].

2 Material and methods

2.1 Tumour cells and animal models

The tumour cells used were N32, which are cells that have been transformed using ethyl-nitrose-urea. These cells produce a weak immune response and grow readily intracerebrally on rats. Well-delineated tumours evolve in nearly 100 % of the rats.

Male Fischer 344 rats weighing 193 to 548 g were used in the experiments. These rats are bred by continuous brother-sister mating, are kept in Macrolon cages, have free access to pellets and water and are kept in a room with normal day and night cycles of illumination.

2.2 Electroporation equipment

For electroporation, an HVIT 1.2 kV unit (Aditus Science AB, Sweden), which produces an exponentially decaying pulse, was used. The electrodes inserted into the brain of the rats consisted of two acupuncture needles 0.3 mm in diameter, placed 5 mm apart and isolated with Epoxy glue except for the distal 5 millimetres.

2.3 Measuring equipment

An LVIT 1 unit and a PC running the ZOK program (both by Aditus Science AB, Sweden) were used to measure the impedance of the brain substance between the electrodes immediately before and after electroporation in the frequency interval of 100 - 100,000 Hz. The results were saved on computer disk for later evaluation.

2.4 Operating procedures in general

For anaesthesia, the rats were given Equities in 165 mg chloral hydrate, 84 mg magnesium sulphate, and 40 mg Pentobarbital per kg body weight intraperitoneally (i.p.), or 5 % Chloral hydrate, 6 ml per kg body weight i.p, plus Buprenorphin, 15 µg/kg body weight i.m. The rats were weighed at inoculation, at treatment and at death.

During operative procedures the rats were fastened into a stereotactic instrument. The skin was incised with a scalpel, holes were drilled in the skull using a dentist's drill, and after inoculation/treatment the holes in the skull were filled with bone wax. The skin was closed with two to three non-resorbable sutures.

2.5 Inoculation

At inoculation a hole was drilled in the skull 1 mm behind the coronary suture and 2 mm right of the sagittal suture. By stereotactic technique, a Hamilton microlitre syringe was inserted into the brain tissue until its point was at a depth of 4 mm below the surface of the skull. 10,000 N32 cells were injected during two minutes, and four minutes later, the syringe was removed slowly, with the purpose of keeping the risk of tumor growth outside the intended area to a minimum.

2.6 Electroporation

Two holes were drilled in the skull, 2.5 mm in front and 2.5 mm behind the inoculation hole, respectively. The electroporation needles were stereotactically inserted 5 mm below the skull surface. The impedance between the

needles was then measured. Eight exponential pulses with field strength of 800 V/cm and capacitance setting of 15 μ F for the first batch of rats and 4.5 μ F for the second batch were given one second apart. Four minutes after the beginning of the first pulse series eight identical pulses were given. The impedance was then measured again.

2.7 Bleomycin

Bleomycin is a cytotoxic drug isolated from the fungus *Streptomyces Verticillus* and exerts its effects by producing single and double DNA strand breaks [15]. The bleomycin molecules are hydrophilic and normally enter cells only marginally through certain cell membrane proteins [16]. This, and the fact that bleomycin has a high renal clearance rate makes it a very useful drug for use in combination with electroporation, since the goal is high uptake of the drug in the area given electric pulses and low uptake elsewhere.

The animals treated with bleomycin were given 1 mg/kg bodyweight injections through a catheter inserted into the femoral vein. The catheter was flushed with normal saline before and after injection. The bleomycin bolus was given three minutes before the start of the first pulse series.

2.8 Sacrifice and perfusion fixation

The rats were sacrificed when showing signs of increased intracranial pressure, such as drowsiness and hemiparesis. After anaesthesia by equitiesin or chloral hydrate, the animals underwent perfusion fixation of the upper body half with a standard buffer solution through a catheter inserted into the aorta. The top of the skull bone was removed, the brain removed and placed in a 5 % formaldehyde suspension. The brain was cut in 5 coronal slices, embedded in paraffin, sectioned at 5 μ m and stained with haematoxylin and eosin

