Magnetoporation: New Method for Permeabilization of Cancerous Cells to Hydrophilic Drugs

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ABSTRACT

Background: In the present study, we investigated the application of pulsed magnetic field (MF) (3.5 T, 1 Hz, 8 square-wave/160 μs) permeabilization on murine breast adenocarcinoma cells when administering bleomycin (BLM) *in vivo*.

Objective: This cross-over study aims to find a noninvasive method to facilitate penetration of hydrophilic anti-cancer drugs through the cancerous cells membrane into the cytosoll in order to minimize the side effects of the chemotherapy treatments of tumors.

Material and Methods: In this cross-over study, a total of 50 female Balb/c mice were tumorized via homograft. After about 2 weeks, magnetic pulses (3.5 T, 1 Hz, 8 square-wave/160 μs) were applied to tumor-bearing mice 3 min after intratumoral BLM solution injection. Tumor volume was measured every 48 h during 22 days.

Results: The results showed that the difference between the BLM plus 3.5 T MF group versus the sham control or sham MF groups was significant. Uptake of BLM molecules by tumoral cells in the BLM plus 3.5 T MF group versus the BLM control group was 7- folds higher that this result was statistically insignificant (P<0.05, SEM=266.8676, analysis of variance).

Conclusion: Significant cell permeabilization to BLM requires greater MF strength or exposure time. Further investigation is necessary.

Keywords

Chemotherapy; Magnetic Fields; Permeabilization; In Vivo; Bleomycin; Balb/C; Adenocarcinoma

Introduction

Magnetochemotherapy is a new method used to increase the permeability of the cell membrane to hydrophilic anticancer drugs such as bleomycin sulfate (BLM).

The physical cornerstone of this method is based on Maxwell's third equation which declares that: "Any alternating magnetic field produces an inductive electric field". The intensity of the induced electric field is directly related to the intensity of the original magnetic field.

Although the intensity of the induced electric field is low, researchers have recently found that pulsed low electric fields (2.5-20 V/cm) can increase cell membrane permeability by up to 10-folds [1, 2]. This

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enhancement in cell membrane permeability leads to the increased uptake of macromolecules by cytosol. This technique is called electroendocytosis.

In an *in vitro* study, based on computerized simulation method, researchers found that the induced electric field intensity caused by an alternating magnetic field at 3.5 T is 7.5 V/cm at a 1cm distance from the probe [3].

In another *in vitro* study, researchers found that a variable magnetic field could enhance the uptake of Lucifer Yellow Dye by CHO cells [4].

In electromagnetism, permeability is the measure of the resistance of a material against the formation of a magnetic field, otherwise known as distributed inductance in transmission line theory.

Hence, it is the degree of magnetization that a material obtains in response to an applied magnetic field. Magnetic permeability is typically represented by the (italicized) Greek letter μ.

The magnetic permeability of the biological matter (e.g. human body tissues) is very close to the magnetic permeability of water (μ=0.99999≈1) [5]. Thus, it can easily pass through human body.

There are many other methods that can be used to deliver hydrophilic drugs into the cytosol of cells, and each of them has its advantages and drawbacks [6].

Considering above facts, and the important limiting factors associated with other parallel methods such as electroporation [7, 8], leads us to examine the possibility of applying magnetic fields rather than electric fields when permeabilizing the cell membrane to hydrophilic drugs.

Material and Methods

In this cross-over study, the following material and methods were used.

Drugs

Bleomycin sulfate (Mylan Pharmaceuticals, S.L.Canonsburg, PA, USA) was purchased from a pharmacy as a crystalline powder. BLM was dissolved in the physiologic solution (0.9% NaCl) at a concentration of 15 mg/mL (2.5U/0.1 mL).

Anesthesia was performed via intraperitoneal injection of a solution containing 4 mL of Saline and 0.5 mL of 10% Ketamine (Alfasan Diergeneesmiddelen B.V., Woerden-Netherlands) and 0.5 mL of 2% Xaylazine (Alfasan Diergeneesmiddelen).

