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Review article

TREATING TUMORAL DISEASES (CANCER) WITH A COMBINATION OF IONIZING RADIATION AND PULSED ELECTRIC FIELDS.

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ABSTRACT

The aim is to review a patented method and apparatus for treating tumor diseases (cancer) with a combination of ionizing radiation and pulsed electric fields that might open a new regime of radiation therapy fractionation with a short intensive treatment with high radiation dose fractions combined with pulsed electric fields.

Sub-optimal radiation treatment was administered separately or in combination with electric pulses of field strength (1200 V/cm) to subcutaneous implanted rat brain tumors on both hind legs. The treatment was repeated four consecutive days and evaluated by recording tumor growth rate and microscopically examination. Tumors were stained for Factor VIII/von Willebrand Factor to investigate effects on the tumor vasculature and for T-lymphocytes CD4 and CD8 to study their infiltration into the treated tumors.

Radiation and pulsed electric fields applied concomitantly resulted in rapid tumor regression with significantly ($P<0.01$ vs. Control) prolonged survival (tumor free >80 days after treatment) and an average cure rate of 67%. Animals treated with either just radiation or just Pulsed electric fields showed no significant enhanced survival. Histological and immunohistochemical examination of tumors treated with RAD and PEF showed instant and severe deteriorating effects on tumor vasculature and enhanced infiltration of T-lymphocytes.

Key words: CD4; CD8; Pulsed electric fields; Radiationtherapy; Rat glioma tumors; Reactive oxygen species (ROS), Tumor vasculature;

General Background

Radiation therapy

Radiation therapy is beside surgery still the most widely used therapy modality for cancer treatment and in developed countries it is given to one out of two patients with cancer (Knöös, 1991, SBU, 2003)

Although a lot of progress is made in development of new methods and techniques for radiotherapy. about half of the curatively intended treatments fail as a result of either distal or local recurrences (DeVita, 1983). Improvements of local radiotherapy are still focused on fractionation and higher absorbed dose to the clinical target volume without exceeding the tolerance of surrounding normal tissue (Suit, 2002, Suit et al., 1988, Suit and Miralbell, 1989). Conventional radiation therapy refers to the therapeutic effect of the direct exposed tumors and to minimize effects on tissues outside the treated target area,

Electrical Pulse therapy

Tumour treatment by applying high voltage pulses can by itself create cellular and sub cellular lesions that cause cell death or apoptosis (Engström et al., 1998a, Engström et al., 1998b). The direct effect of electrical pulses PEF on cell tissue is mainly by dielectric breakdown of the cell membrane (Weaver, 2003, Weaver and Chizmadzhev, 1996).

There is also an indirect due to formation of a variety of reactive oxygen species. Lipid per-oxidation induced in the membrane area being permeabilized appears to be correlated to cell survival (Maccarrone et al., 1995a, Maccarrone et al., 1995b).

PEF induces reactive oxygen species that are associated with the part of the membrane that is permeabilized (Gabriel and Teissie, 1994). The technique of detecting free radicals based on chemo-luminescence has shown that cell survival is directly correlated to the extent of oxidative stress. In biological systems the formation of hydroxyl radicals - from hydrogen peroxide follows the Haber-Weiss reaction. These radicals enable single strand breaks in DNA (Mello-Filho and Meneghini, 1984). Such DNA damage induced by PEF has been experimentally verified by the formation of increased number of strand breaks with increased charge of the applied pulses (Meaking et al., 1995, Meldrum et al., 1999;). The field strength and pulse length seems to act independently in the creation of DNA strand breaks. The observed absence of a reduction in DNA breaks with time indicates that there is little, if any repair of PEF-induced DNA damage. Hoffmann et al. have recently demonstrated the induction of apoptosis and activation of caspases from single exponential decaying electric pulses of high field strength (Hofmann et al., 1999). The similarity of survival curves of both cells and in vivo tumours treated with radiation or pulsed electric fields indicate a similar killing mechanism (Danfelter et al., 1998, Baureus-Koch et al., 2004).

Pulsed Electric field therapy causing irreversible electroporation has shown promise as a therapy for soft-tissue neoplasms, using minimally invasive instrumentation and allowing for treatment monitoring with routine clinical procedures (Al-Sakere et al., 2007, Lee et al., 2007, Maor et al., 2010, Maor et al., 2009, Rubinsky et al., 2007, Rubinsky et al., 2008).

Pulsed Electric Field treatment for malignant Glioma

Tumour treatment witb Combined Radiation "RAD" and Pulsed Electric Field Exposure "PEF"

In previous studies it was found that a combination of chemotherapy and PEF increased the survival rate of rats with RG2 brain tumours implanted in the brain (Salford et al., 1993). In 1965 I got the idea to compare the effect with PEF and radiation therapy (RAD) and their combination. The combined treatment of V-79 fibroblasts with a combination of radiation with an absorbed dose of 2 Gy and exponential shaped pulsed electric fields with an amplitude corresponding 1600 V/cm and a time-constant of 1 ms with 1s repetition resulted in remarkable effective killing of the cells (Danfelter et al., 1998).

