

Chapter 11

Drug and Gene Electrotransfer to the Brain

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Abstract Applying drugs and genes to brain tissue using electroporation is a new and upcoming field of research. Because of the blood brain barrier (BBB), the access of agents from the blood into brain tissue is restricted. This could theoretically be overcome by the use of electroporation. In the early 1990s, the first pre-clinical experiments using electrochemotherapy in brain tissue were conducted in rats with inoculated brain tumours; however, the technique has not yet been further explored and applied in humans. This chapter will focus on the pre-clinical experience with drug and gene electrotransfer in the brain, the clinical experience with use of bleomycin in the brain, and the following clinical perspectives. Further discussion of central issues, such as safety and the BBB, will also be discussed.

Keywords Brain • Tumour • Bleomycin • Blood brain barrier • Electrode • Pre-clinical • Electrochemotherapy • Gene electrotransfer

Introduction

This chapter will address the pre-clinical experience with drug and gene electrotransfer in the brain and the following clinical perspectives.

Primary and Secondary Brain Cancer

In the US, around 170,000 cancer patients each year will suffer from brain metastases [1]. We have estimated that in the European Union 72,000 patients per year will develop primary brain cancer and 240,000 patients per year will develop brain metastases. Patients with lung cancer, breast cancer and malignant melanoma are at most risk for developing metastases to the brain. Clinical-, imaging- and autopsy series have shown that about half of brain metastases will be solitary and

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half will be multiple [1]. The most common symptoms of brain metastases are headaches, altered mental status and focal weakness, but seizures and gait ataxia are also observed [1]. The prognosis is dependent on factors such as performance status, control of primary cancer, age, number of metastases and presence of midline shift and post-WBRT response. WBRT, short for whole-brain radiotherapy, is the treatment of choice for patients with multiple brain metastases. Unfortunately, WBRT is a palliative treatment, and the median survival time is 4–6 months. About half of the patients experience an improvement in their neurological symptoms after WBRT [1]. Selected patients can be treated with surgery and stereotaxic radiotherapy. Still, the majority of patients do not achieve local control and frequently succumb to the progressive brain disease [1]. Therefore, there is a desperate need for new treatments that can provide better results.

Brain Diseases

Diseases of the brain cause considerable morbidity, at great cost for the individual patient and for society as a whole [2]. Gene therapy is one of the avenues being investigated for brain diseases, and one of the most investigated disease targets has been Parkinson's disease [3]. The first clinical results of gene therapy with viral vectors show promising results [4]. However, it would be an advantage to perform non-viral gene therapy from a patient safety, treatment cost and an environmental point of view. Since treatment of, e.g. Parkinson's disease involves transfer to a defined target region (the substantia nigra), it would be a valid goal for the use of gene electrotransfer.

There is no clinical experience with the electrotransfer of drugs or genes to the brain yet, thus, the following paragraphs will summarize the pre-clinical experience in this area.

Electrochemotherapy to Treat Brain Tumours: In Vivo

Electrochemotherapy is dealt with in detail in other chapters (Chaps. 6, 8 and 9). In essence, the cytotoxicity of the anti-cancer drug, bleomycin, may be augmented more than 300 times by enabling the permeability of the membrane by application of electric pulses [5–7].

Pre-Clinical Studies on Electrochemotherapy in the Brain

In 1993, the first reported pre-clinical experiments were made by using the principles of electroporation to achieve improved uptake of bleomycin into brain tumours in vivo. The experiments were performed in Fischer 344 rats inoculated with tumour cells and treated with electrochemotherapy. The end point was survival time to observation of symptoms of severe tumour growth (e.g. the rats moving in circles, hemiparesis or drowsiness), and consequently termination after appearance of symptoms. This association study indicated that rats treated with electrochemotherapy (bleomycin) had a better mean survival time (days \pm SD) of 10.3 ± 4.7 ($n = 17$, $p = 0.015$) than untreated 6.3 ± 3.2 ($n = 13$, $p = 0.005$) [8].

Electrochemotherapy has also been given to Fischer 344 rats inoculated with brain tumour cells, with the end point to look for pathological necrotic tissue in the target area suggesting successful elimination of target cells in the treated area [9]. We have experience with the treatment of brain tissue and inoculated brain tumours in Fischer 344 rats with electroporation, bleomycin and electrochemotherapy. In initial studies, nine out of ten rats treated with an eight-electrode device had severely affected and necrotic brain tissue in the target areas. Post-treatment rats showed no obvious

adverse behavioural effects. Some of the rats were monitored *in vivo* by magnetic resonance imaging (MRI) to confirm presence of tumour and treatment [9].