3 Results and discussion

3.1 Whole blood and blood plasma

Rats of the first batch were treated on the 14th day after inoculation and rats

in the second batch on the 10th day after inoculation. The first batch consisted of seven rats receiving combined electroporation and bleomycin treatment, seven controls and the five animals receiving electroporation alone. Capacitance was set to 15 μF for these animals. In the second batch, five rats received combined electroporation and bleomycin treatment, and four were controls. The capacitance was set to 4.5 μF . The other parameters were equal. In the first batch survival rates were: Among the rats treated with electroporation and bleomycin 10.9 days (SD +/- 7.1 days); among the control rats 12.7 days (SD +/- 2.4 days) and among the rats receiving electroporation alone 11.8 days (SD +/- 5.6 days) (Fig. 1 a). Three of the animals given the combined treatment and one of the ones treated with only electroporation died within three days after treatment. If those animals are excluded, survival rates were: Among the rats treated with electroporation and bleomycin 16.5 days (SD +/- 1.0 days) and among the rats receiving electroporation alone 14.0 days (SD +/- 3.2 days), thus the rats treated with bleomycin and electroporation lived an average of 3.8 days longer than controls ($p < 0.01$).

In the second batch the survival rates were 15 days (SD +/- 2.2 days) for the treated rats and 12.5 days (SD +/- 2.9 days) for controls (Fig. 1 b), i.e. treated rats lived an average of 2.5 days longer than the controls ($p = 0.21$)

If survival times from animals in both batches (not counting those animals that died within three days of treatment) are put together, rats treated with electroporation and bleomycin lived an average of 3,1 days longer than controls ($p < 0.01$) first batch.

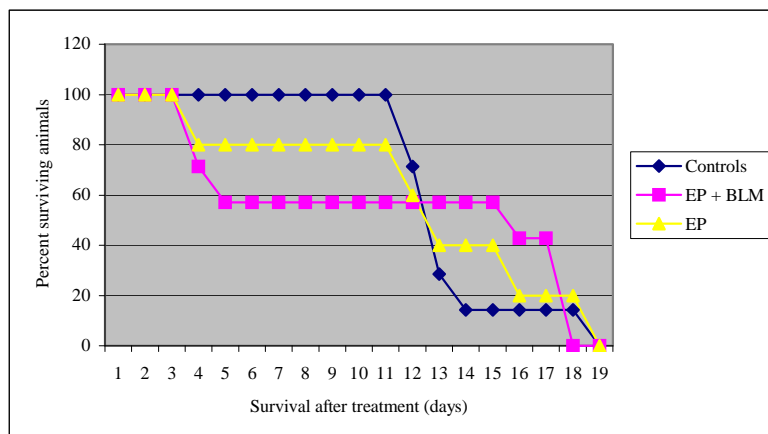


Fig.1 a, Survival after treatment, first batch

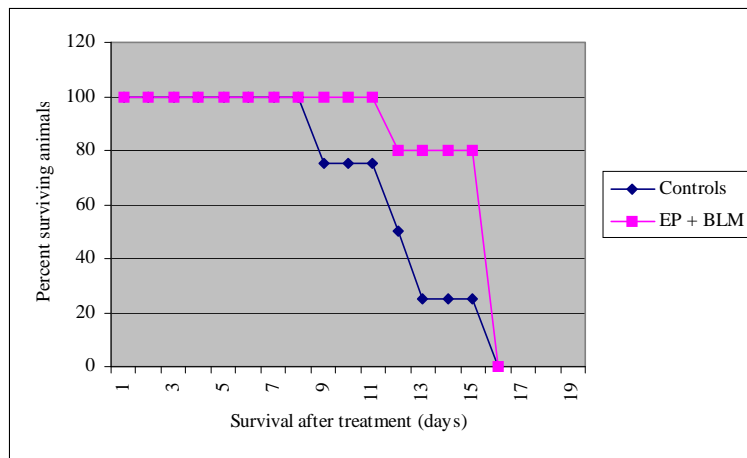


Fig. 1 b, Survival after treatment, second batch

The results of the impedance measurements can be seen in fig. 2 a for the and fig. 2 b for the second batch.