Radiation therapy alone is known to have limited therapeutic effects on brain tumours but the combination with high voltage pulses has never been studied before. Influenced by the encouraging results from V79 the combined treatment PEF+RAD was applied in a rat tumour model with implanted tumours. Combined daily treatments doing 4 days with ^{60}Co-gammaradiation (2 Gy) and pulsed electric field (1300 V/cm, 16 exponential pulses, τ=1 ms) was given to Fischer rats with N32 tumours implanted on the flank of Fischer rats. Each experiment was performed on four different groups with 6 animals in each:

- Untreated Controls; (Ctrl)
- Radiation therapy only; (RAD)
- Pulsed electric field only (PEF)
- Combined Radiation therapy and Pulsed electric field. (RAD + PEF)

The radiation therapy vas given an SSD of 80 cm and the tumour was marked off with lead blocks to avoid irradiating fresh tissue. The Pulsed electric field was applied around the tumour by using an isolated slide-calliper with plane electrodes (15x15 mm) mounted on the blades. Tumour response was studied by measuring the length, width and thickness with the slide-calliper and estimating the tumour volume as an ellipsoid.

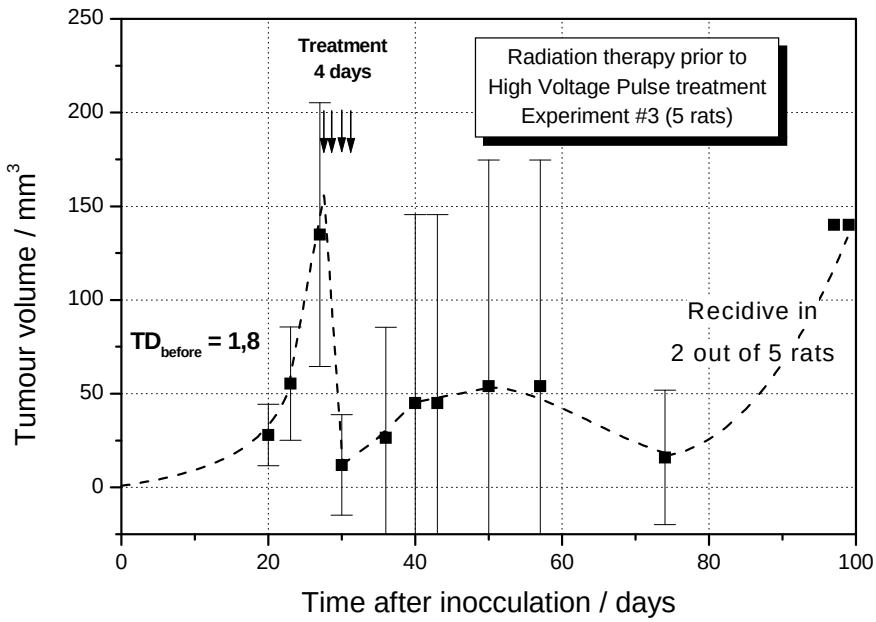

Figure 1. Effect of combined treatment in 4 consecutive days first with ^{60}Co-radiation 5 Gy and then 16 High Voltage Pulses (1300 V/cm, 1 m).

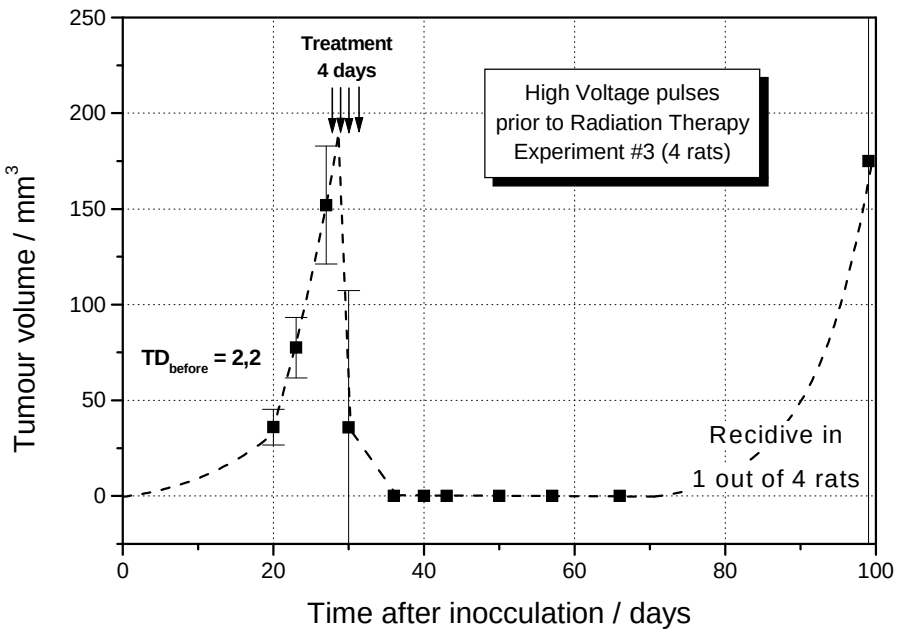

Figure 2. Effect of combined treatment in 4 consecutive days first with 16 High Voltage Pulses (1300 V/cm, 1 ms) and then with ^{60}Co-gamma radiation 5 Gy.