Mice and Tumors

A total of 50 healthy inbred female Balb/c mice aged 6–8 weeks and weighing 18-20 g, were purchased from the Pasteur Institute, (Tehran, Iran). The mice were maintained at 22 °C with a natural day/night light cycle for 10 days to ensure adaptation. A spontaneous mouse mammary tumor (SMMT), i.e., an invasive ductal carcinoma, was obtained from the Immunology Department of Tarbiat Modares University (Tehran, Iran) and transplanted by implanting a 4 mm^3 fragment into the right flank of each anesthetized mouse by homograft surgery. Approximately 2 weeks after tumor transplantation, when the tumor's largest diameter was between 5-10 mm (as measured by digital calipers), the mice were randomly divided into experimental groups (15 animals in each of the control and treatment groups).

Instruments

To expose the mice to the magnetic field, we used a magnetic stimulator (MAGSTIM® Rapid U.K. Pat.-ent No. GB2298370B). This device is routinely used when stimulating nerves in the treatment of epileptic patients.

Treatments

After 2-3 weeks following the homograft, the tumor reached a curable size. Depending on the assigned group, appropriate treatment was administered to the mice. Mice in the experimental group were exposed to the 3.5 T magnetic field by putting them in a hand-made

device.

Drug Preparation and Injection

One milliliter of injectable saline was added to one BLM vial that contained 15 mg of crystalline powder of BLM. As each unit (U) contains 0.56-0.66 mg of BLM, our solution had ~25 U of BLM. Thus for each 0.1 mL, there were 2.5 U of BLM present. Depending on the tumor size, the appropriate BLM dose was injected directly into the tumor. In order to better spread the drug into the tumor based on tumor volume, the injection was performed at two steps at two opposite points of the tumor.

For each gram of the mouse's weight, 0.01 mL of anesthesia solution was injected intraperitoneally.

Mouse Exposure

Each anesthetized mouse was put in contact with the magnetic stimulator probe 3 minutes following the BLM injection and treatment exposure was performed as follows: (3.5 T, 1 Hz, 8 square-wave/160 μs).

Experimental Groups

In this study, the mice were divided into one of five experimental groups:

i. Sham control group (Sham Cont.): only 0.1mL of distilled water, as per the described protocol, was injected into the tumor. No drug or irradiation was used.

ii. BLM control group (BLM Cont.): only 0.1 mL of BLM solution, under the mentioned protocol, was injected into the tumor. No irradiation was used.

iii. BLM plus 3.5 T magnetic field (MF) group (BLM plus 3.5 T MF): 0.1 mL of BLM solution was injected into the tumor at two opposite points. After a 3-minute delay the drug was spread between the tumor cells. The tumor was then put in contact with the magnetic stimulator probe. Eight pulses were applied to the tumor at a frequency of 1 Hz. The duration of each pulse was 160 μs and the intensity of each pulse was 3.5 T as previously mentioned Sham magnetic field group (Sham MF): the mouse, which had a curable-sized tumor, was put in contact with the probe, but no field was applied and neither drugs nor water were injected into the tumor. The time of the treatment for each of the cases was 8 seconds.

iv. Only magnetic field group (Only MF): the mouse, which had a curable-sized tumor, was put in contact with the probe. Eight pulses were applied. Neither drugs nor water were injected into the tumor. The time of the treatment for each of the cases was 8 seconds.

Tumor Monitoring

The tumor diameter was measured every 48 h using a 0.02 mm digital caliper along the two largest diameters. Each diameter was measured three times and the average was used for the calculation. Tumor volume was calculated using a standard formula. The formula most often used to measure tumor volume was $V=ab^2\pi/6$, in which (a) is the longest diameter and (b) is the next longest diameter perpendicular to (a) [9].