Bleomycin and the Blood Brain Barrier

The blood brain barrier (BBB) is made of non-fenestrated endothelial cells, which makes it highly impermeable, allowing only the passage of small, hydrophobic and uncharged molecules such as water. The BBB is disrupted to different degrees by pathological processes, such as in instances of stroke, a malignant brain tumour, infection, or trauma. After cranial irradiation (WBRT), the BBB is damaged for weeks to months [10].

Bleomycin has been used in the treatment of human brain tumours for several years, but with limited effectiveness. If bleomycin is to be given intravenously, it is essential that the drug passes the BBB and leaves the vascular system, in order to obtain an effect in the target area of the brain. Bleomycin, at its best, is inadequately effective, and at worst is not capable of passing the BBB under normal circumstances, because it is a large, hydrophilic and charged molecule. Electroporation may cause an increased BBB permeabilization. An improved mean survival time was reported for intravenously administered bleomycin and electroporation in rats [8], suggesting that the BBB can be altered to allow penetration of bleomycin through the simultaneous process of electroporation. The observations from intra-cranially administered bleomycin and electroporation in rats [9] suggest that for bleomycin to be effective, it is necessary to use additional methods to alter the BBB. Intra-cranially administered bleomycin had no effect on target brain tissue unless electroporation was also performed.

Particular Considerations for Electrodes Used in the Brain

Previous chapters have dealt in detail with questions on electric field distribution and electrode design (Chaps. 4 and 5). What needs to be mentioned here is that the anatomical constraints associated with electrotransfer in the brain present some additional challenges to electrode versatility.

It is desirable that the electrode may be inserted with minimal damage to healthy tissues. This includes making insertion through a burr hole if possible, and using a relatively small calibre for insertion through normal tissue between the brain surface and the target region (Fig. 11.1).

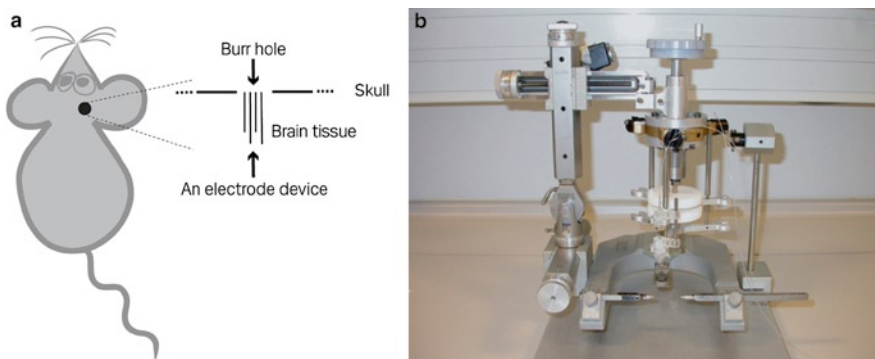


Fig. 11.1 Experimental setup for electroporation in a rat brain. (a) Sedated rat with a burr hole in the skull for intra-cranial injection of tumour cells, drugs and genes, and for insertion of an electrode device for electroporation in soft tissue. (b) Stereotaxic equipment for precise and accurate intra-cranial placement of needles and electrodes in an electroporation setup. The stereotaxic equipment is supplemented by a carousel with three working positions for needles or electrodes useful for electroporation with drug or genes

For example, when treating skin metastatic lesions, treatment failures can easily be handled by re-treatment. Since electrotransfer to the brain is an invasive procedure, one-time-only treatment is the goal, and therefore optimal field distribution for the intended procedure is crucial. Additionally, field distribution should be adequate with a high level of predictability. Accurate positioning of the electrode device is possible by mounting on stereotaxic frames, as those used in neurosurgery, and the equipment will have to be put in neurosurgical units.

Gene Electrotransfer in the Brain

In Vivo Pre-Clinical Studies

Pre-clinical studies have shown that the electroporation technology can be used for gene transfer in vivo [3, 9–15] [17]. The results are obtained at many different conditions depending on the choice of electrode device (invasive or non-invasive, electrode configuration etc.), injection of the gene, pulse generator features, voltage amplitude, and the frequency, number and duration of applied pulses. Results also vary with the choice of species (mice, rats, chickens or hamsters) and the state of the animal (newborn or adults). It is also dependent on the procedures used to evaluate transfection efficiency (fluorescence imaging, protein expression, immunohistochemical staining). As expected within a new scientific field, data is still limited.

In gene electrotransfer studies so far, there have mainly been reports of the green fluorescent protein (GFP) reporter gene transfected into brain tissue by electrotransfer [3, 9–12, 14, 15] [17]. The results were qualitative and, in a few cases, also quantitative based on the expression of GFP measured by fluorescent imaging. The positive results demonstrating expression of GFP signals that the involved cells have been transiently permeabilized, and that the plasmid with the GFP gene has gained access to the cell cytosol and further to the cell nucleus to be expressed. Alterations in the cell membrane must only have been temporary in order to achieve expression of the protein GFP (see Chap. 2).