In another experiment the animals were arranged into five groups. One group of 8 controls

(Ctrl) received no treatment. Treatment with radiation (4×6 Gy) was given to 5 rats (RAD) and treatment pulsed electric fields (4× 8 pulses of 1400 V/cm) to 4 rats (PEF). The combined treatment was done in two groups, 4 rats were given pulsed electric field followed by radiation (PEF+RAD) and 5 rats radiation treatment followed by pulsed electric field treatment (RAD+PEF). The total absorbed radiation dose given to the tumours was 20 Gy, given in 4 fractions of 5 Gy each day in four consecutive days. In the combinations the second treatment was conducted 10 to 15 minutes after the first treatment. The whole combined treatment session was finished after 4 to 5 minutes.

Animals (single-treated and controls) were sacrificed when the tumour had reached a size of 5 cm^3. The average time to this event was defined as "survival time" and is given in table 1 as average for the various groups. There was a significant difference ($p<0.05$) in survival time between the combined treatment and the controls.Fisher's exact probability test of 2×2 contingency tables was used to calculate p-values of significant differences. The number of cures was significantly larger for the EP+RT group compared to the control group ($p=0.009$) and the RAD group ($p=0.03$). There were no significant differences in number of cures for PEF vs. controls or PEF vs. RAD.

Table 1. Treatment data, summary of results and statistics

Treatment	*Survival (days) excl. cures*	TGD (%) *of non-cured*	*N*	*Number of cures (CR>80 d post treatment)*	*Fisher's exact probability test compared to the control group*
Control	41.5 ± 1.1	0	8	0	
RAD	50 ± 1.1	21	5	0	NS
PEF	70 ± 14.6	69	4	1	NS
PEF+RAD	98.3 ± 0.7	137	9	6	P<0.01 (vs.Control) P<0.05 (vs. RAD)

Results from combined treatment with ^{60}Co-gamma radiation therapy (RAD) and pulsed electric field therapy (PEF) show that the average time when the tumour had reached a size of 5 cm^3:

> 94 days; when treated first with RAD then PEF
>100 days; when treated first with PEF then RAD

All treated animals showed an initial partial or complete tumour remission within a few days after the end of the 4-day treatment. For the combination radiation RAD: 4×5Gy and then PEF: 4×(1300 V/cm), Three out of the 11 treated animals had complete remission lasting more than 100 days. For the combination 4×PEF (1300 V/cm) and then radiation 4×5Gy, 3 out of the 4 treated animals had complete remission >100 days. The outcome of this preliminary study of combining pulsed electric field and radiation therapy suggests an astonishingly effective therapeutic response (Engström et al., 1999a, Engström et al., 1998a, Engström et al., 1998b, Engström et al., 2001, Engström et al., 1999b) It is thus worthwhile to further explore this combined type of treatment to improve the therapy of resistant tumours. It might be of particularly interest to apply high voltage impulses combined with brachy-therapy where the radioactive source (i.e. iridium) can serve as electrode.

T-lymphocyte infiltration

Infiltration of T-cells CD4+ and CD8+ have been studied in subcutaneous implanted N29 tumours after various type treatments. The tumours was excised after treatment and embedded in paraffin. The microscopic slides were scanned in a CCD scanner and the degree of staining was evaluated by image processing (Image Pro 5). The scanned images are shown in **Fig**. **3** and **Fig**. **4** below.

