USING ALTERNATING ELECTRIC FIELDS TO INCREASE PERMEABILITY OF THE BLOOD BRAIN BARRIER

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This Application claims the benefit of US Provisional Applications 63/015,099 (filed April 24, 2020) and 63/071,748 (filed August 28, 2020), each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Ordinarily, cerebral microvessels strictly regulate the transfer of substances between the blood and the brain tissue. This regulation by cerebral micro-vessels is called the blood–brain barrier (BBB), and is due to intercellular tight junctions (TJs) that form between brain capillary endothelial cells. In cerebral capillaries, TJs proteins are expressed 50–100 times more than in peripheral microvessels. TJs are formed by an intricate complex of transmembrane proteins (claudin and occludin) with cytoplasmic accessory proteins (ZO-1 and -2, cingulin, AF-6, and 7H6). By linking to the actin cytoskeleton, these proteins form a strong cell–cell connection. Brain endothelial cells, which form the endothelium of cerebral microvessels, are responsible for about 75–80% of the BBB's resistance to substances, and other cells such as astrocytes and pericytes provide the remainder of the resistance.

[0003] The BBB consists of tight junctions around the capillaries, and it ordinarily restricts diffusion of microscopic objects and large or hydrophilic molecules into the brain, while allowing for the diffusion of hydrophobic molecules (transcellular instead of paracellular transport).

[0004] In healthy people, the BBB serves a very important function because it prevents harmful substances (e.g. bacteria, viruses, and potentially harmful large or hydrophilic molecules) from entering the brain. There are, however, situations where the action of the BBB introduces difficulties. For example, it might be desirable to deliver large or hydrophilic drug molecules to treat a disease in the patient's brain. But when the BBB is operating normally, these drugs are blocked from entering the brain by the BBB.

SUMMARY OF THE INVENTION

[0005] One aspect of the invention is directed to a system~~first method~~ for delivering a substance across a blood brain barrier of a subject's brain. The system~~first method~~ comprises

a controller; and an alternating current (AC) voltage generator that is coupled to the controller, wherein the controller is configured to control the AC voltage generator to: apply~~ing~~ an alternating electric field to the subject's brain for a first period of time, wherein application of the alternating electric field to the subject's brain for the first period of time increases permeability of the blood brain barrier in the subject's brain, and wherein the increased permeability of the blood brain barrier allows a first substance to cross the blood brain barrier;~~. The first method also comprises administering a first substance to the subject after the first period of time has elapsed, wherein the increased permeability of the blood brain barrier allows the first substance to cross the blood brain barrier. The first method also comprises~~ discontinu~~e~~ing the application of the alternating electric field for enough time to allow the blood brain barrier to recover; and~~. The first method also comprises,~~ after the blood brain barrier has recovered, apply~~ing~~ the~~an~~ alternating electric field to the subject's brain for a second period of time,~~.~~ wherein ~~A~~application of the alternating electric field to the subject's brain for the second period of time increases permeability of the blood brain barrier in the subject's brain, and wherein~~. The first method also comprises administering a second substance to the subject after the second period of time has elapsed, wherein~~ the increased permeability of the blood brain barrier allows a~~the~~ second substance to cross the blood brain barrier.

[0006] In some instances of the system~~first method~~, the alternating electric field is applied at a frequency between 75 kHz and 125 kHz. In some instances of the system~~first method~~, the alternating electric field is applied at a frequency of at least 50 kHz. In some instances of the system~~first method~~, the second period of time is at least 24 hours. In some instances of the system~~first method~~, the second period of time is at least 48 hours. In some instances of the system~~first method~~, the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.

[0007] In some instances of the system~~first method~~, the alternating electric field is applied at a frequency between 75 kHz and 125 kHz, the second period of time is at least 24 hours, and the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.

[0008] As disclosed~~In some instances of the first method~~, the administering of the second substance is performed intravenously. ~~In some instances of the first method, t~~The administering of the second substance is performed orally. ~~In some instances of the first method, t~~The first substance and the second substance are identical. ~~In some instances of the first method, a~~At least one of the first substance and the second substance comprises paclitaxel.

[0009] Another aspect of the invention is directed to a system~~econd method~~ for delivering a substance across a blood brain barrier of the subject's brain. The system~~econd method~~ comprises a controller; and an alternating current (AC) voltage generator that is coupled to the controller, wherein the controller is configured to control the AC voltage generator to: apply~~ing~~ a first alternating electric field at a first frequency to the subject's brain for a first period of time, wherein application of the first alternating electric field at the first frequency to the subject's brain for the first period of time increases permeability of the blood brain barrier in the subject's brain; and~~. The second method also comprises, subsequent to the first period of time,~~ apply~~ing~~ a second alternating electric field at a second frequency to the subject's brain for a second period of time,~~.~~ wherein ~~T~~the second frequency is different from the first frequency,~~ and~~ wherein the second alternating electric field at the second frequency operates to maintain permeability of the blood brain barrier, and wherein~~. The second method also comprises administering the substance to the subject at least 24 hours after the first period of time has elapsed, wherein~~ the maintained permeability of the blood brain barrier allows the substance to cross the blood brain barrier.