Statistical Analysis

Using Microsoft Excel (Microsoft Office 2007; Microsoft Corporation, Redmond, WA, USA), the data were processed and the graph for each tumor growth curve of each treatment group was delineated and rendered. Statistical analyses were performed using SPSS for Windows version 18 (IBM Corporation, Armonk, NY, USA). We performed one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) method. $P<0.05$ was considered significant in the rejection of the null hypothesis.

Analysis of the data with SPSS showed that there were no significant differences between the experimental groups on treatment day ($P<0.05$).

Results

In this study, our results showed that the application of a magnetic field enhances the pen-

etration of BLM through the tumor-cell membrane *in vivo*. The growth curve of the tumor in each experimental group between the treatment day and 22 days after treatment is shown in Figure 1.

Statistical analysis of the data showed that the mice in the Sham Cont. group demonstrated a statistically significant difference when compared with mice in the BLM plus 3.5 T MF group (P=0.008; SEM=266.8676, ANOVA) and those in the BLM Cont. group (P=0.059).

Conversely, the difference between the results of the mice in the BLM Cont. and Sham Cont. groups was not significant (P<0.05).

The difference between the mice in the BLM plus 3.5 T MF group and those in the Sham MF and Only MF groups was statistically significant (P=0.015 and P= 0.089 respectively).

Comparing the significance level of the BLM Cont. and BLM plus 3.5 T MF groups versus that of the Sham Cont. group revealed that the application of the magnetic field 3minutes after the BLM injection resulted in the tumor-cell membrane being about 7.4 times more permeable. However, this finding was not enough to yield a significant difference between the BLM Cont. and BLM plus 3.5 T MF groups at the P<0.05 level.

Discussion

According to Maxwell's third law, the pulsed magnetic field induced pulsed low electric fields. Pulsed low electric fields have two distinct effects on tumor cells:

1) They can result in cell membrane polarization, which can alter the membrane's cross potential; and

2) They can cause tangential electrophoresis at the site where polarized proteins and lipids can be found on the cell surface (1; 2).

The first effect does not occur in a solid tumor; however, the second can, and it may also lead to molecular uptake enhancement in the inter-cellular environment.

In the plasma membrane of an unexposed cell, the charged elements (proteins and phospholipids) are evenly distributed. After a short

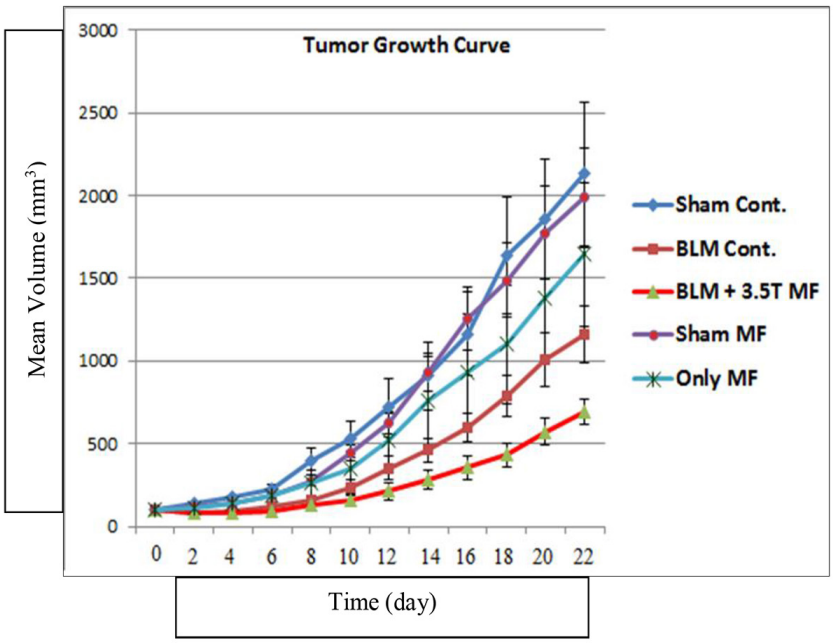

Figure 1: The growth curve of the tumor in each experimental group (n=10) bearing tumors of murine breast adenocarcinoma: Sham Cont., ♦; Bleomycin Cont., ■; BLM+3.5TMF, ▲; Sham MF, ●; Only MF, *. Standard error bars are also shown.

period of exposure to a pulsed low electric field, the distribution of the charged elements differs; proteins and phospholipids receive opposite charges and subsequently attract one another. Therefore, the gap between adjacent phospholipids expands. This leads to the creation of a path through which BLM can diffuse into the cytosol [2].