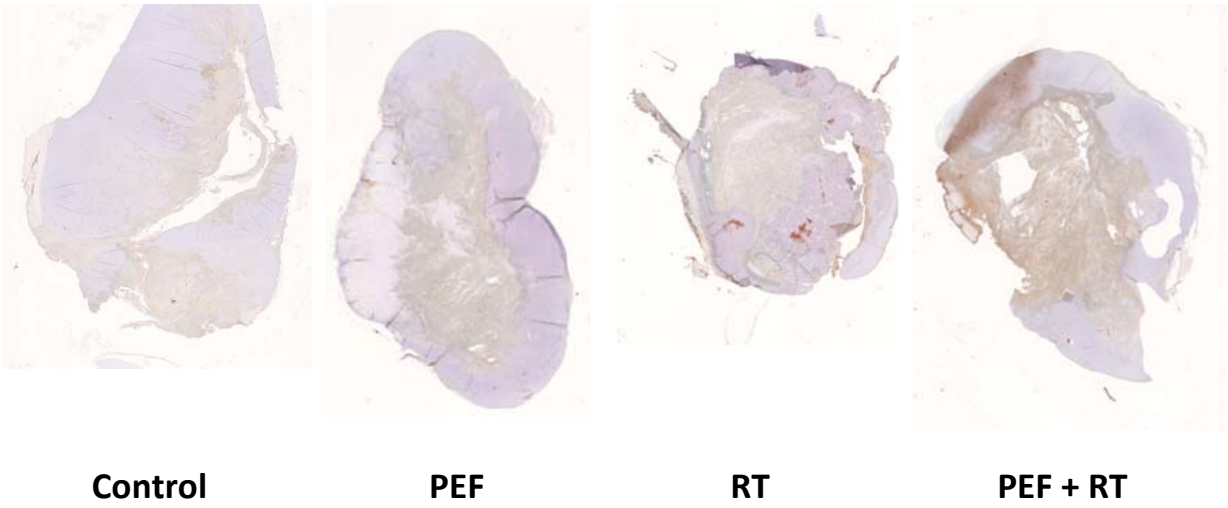

Figure 3. CD4+ infiltration in tumours of various treatment: Controls, Pulsed electric fields PEF (1400 V/cm), radiation therapy RT (4x5 Gy) and the combination PEF+RT

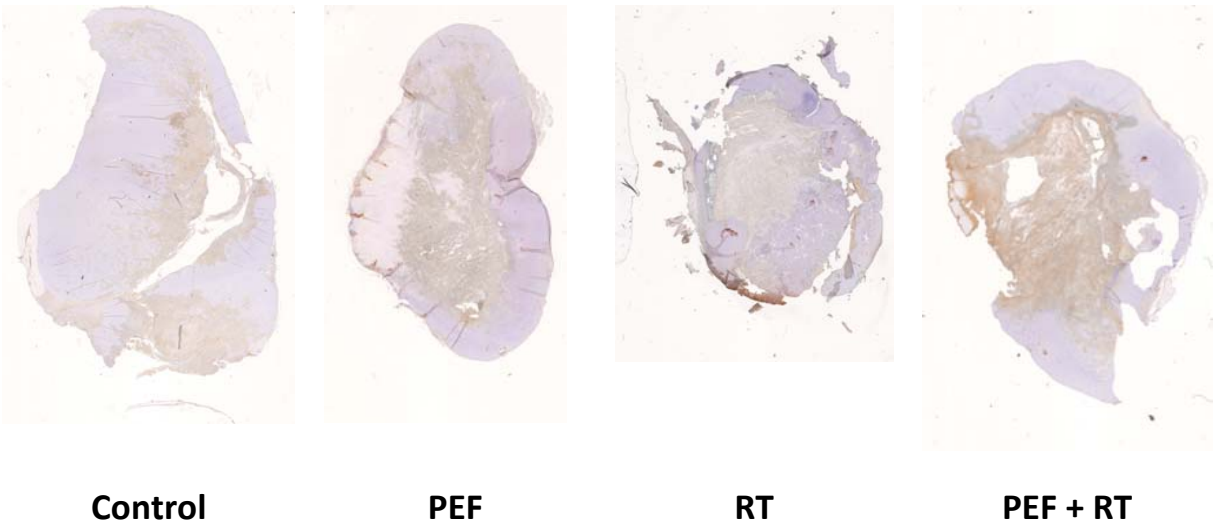

Figure 4. CD8+ infiltration in tumours of various treatment: Controls, Pulsed electric fields PEF (1400 V/cm), radiation therapy RT (4x5 Gy) and the combination PEF+RT

In **Fig**. **5** and **Fig**. **6** are given the average results of the area of tumour stained for CD4+ CD8+ respectively after various number of different treatments for each group of treatment..

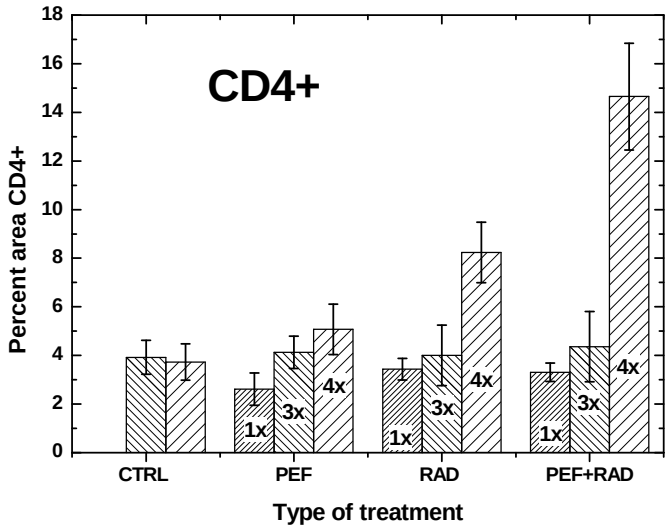

Figure 5. Percent area of tumour stained for CD4+ after various number of different treatments: Controls (CTRL), Pulsed electric fields (PEF 1, 2 and 4x 8 pulses of 1400 V/cm), radiation therapy (RAD 1, 3 and 4x5 Gy) and the combination (PEF+RAD 1, 3 and 4 times of each).