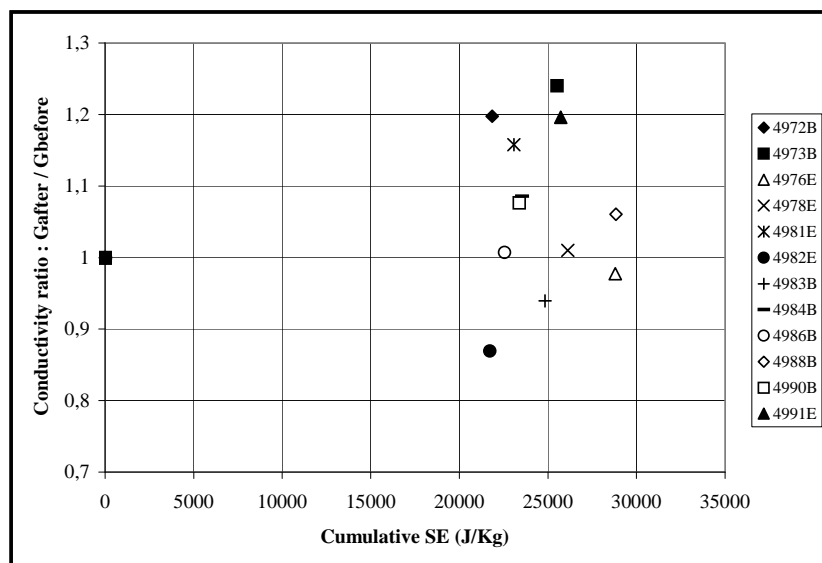


Fig. 1 a, Changes in conductivity, first batch.

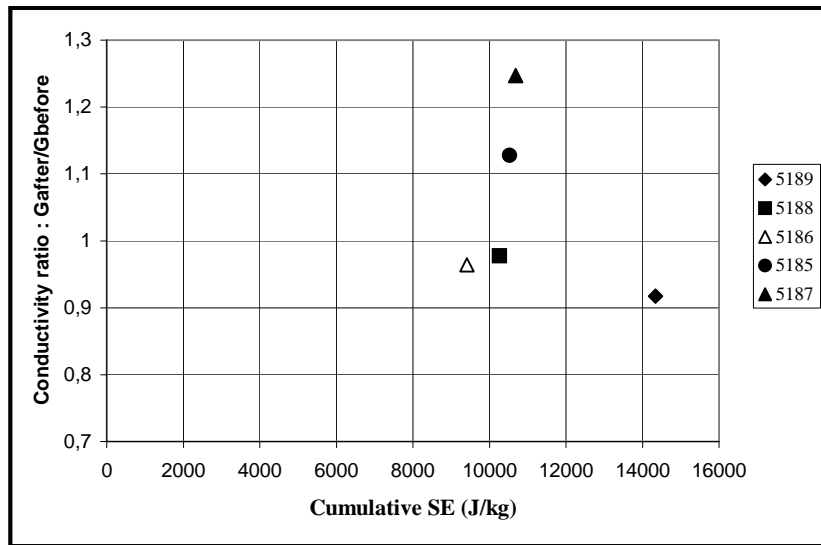


Fig. 2 b, Changes in conductivity, second batch.

4. Discussion and Conclusion (L 14:24/14)

During these experiments, the treated rats in the first batch had tumors grow for 14 days and were then given electroporation with 8 + 8 exponential pulses at 800 v/cm, and 15 μ F. Three of the animals given electroporation and bleomycin and one of the animals given electroporation alone died within three days of treatment. The reason for this may be oedema and increased intracranial pressure due to the large amount of energy delivered to the brain. In the second batch of rats given electroporation with 8 + 8 exponential pulses at 800 v/cm but with the lower capacitance setting of 4.5 μ F, none of the treated rats died within a week after treatment. Among these rats, the tumors had also grown for only 10 days, thus further decreasing the risk of critical increases of the intracranial pressure directly after treatment.

Interpretation of the impedance scans made before and after is made difficult due to factors such as possible short-circuiting of electrodes due to bleeding in the operating area.

Not counting the rats in the first batch that died within three days of treatment, the rats treated with electroporation had a significantly prolonged life compared to controls. These results combined with results of the prior study of treatment of brain tumors in rats with electroporation and

bleomycin [3] suggest that this form of treatment may prolong the life of patients with brain tumors.

Future studies could include the use of intralesional administration of bleomycin. Also, studies could be made with this type of treatment combined with drugs that decrease intracranial oedema such as corticosteroids.

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