Although it seems as though the pulsed low electric field that is induced by the magnetic field is unable to create pores in the cell membrane, it is actually capable of enhancing the uptake of macromolecules by the cytosol. This increased uptake of macromolecules can thus result in increased aggregation of the BLM molecules into the cytosol.

The number of BLM molecules in the cytosol assigns and determine the method of cell death or destruction. If only a few thousands BLM molecules are present in the cytosol, the cell will arrest in the G2-M phase and become enlarged; at this point, polynuclei and micronuclei can be observed. Then, the cells will die slowly in a process that lasts about three doubling times. If, however, the cell contains several millions BLM molecules, it will be killed within a few minutes via pseudoapoptosis. When this occurs, BLM can induce a characteristic form of DNA fragmentation. This is followed by cell shrinkage, membrane blebbing and chromatin condensation [10, 11].

By using electroporation, real pores will form in the cell membrane, and these may be permanent. This leads to the entrance of several million BLM molecules into the cytosol, meaning that the cell will be killed within a few minutes. Conversely, by using an alternating magnetic field that leads to the formation of a pulsed low electric field, a disturbance will occur in the arrangement of phospholipids and protein molecules within the cell membrane's structure. This effect is called electroendocytosis and we suggest this phenomenon is responsible for the increased uptake of BLM in the BLM plus 3.5 T MF group [1, 2]. The cell membrane disarrangement occurs long enough (about 1 h) to pass enough BLM molecules through the cell membrane into the cytosol [1, 2].

Electroendocytosis can facilitate the uptake of macromolecules in the range of 1-200 kD and smooths the diffusion of this range of macromolecules across the cell membrane along their electrochemical gradients [9]. BLM is a macromolecule that consists of 11 glycopeptides and has, a molecular weight of 1500Da [12]. Given these properties, BLM is suitable for passage into the cells via electroendocytosis.

Although magnetoporation passes fewer molecules into the cells when compared with electroporation, none of the limitations of electroporation apply in the case of magnetoporation. Therefore, repeated application of a magnetic field following BLM injection may yield the desired results when treating many solid tumors.

The significance level observed for the Only MF versus Sham Cont. (P=0.318) groups when compared with that of the Sham MF versus Sham Cont. (P=0.822) groups was 2.59 times more significant. This reveals that the magnetic field itself has a prohibiting effect on cell division; in this way, the application of a magnetic field may be considered as a cancer-control method.

Furthermore, the difference between the Only MF versus BLM plus 3.5 T MF groups was fairly significant (P=0.089) nevertheless, the difference between the Sham MF versus BLM plus 3.5 T MF groups was significant (P=0.015). This fact also supports the notion that the magnetic field has inhibitory effects on cell division. Base on the findings of this report, additional research investigating the observed effects is warranted.

Conclusion

Many of anticancer drugs (e.g. BLM) that prescribed to treat the solid tumors are hydrophilic substances and main problem for the efficiency of the treatment is the passage of drug

through the lipid layer of the cell wall into the cytosol.

The results of present study show that the magnetic field (3.5 T, 1 Hz, 8 square-wave/160 μs) can perform an important role in the future of noninvasive treatments of the solid tumors. Applying of magnetic field is noninvasive and the induced electric field performs the main duty to make cells permeable to hydrophilic drugs by a phenomenon called "electroendocytosis". Since more intensity or exposure time of the magnetic field may be required to achieve better results, future investigation is necessary.

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Conflict of Interest

None

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