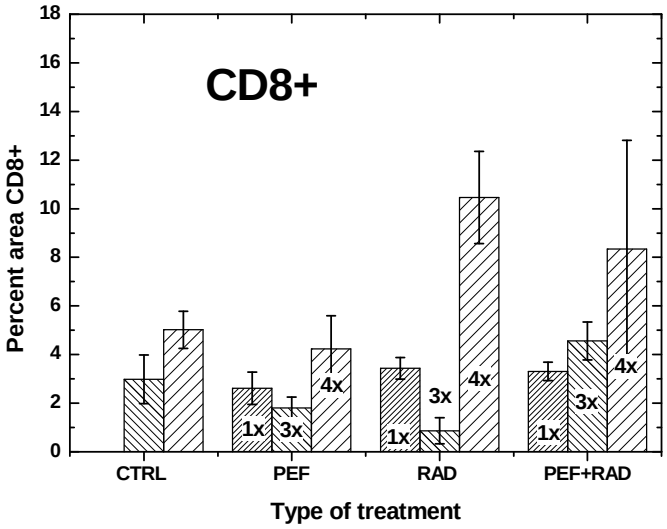

Figure 6. Percent area of tumour stained for CD4+ after various number of different treatments Controls, Pulsed electric fields PEF (1, 2 and 4x 8 pulses of 1400 V/cm), radiation therapy RT (1, 3 and 4x5 Gy) and the combination PEF+RT.

Elevated sphingomyelinase activity and Ceramide.

Ceramide is a bioactive compound involved in regulation of apoptosis as well as differentiation of the cell. It is produced by the enzyme sphingomyelinase (SMase) which is acting on sphingomyelin, a constituent of the cell membrane. Ionizing radiation and pulsed electric fields acting directly on the cell membrane induce ROS that stimulate the enzyme sphingomyelinase to hydrolyze sphingomyelin which induce production of ceramide. Thus ceramide generated by interaction of ionizing radiation with cellular membranes cause apoptotic signalling which create an alternative to DNA damage for radiation-induced cell death (Fuks et al., 1994, Garcia-Barros et al., 2003, Haimovitz-Friedman et al., 1996, Paris et al., 2001).

Recent studies have shown that a protein product of Acid SMase, named secretory sphingomyelinase S-SMase is secreted into the circulation under inflammatory conditions (Roth et al., 2011, Wong et al., 1999, Marathe et al., 1998, Marathe et al., 1999). S-SMase is activated during radiation therapy at high absorbed dose fractions and cause elevation of ceramide in low-density lipoprotein (LDL) particles present in the serum of the patients (Sathishkumar et al., 2005). The potential significance of these increases has been shown by isolating and analyzing low-density lipoprotein (LDL) particles from the serum of patients undergoing radiation therapy. These ceramide rich LDL particles also sensitize human micro vascular endothelial cells to apoptosis in response to conventional dose fractions of radiation.

Exponential decaying electric pulses of high field strength produce ROS that cause an inflammatory tissue reaction that produce S-SMase that increase of ceramide in circulating LDL particles which activate caspases and induce apoptosis in endothelial cells (Hofmann et al., 1999, Pinero et al., 1997).

An immunohistochemical study of vascular integrity was performed to investigate the possible role of the vasculature in the tumor necrotic effect after PEF treatment. Immuno-histochemistry was applied using a fluorescent antibody to factor VIII-related antigen (the von Willebrand Factor, vWF) as a marker for endothelial cells in micro vessels (Engström et al., 1999a, Engström et al., 2001, Engström et al., 1999b). A tumor treated with radiation only, excised 36 hrs post treatment show clearly demarcated endothelial walls of apparently unaffected blood vessels. After PEF + RAD after 2 hours endothelial cells of the vasculature in the *tumor* rim show intense expression of vWF. After 36 hours post PEF +RAD treatment, however, the major vessels appear fragmentarily stained indicating a commenced breakdown of the vasculature. The smaller vessels are still clearly stained and appear vital. Stained areas of endothelial cells three days (72 hours) after PEF treatment are shown shows no intact vessels and only scarce, fragmented areas of stained endothelial cells.

These results thus suggest that radiation and pulsed electric fields stimulate S-Mase forming ceramide that may contribute to the improved clinical response by anti-angiogenesis and imply that abscopal effects may be involved (Persson et al., 2010, Baureus-Koch et al., 2004).

Electro chemotherapy

Electro chemotherapy of solid tumors is potentiated by increased delivery of therapeutic drugs into tumors by means of PEF. The principal mechanism of this therapy is increased permeabilization of cell membranes, resulting in increased inflow of the chemotherapeutic drugs such as bleomycin and cisplatin to the cell interior (Belehradek Jr. et al., 1994, Belehradek et al., 1991, Cemazar et al., 1998, Kanduser et al., 2006). In clinical settings electro chemotherapy demonstrate excellent antitumor effects in different coetaneous and subcutaneous tumors, predominantly in palliative care, resulting high fraction (up to about 80%) objective responses of the treated tumor nodules (Gehl et al., 2006, Andre et al., 2006, Campana et al., 2009, Quaglino et al., 2008, Andre et al., 2008).