[0010] In some instances of the system~~econd method~~, the first frequency is between 75 kHz and 125 kHz.

[0011] In some instances of the system~~second method~~, the first frequency is between 50 kHz and 190 kHz. Optionally, in these instances, the second frequency is at least 190 kHz.

[0012] In some instances of the system~~econd method~~, the first frequency is at least 50 kHz and the second frequency is higher than the first frequency. In some instances of the system~~econd method~~, the first period of time is at least 24 hours. In some instances of the system~~econd method~~, the second period of time comprises a single uninterrupted interval of time that is at least one week long.

[0013] In some instances of the system~~econd method~~, the second period of time comprises a plurality of non-contiguous intervals of time during which the second alternating electric field at the second frequency is applied to the subject's brain, wherein the plurality of non-contiguous intervals of time collectively add up to at least one week.

[0014] Another aspect of the invention is directed to a systemthird method for delivering a substance across a blood brain barrier of a subject's brain. The systemthird method comprises a controller; and an alternating current (AC) voltage generator that is coupled to the controller, wherein the controller is configured to control the AC voltage generator to: applying an alternating electric field to the subject's brain for a period of time, wherein application of the alternating electric field to the subject's brain for the period of time increases permeability of the blood brain barrier in the subject's brain, and. The third method also

~~comprises administering paclitaxel to the subject after the period of time has elapsed,~~ wherein the increased permeability of the blood brain barrier allows ~~the~~ paclitaxel or at least one metabolite thereof to cross the blood brain barrier.

[0015] In some instances of the system~~third method~~, the alternating electric field is applied at a frequency between 75 kHz and 125 kHz. In some instances of the system~~third method~~, the period of time is at least 24 hours. In some instances of the system~~third method~~, the period of time is at least 48 hours. In some instances of the system~~third method~~, the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.

[0016] In some instances of the system~~third method~~, the alternating electric field is applied at a frequency between 75 kHz and 125 kHz, the period of time is at least 24 hours, and the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.

[0017] ~~Optionally~~According to the disclosure~~, in any of the instances of the first, second, or third methods described above~~, the substance has a molecular weight of at least 4 kDa or a molecular weight of at least 69 kDa.

[0018] According to the disclosure~~Optionally, in any of the instances of the first, second, or third methods described above~~, the substance has at least one characteristic that ordinarily impedes the substance from crossing a non-leaky BBB.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1 depicts an exemplary setup for in vitro experiments in which imortilized murine brain capillary endothelial cells (cerebEND) were grown on coverslips and transwell inserts to create an artificial in vitro version of the BBB.

[0020] FIGS. 2A and 2B depict the results of integrity and permeability testing, respectively, on the artificial BBB.

[0021] FIGS. 3A and 3B depict data showing that the increased permeability of the artificial BBB is not caused by cell death.

[0022] FIG. 4 depicts the positions at which rats' brains were sliced for an in vivo experiment.

WHAT IS CLAIMED IS:

1. A ~~method~~system for delivering a substance across a blood brain barrier of a subject's brain, the ~~method~~system comprising:

a controller; and

an alternating current (AC) voltage generator that is coupled to the controller, wherein the controller is configured to control the AC voltage generator to:

apply~~ing~~ an alternating electric field to the subject's brain for a first period of time, wherein application of the alternating electric field to the subject's brain for the first period of time increases permeability of the blood brain barrier in the subject's brain, and wherein the increased permeability of the blood brain barrier allows a first substance to cross the blood brain barrier;

~~administering a first substance to the subject after the first period of time has elapsed, wherein the increased permeability of the blood brain barrier allows the first substance to cross the blood brain barrier;~~

discontinu~~e~~~~ing~~ the application of the alternating electric field for enough time to allow the blood brain barrier to recover; and

after the blood brain barrier has recovered, apply~~ing~~ the~~an~~ alternating electric field to the subject's brain for a second period of time, wherein application of the alternating electric field to the subject's brain for the second period of time increases the permeability of the blood brain barrier in the subject's brain, and wherein the increased permeability of the blood brain barrier allows a second substance to cross the blood brain barrier.~~; and~~

~~administering a second substance to the subject after the second period of time has elapsed, wherein the increased permeability of the blood brain barrier allows the second substance to cross the blood brain barrier.~~

~~2. The method of claim 1, wherein the alternating electric field is applied at a frequency between 75 kHz and 125 kHz.~~

2~~3~~. The system~~method~~ of claim 1, wherein the alternating electric field is applied at a frequency of at least 50 kHz.

34. The system~~method~~ of claim 1, wherein the second period of time is at least 24 hours.

45. The system~~method~~ of claim 1, wherein the second period of time is at least 48 hours.

~~6. The method of claim 1, wherein the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.~~

57. The system~~method~~ of claim 1, wherein the alternating electric field is applied at a frequency between 75 kHz and 125 kHz, the second period of time is at least 24 hours, and the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.