The gene electrotransfer results were achieved by injection of 0.5–40 μg DNA and using 1–5 pulses at 100–1,000 V/cm for 2–50 ms at 1–10 Hz, and by using invasive as well as non-invasive electrodes. All these heterogeneous parameters suggest that gene electrotransfer can be achieved under several different conditions. From a clinical point of view, the invasive technology may be the most relevant, as treatment of a specific region of the brain will not be achievable with external electrodes. Promising reports have also been shown using genes other than GFP, where gene expression from the gene of interest is obtained [18, 19]. It is also possible to see a “dose-response” relationship between applied voltage and the efficiency of GFP transfected into brain cells [9].

Which Brain Cells Were Susceptible to Gene Electrotransfer?

The different cell types in the brain may have slightly different thresholds for transient permeabilization due to cell size, shape, and basic resting membrane potential. So far the aim of most studies is fulfilled simply by successful gene electrotransfer, but further studies may show evidence of cell type sensitivity and specificity when variable electroporation parameters are used. Meningeal cells and oligodendrocytes [11], neurons and microglia [11], neuronal cells [17], cells in the suprachiasmatic nucleus [12], ependymal cells (in ventricles), putative neural stem or progenitor cells and neuroblasts [9] have all been listed as brain cell types suitable for gene electrotransfer in animal models.

Transgene Expression over Time

The brain tissue is known for its many cells that, once differentiated, principally survive the entire lifetime of the organism. It is also known for its low mitotic activity. Compared to other body cell types like skin and muscle, the brain has the potential for long-term expression of transfected genes beyond weeks, months or even a few years. However, the anti-pathogenic systems may have to be circumvented to avoid elimination of the gene and immediate degradation of the gene product. Among the reports using invasive electrodes for gene electrotransfer, gene expression was shown to last up until 45 days from electrotransfer [12].

Since most papers report visual observations of GFP after termination of the animal, one would hope for new future long-term studies using the available *in vivo* fluorescent technologies.

Safety Issues

For gene electrotransfer in the brain tissue to become a successful technology, there are several safety issues that have to be met. The use of invasive electrodes raises the risks of bleeding and infection. When the electrodes are inserted, there is risk of damage to healthy tissue. There are also the toxic effects of DNA to consider. As an example, when targeting dopamine-producing cells in the substantia nigra of a Parkinson's disease patient with gene electrotransfer, the consequences of non-target cell damage could cause clinical deterioration.

In pre-clinical studies, photomicrographs and pathological staining for gliafilaments, neurofilaments and necrotic cells have revealed that the physical damage as a result of the needles and electrodes is in fact relatively small and basically restricted to areas where these items have passed through brain tissue on their way to the target area. Not surprisingly, smaller electrodes make less damage [11]. Necrotic cells are found along the electrode traces [11], as well as astrocytes and lymphocytes [13]. Gliafilaments are more affected by electrochemotherapy than neurofilaments, as the effect on neurofilaments is restricted to the target area, whereas it goes beyond the target area for the gliafilaments [9]. Behavioural observations in a single pre-clinical study showed no signs of adverse behavioural effect after application of electroporation and inoculation of the gene [3].

Only a few of these safety issues concerning electroporation of brain tissue are relevant to virus-mediated gene therapy, which has other major safety concerns, such as strong immune response to *vira*, toxicity and limitations in restricting *vira* to the target area.

Clinical Perspectives on Drug and Gene Electrotransfer in Brain Tissue

Where Are We Now

The future holds a lot of challenges for electrotransfer of drugs and genes to the brain. Among these are the electrodes. Electrodes need to be physically flexible in length, shape and selection (see Chap. 5). For gene electrotransfer, the demands of the electrodes primarily focus on their ability to be selective, in order to obtain gene transfer in highly restricted areas of the brain.

In the clinic, neurosurgeons have the necessary technology available to target any area for gene electrotransfer by coordinating using stereotaxy. Only the prospect of severe and non-recoverable brain damage made in the attempt to reach the target by the electrodes may withhold the surgeons from using the available technology. Figure 11.2 shows a schematic view of the Ellisphere brain electrode in action in the clinical setting.