Radio sensitization of tumors by bleomycin or cisplatin is used clinically in the treatment of locally advanced esophageal cancer and of head and neck tumors (Kleinberg and Forastiere, 2007, Fontenot et al., 2003, Chi et al., 2003, Budihna et al., 2005, Strojan et al., 2008, Suntharalingam et al., 2001). Thus evidently electro chemotherapy with bleomycin or cisplatin preceding single-dose radiation therapy significantly enhances the therapeutic effect. This effect has been demonstrated in animal tumor models as well as in a clinical case (Cemazar et al., 2003, Kranjc et al., 2005, Cemazar et al., 2009, Cemazar et al., 2001, Andre et al., 2008). Radiation therapy combined with electroporation and a low concentration of doxorubicin hydrochloride also enhanced significantly the antitumor effect (Shil et al., 2006, Shil et al., 2005, Cemazar et al., 2003).

The combination of electro chemotherapy with bleomycin and fractionated radiation therapy has been studied in two murine tumor models with different histology and radio sensitivity and compared it to single-dose irradiation. Skin damage in the irradiated area was also evaluated. (Cemazar et al., 2003, Kranjc et al., 2005, Cemazar et al., 2009). The result of all those studies brings electro chemotherapy combined with radiation therapy close to clinical use. The aim of electro chemotherapy is, however, still local increase of drug uptake and is mainly used for palliative treatments.

Patent History

Based on the remarkable effect of the combination of radiation and pulsed electric field treatment a Swedish patent entitled "Devices for electrodynamics radiation therapy of tumor diseases" was applied for at July 18 in the year 1996. It was written in Swedish and thus not easy accessible abroad (Persson, 1998a).

International application entitled "A METHOD AND AN APPARATUS FOR TREATING TUMORAL DISEASES (CANCER)" was published 1998 April 9, under the patent cooperation treaty (PTC WO9814238 (A1)) (Persson, 1998b). In the **Fig**. **7** below the block scheme describe the device as an apparatus (#40) that according to the invention includes means (#34) for ionizing radiation and a high voltage generator (1) for generating brief voltage pulses for voltage application of electrodes (#6, 15, 16, and #24) included in the apparatus.

The electrodes are designed to be secured at or introduced into tissue in a restricted region of a human or an animal and to form between them an electric field in the tissue. The means (34) are provided to emit ionizing radiation to a tumor in the tissue in that region which is to be treated, while the electrodes (6, 15, 16, 24) are disposed to be placed in or at the tumor in order that the electric field pass through the tumor.

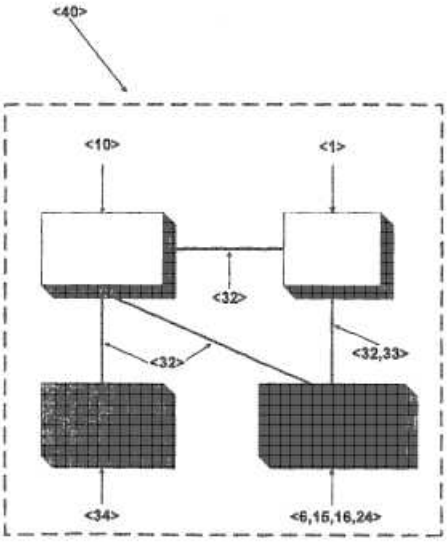

Figure 7. Block scheme describing the device as an apparatus (#40) including means (#34) for delivering ionizing radiation and a high voltage generator (#1) for generating brief voltage pulses for application of electrodes (#6, 15, 16, and #24) included in the apparatus.

United States patent was applied for in 1997 October 1 (Persson, 2001). It was, however, difficult to convince the examiners of the patent application that this was an electric field exposure device and not primary a magnetic field exposure device according to Liboff (US Patent 5,211,622 Liboff Method and apparatus for the treatment of cancer) (Liboff et al., 1995).

The Liboff´s method use an external magnetic field generated by electric current in windings, while with the present device an internal strong electric field is applied through electrodes inserted into the tissue of the subject. Thus the two methods are based on quite

different mechanism. Liboff's method relies on modulation of specific ions by applying external magnetic field of a frequency tuned to the cyclotron resonance of the ion in question.

While in the present method, however, internal electric fields are applied from electrodes inserted in the tissue that produce reactive oxygenized species "ROS" in the exposed tissue. The biological effect of these oxygenized species on the tissue is further enhanced by free radicals from ionizing radiation exposure. At the time of the patent application the use of pulsed electric field in combination with radiation was not mentioned at all in any other patent or publication. Thus it does not seem obvious to combine the use of pulsed electric fields and ionizing radiation for cancer treatment. Further the method described in Liboff's patent is just speculative and not proven to be effective for treatment of cancerous tumors by *in vivo* experiments. The electric field method, however, has been proven to be extraordinary efficient for treatment of brain tumors implanted on rats. These tumors are one of the most severe types of cancer, and are extremely difficult to treat. By using the method described in the patent, however, we obtain 85% complete remissions in this otherwise incurable tumor. That's make the electric field method unique concerning the tumor treatment effect.