~~8. The method of claim 1, wherein the administering of the second substance is performed intravenously.~~

~~9. The method of claim 1, wherein the administering of the second substance is performed orally.~~

~~10. The method of claim 1, wherein the first substance and the second substance are identical.~~

~~11. The method of claim 1, wherein at least one of the first substance and the second substance comprises paclitaxel.~~

612. A system~~method~~ for delivering a substance across a blood brain barrier of the subject's brain, the system~~method~~ comprising:

a controller; and

an alternating current (AC) voltage generator that is coupled to the controller, wherein the controller is configured to control the AC voltage generator to:

apply~~ing~~ a first alternating electric field at a first frequency to the subject's brain for a first period of time, wherein application of the first alternating electric field at the first frequency to the subject's brain for the first period of time increases permeability of the blood brain barrier in the subject's brain; and

~~subsequent to the first period of time,~~ apply~~ing~~ a second alternating electric

field at a second frequency to the subject's brain for a second period of time, wherein the second frequency is different from the first frequency, ~~and~~ wherein the second alternating electric field at the second frequency operates to maintain the permeability of the blood brain barrier, and wherein the maintained permeability of the blood brain barrier allows the~~a~~ substance to cross the blood brain barrier.~~; and~~

~~administering the substance to the subject at least 24 hours after the first period of time has elapsed, wherein the maintained permeability of the blood brain barrier allows the substance to cross the blood brain barrier.~~

7. The system of claim 6, further comprising:

a relay that is coupled to the AC voltage generator, wherein the relay is configured to adjust an output of the AC voltage generator between the first alternating electrical field at the first frequency and the second alternating electrical field at the second frequency.

8. The system of claim 6 or 7, wherein the controller is configured to control a control input between a first state or a second state, wherein when the control input is in the first state:

the AC voltage generator outputs the first alternating electric field at the first frequency; and

when the control input is in the second state:

the AC voltage generator outputs the second alternating electric field at the second frequency.

9~~13~~. The system~~method of~~as in any of claims 6 to 8~~12~~, wherein the first frequency is between 75 kHz and 125 kHz.

10~~14~~. The system~~method of~~as in any of claims 6 to 8~~12~~, wherein the first frequency is between 50 kHz and 190 kHz.

11~~5~~. The system~~method~~ of claim 10~~14~~, wherein the second frequency is at least 190 kHz.

12~~6~~. The system~~method of~~as in any of claims 6 to 8~~1~~, wherein the first frequency is at least 50 kHz and the second frequency is higher than the first frequency.

137. The system~~method~~ ~~of~~as in any of claims 6 to 8~~12~~, wherein the first period of time is at least 24 hours.

148. The system~~method~~ ~~of~~as in any of claims 6 to 8~~12~~, wherein the second period of time comprises a single uninterrupted interval of time that is at least one week long.

159. The system~~method~~ ~~of~~as in any of claims 6 to 8~~12~~, wherein the second period of time comprises a plurality of non-contiguous intervals of time during which the second alternating electric field at the second frequency is applied to the subject's brain, wherein the plurality of non-contiguous intervals of time collectively add up to at least one week.

16~~20~~. A system~~method~~ for delivering a substance across a blood brain barrier of a subject's brain, the system~~method~~ comprising:

a controller; and

an alternating current (AC) voltage generator that is coupled to the controller, wherein the controller is configured to control the AC voltage generator to:

apply~~ing~~ an alternating electric field to the subject's brain for a period of time, wherein application of the alternating electric field to the subject's brain for the period of time increases permeability of the blood brain barrier in the subject's brain,~~;~~ and ~~administering paclitaxel to the subject after the period of time has elapsed,~~ wherein the increased permeability of the blood brain barrier allows ~~the~~ paclitaxel or at least one metabolite thereof to cross the blood brain barrier.

17~~21~~. The system~~method~~ of claim 1 or 16~~20~~, wherein the alternating electric field is applied at a frequency between 75 kHz and 125 kHz.

18~~22~~. The system~~method~~ of claim 16~~20~~, wherein the period of time is at least 24 hours.

~~23. The method of claim 20, wherein the period of time is at least 48 hours.~~

19~~24~~. The system~~method~~ of claim 1 or 16~~20~~, wherein the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.

20~~25~~. The system~~method~~ of claim 16~~20~~, wherein the alternating electric field is applied at a

frequency between 75 kHz and 125 kHz, the period of time is at least 24 hours, and the alternating electric field has a field strength of at least 1 V/cm in at least a portion of the subject's brain.

~~26. A method as in any of claims 1-25, wherein the substance has a molecular weight of at least 4 kDa.~~

~~27. A method as in any of claims 1-25, wherein the substance has a molecular weight of at least 69 kDa.~~

~~28. A method as in any of claims 1-25, wherein the substance has at least one characteristic that ordinarily impedes the substance from crossing a non-leaky BBB.~~