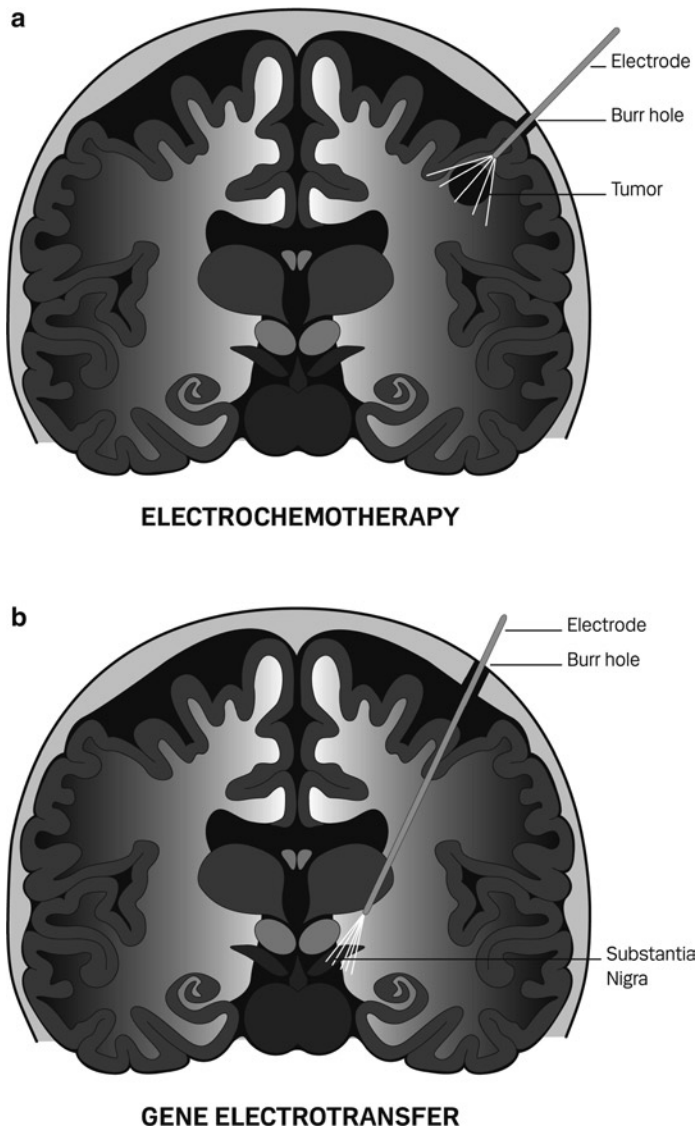


Fig. 11.2 Schematic drawing of methods for applying electrochemotherapy (**a**) and gene electrotransfer (**b**) to brain tissue in the clinical setting. A more detailed description of the concept behind this electrode device can be found in Chap. 5, Sect. 5.2, the Ellisphere system

With regard to electrochemotherapy, there are relevant data from clinical experience with bleomycin directly injected into a brain tumour or cyst. Bleomycin is a drug often used for electrochemotherapy, and has been used in the treatment of brain tumours for selected cases for more than 30 years as single drug injections. This experience can be used to evaluate the toxicity of bleomycin in brain tissue for future use of electrochemotherapy in the brain. Currently, there are two major indications for the use of bleomycin in the brain: solid tumours, such as glioblastoma, and cystic tumours in the form of craniopharyngiomas [14]. Unfortunately, the clinical benefit has been limited, but that is probably due to the nature of bleomycin and the BBB. Bleomycin is a hydrophilic and charged molecule, which poorly passes the intact plasma membrane [15]. The BBB is

nonpermeable to many types of chemotherapy, and that is certainly true for a large molecule such as bleomycin with characteristics as mentioned above. This is reflected by the clinical results, because local bleomycin treatment works better on a thin-walled cyst and practically not on solid tumours [23–26]. The clinical effect on brain tissue is also dose dependent, which is also true for the adverse effects. In a review of the adverse effects of bleomycin used in the treatment of solid and cystic brain tumours, there were only a few cases of serious adverse effects [14].

A future clinical trial is planned, with the purpose of investigating the efficacy and safety of electrochemotherapy using bleomycin in the treatment of secondary brain tumours. The rationale behind this protocol is that clinical trials have shown improvement in neurocognitive function and survival with tumour volume reduction [27–29]. The total tumour volume is therefore an important clinical parameter and could be diminished by electrochemotherapy.

No clinical studies have yet been performed for brain tissue gene therapy, but Parkinson's disease is an obvious candidate for gene therapy. It represents a disease where the dopamine production is disturbed, and where dopamine level restoration is an acknowledged and eligible treatment of Parkinson's disease symptoms [3]. The target of gene therapy would be the substantia nigra, to attain regeneration and control of the dopamine production *in vivo* (Fig. 11.2b). The ability to target specifically with gene electrotransfer may therefore make it the method of choice for gene therapy in highly restricted and small target areas.

Treatment of brain disorders is a major challenge, and it will be highly interesting to see the future research in the area of gene and drug electrotransfer to the brain.

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