Neither electric field nor ionizing radiation, that is the main issue of the present method, is mentioned in Liboff´s patent 5,183,456. Liboff´s patent 5,183,456 describes the applying and measurement of magnetic fields, using coils and magnetic sensors (Hall element). This control is done to adjust for the temperature changes of the resistivity of the windings in the coil. To stay at resonance condition it is important to have a highly constant magnetic field in the tissue. In the present device electric fields are applied through applied electrodes or inserted needles. The electric field in tissue that creates the effect is highly variable. The effect of the electric field can be recorded by measure the change in the electrical impedance of the tissue between or during the electric pulses. These measurements can be used as feedback control of the applied voltage to optimize the effect of treatment (Glahder et al., 2005).

Another patent by Takanaka ("Beam Supply device" by Masao Takanaka) concerns a device for supplying particle radiation beam to therapy by using rotatable electromagnet. No electric fields are applied to the patient in the fashion described by our method. The claims of patent 5,349,198 concerns the beam transport of charged particles and not gamma radiation as apply to our method. Further patent 5,349,198 does not contain any method of modulating the radiation therapy effect by using pulsed electric field as claimed by our method.

The object is a beam supply for charged particles and devices for deflecting the beam. This has no concern at all in our patent application. The radiation can be generated by any radiation source that can be either an accelerator or radioactive material and administered externally or internally in the patient. In this paragraph is not mentioned anything about electric field that is the main issue of our patent to be combined with the ionizing radiation.

The US Patent 5,585,643; by Johnson concern a method and apparatus for directing electron radiation to subcutaneous cells. This is a generally description of radiation therapy not mentioning any combination with pulsed electric fields that is related to our invention. A miniaturized copy of the technique is described and it is obvious that a miniature device intraoperative radiotherapy is more inexpensive than a large accelerator. It does, however, not mention any combination of that device with applied electric fields. The method and apparatus described in patent 5,585,643 by Johnson might as well be equipped with an organ for applying electric fields but that is not mentioned by him and thus not an obvious alternative.

Intra operative radiotherapy is one of many radiation therapy alternatives by which our method can be applied. There is a special electrode applicator is described in the present patent (**Fig**.7) that is aimed to be used by the surgeon in connection with intra operative

radiation therapy procedures and treatment of superficial nodules (Persson, 1998a). It consists of two shanks with electrodes which grip the tumor nodule. The field strength between the electrodes is adjusted with the aid of an integrated distance sensor connected to the computer controlling the device.

Since Liboff's patent apply to magnetic fields it might be obvious to apply this for radiation therapy according to Tanaka. Our invention, however, is based on the use of pulsed electric fields and there has not been any rational of doing this before the discovery made by the inventor and thus it is not obvious.

The description of the arrangements of the electrodes is necessary to give a complete understanding of the action of the device. It is, however, by no means obvious how to apply the electrodes to give the optimal field distribution. In clinical practice there will be a dose-planning procedure involved that gives the best pattern for placing and exciting the pulsed field. The matrix applicator described in our patent gives the best flexibility for treating tumors of various size and shape. Nothing similar has ever been described for the purpose of a combined treatment with pulsed electric fields and radiation.

If the suggestions made by the examiner about the accuracy of the electric field and the use of insulated electrodes are correct, they are, however, not obvious to us. In contrary to the reviewers comment, un-insulated electrodes do create a stronger more directional field in tissue than insulated electrodes. The purpose of mention the alternative with insulated electrodes was just to exclude possibilities to get around the claims.

The electrode applicators for use in cavities are not of an obvious design. Since these electrodes apply to the interior surface of the cavity, the applicators are designed with electrodes at different distances (as shown in the patent) that give the possibility to modulate the depth of action by applying different potential on the various pair of electrodes. Thus the ways the electrodes are designed are by no means obvious (Persson, 1998a, Persson, 1998b).

The statement made by the examiner that electric field generated through the tumor reduce or sterilize tumors is one of the original observations made by the inventor and is not obvious at all. In order to be effective it requires the special amplitude, pulse length and pulse shape mentioned in the patent. It is even less obvious to combine this finding with radiation from brachytherapy sources or radioactive seeds.

The claim of having electrodes of porous material to include therapeutic substances is not an obvious since it requires no other way of administration the therapeutic agent. By using this type of electrodes a minimal amount of agent is administered and the efficiency of uptake in the cells is high due to the focused electric field distribution close to the electrode. Thus by using this technique low amplitude pulses can be used for the benefit of the patient. In case the therapeutic substance is plasmid DNA this technique also decrease the detrimental effect of the electric field on the DNA. Thus this type of electrode is not obvious at all.

DISCUSSION AND CONCLUSIONS

Radiation therapy (RAD) in combination with pulsed electric fields (PEF) was discovered to be an efficient therapy particularly for highly resistant tumors (glioma). A distinct efficient antitumor effect of the combined treatment of electric pulses and radiation treatment was observed while the separate treatments were not effective one by one. This is probably due to a synergistic effect of PEF and RAD on the endothelial cell membranes caused by enhanced ceramide level that initiate apoptosis of endothelial cells and collapse of the microcirculation (Engström et al., 2001, Engström et al., 1999b, Persson, 1998a, Persson, 2001, Persson et al., 2004).

The discovery was made when sub-optimal radiation treatment was administered separately or in combination with electric pulses of high field strength to subcutaneous implanted rat brain tumors. The treatment was repeated during four consecutive days and evaluated by tumor growth delay TGD and microscopic histopathologically examination. The effect on the tumor vasculature was confirmed by staining the excised tumors for Factor VIII/von Willebrand Factor. The infiltration of T-lymphocytes in the tumor after the combined treatment was enhanced compared to the single treatments.

Animals treated with PEF and/or RAD tolerated the treatment well. In some cases PEF caused a temporary stiffness of the treated leg due to partial exposure to the thigh muscle. The PEF treatment were administered transferral using plate electrodes, which results in a strong electric field in the skin due to the high resistivity of especially the epidermis (Prausnitz, 1996). Occasionally, a small-blackened crust was formed over the tumor after the pulse treatment, with subsequent growth of new skin beneath the crust.

The overall picture gained from the histopathological results and the von Willebrand (factor VIII) staining, is that tumors in Controls and RAD groups are clearly more vital than in the PEF and RAD + PEF groups. Radiation alone had only minor effects on tumor growth. The application of electric pulses clearly affected *tumor* vitality and is suspected and the sole cause of changes in group PEF and that these changes develop and mature over time. Tumors excised early (2 to 6 hours) after electric pulse treatment show fresh hemorrhages and edema in the vital part of the tumor, which indicate almost immediate vascular damage. However, the rim cells of these tumors were vital with no apparent sign of karyolysis or membrane disruption. A clear correlation was found between increasing signs of necrosis development and time after treatment. After 72 hours, the histology of the tumor cells was markedly different compared with the early excisions.

Oxidative stress is one of several factors known to induce programmed cell death, i.e. apoptosis (Brielmeier et al., 1998). Impaired oxygen supply due to extensive vascular damage or ischemia also cause apoptotic tumor cell death. Noticeable and long lasting (> 24 hrs) decrease in tumor blood flow after application of high-voltage electric pulses (Sersa et al., 1999). and this ischemic effect has been observed also in other studies (Ramirez et al., 1998).

The main issue of the patent is that pulsed electric field can be used as a physical radio-sensitizing agent. This action is not described in any of the patents that are referred to by the examiner as reason to reject the claims. Other referred patents do not mention electric fields at all. They describe the use of external magnetic fields either to modulate specific ions or deflecting the beam of electron or proton radiation.

The combination of pulsed electric fields and radiation for cancer treatment has not been mentioned in the literature over the past 100 years when radiation therapy has been in use for cancer treatment and thus this combination cannot be an obvious invention. On the contrary there has been no rational of doing this combination before the original discovery made by the patent holder. The original experimental finding on which the invention is described is included in the patent and such an experiment has not been published so far. In 2001 June 19 the patent was approved with patent no: US 6,248,056 B1 (Blad et al., 2001). In 1997 October 01 patent was applied for in Japan which was published in 2001 February 6 with patent number JP 2001501512(T) (Blad et al., 2001).

The method and device used for combined treatment of pulsed electric fields and radiation therapy was found to be and unique discovery and invention. Still no other similar patent has been suggested. But there are still very few investigators whom have considered this very promising way to treat tumors. One way to develop treatment strategies that inhibit tumor growth is combining radiation, angiogenesis inhibitors, and gene-immunotherapy (Kamensek and Sersa, 2008, Gupta et al., 2009, Persson et al., 2010). Angiogenesis inhibitors

can destroy tumor vasculature and enhance the effects of radiation which also favorably altering the immunologic tumor micro-environment. Since treatment with pulsed electric field eradicates the microcirculation it seems to act as a pseudo angiogenesis inhibitor. The combination of radiation, immunotherapy, and pulsed electric fields could be an effective method in the management of cancer (Gupta et al., 2009). By using the presented system for treating tumoral diseases (cancer) with a combination of ionizing radiation and pulsed electric fields a new regime of radiation fractionation is possible. A short intensive treatment with high radiation dose fractions combined with pulsed electric fields followed by a longer resting interval allowing the patient own immune system to be effectual. The intensity of following therapy session may depend on the outcome of the previous. Since the total radiation dose is low it might be possible by this method to treat recurrent tumors already given full dose with conventional therapy by using PEF combined with a few radiation fractions up to a total absorbed dose less than 15 Gy.

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