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Technologies for controlling ultrasound targeted drug delivery in brain using animal models

Mestrado Integrado em Engenharia Biomédica e Biofísica
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Audaces Fortuna Iuvat - "A sorte protege os audazes."
Virgílio, Eneida

ABSTRACT

The development of methods for Central Nervous System drug delivery is still a challenge, due to the difficulty to deliver drug molecules across the Blood-Brain barrier that prevents the access of 100% of large-molecule drugs and 98% of small-molecule drugs.

Different methods have been tested with the goal to bypass the Blood-Brain Barrier, but they are either invasive, non-targeted or require the formulation of new drugs.

We hypothesized that the combination of two methods, Focused Ultrasound and Intranasal delivery, can reduce the disadvantages that other methods present and result in greater overall delivery, improved tissue penetration, and possibly enrichment of the therapeutic agent (i.e. targeting) in the desired location(s) in brain.

This pilot study was designed to test the performance characteristics of study designs, measures, procedures, recruitment criteria and operational strategies.

By doing an animal model with 12 rats, we achieved a simple and efficient experimental method to apply Focused Ultrasound and Intranasal delivery combined and the only changes done between subjects were related to the quantity given, timings and concentration.

Gadolinium enhancement was detected along the olfactory pathway, reaching the main olfactory epithelium in all the rats and it was possible also to visualize flux through the main olfactory bulb also. We also proved that all the intranasal delivered model drugs using Focused Ultrasound (Trypan Blue and Dextrans) bypassed the Blood-Brain Barrier, being in that case delivered in important areas in the brain, in the level of the *Rostral Striatum* and the *Substantia Nigra,* targets for Parkinson's disease.

The findings can corroborate the idea that Focused Ultrasound can enhance the delivery of drugs in the brain using Intranasal administration, providing a target outcome and more sensitive, which is valuable in brain applications.

KEY-WORDS: Focused Ultrasound, Intranasal Delivery, Blood-Brain Barrier, Brain Drug Delivery, Parkinson's disease.

RESUMO

As doenças do sistema nervoso como as doenças de Alzheimer e Parkinson fazem parte das principais causas de incapacidade e morte, a nível mundial, estando a incidência das mesmas a aumentar associado a factores como o envelhecimento da população. Existe assim um enorme problema relacionado com a libertação de drogas para processos de controlo de doença nestes pacientes, estando sujeitos à existência de diversos mecanismos de protecção das estruturas inerentes ao sistema nervoso central. Um dos mais importantes é a barreira hematoencefálica que constitui um desafio a ultrapassar aquando da entrega de drogas terapêuticas, impedindo a entrada de cerca de 100% das substâncias com maior peso molecular e de 98% para as restantes. Diversos métodos têm sido desenvolvidos de modo a contornar esta barreira, apresentando porém limitações relacionadas com o facto de serem invasivos, terem uma baixa especificidade, alta toxicidade, sendo assim necessária a procura de outras técnicas.

A utilização de ultra-sons em medicina é vasta a nível de diagnóstico e terapêutico. São vários os efeitos biológicos associados à utilização de ultra-sons de alta frequência, sendo estes capazes de causar uma abertura temporária da barreira hematoencefálica de forma localizada, permitindo a entrega de drogas em regiões de interesse clínico. Este fenómeno deve-se às interações mecânicas causadas pelas ondas ultra-sónicas entre as estruturas vasculares e o tecido endotelial. Esta técnica é não invasiva, sendo um método transiente.

Outro método não invasivo utilizado terapeuticamente é a entrega de drogas por via intranasal, já que estes por esta via de administração, irão passar diretamente para o sistema nervoso central, por meio dos nervos olfactório e trigeminal. Porém esta técnica apresenta uma baixa eficiência, uma vez que apenas uma pequena fração da dose administrada chega ao local de interesse.

O projeto experimental teve por base a combinação destes dois métodos não invasivos, ultra-sons de alta frequência e administração intranasal, considerando que por hipótese, este novo processo resultaria numa entrega mais eficiente em regiões de interesse no sistema nervoso central para a doença de Parkinson, ultrapassando as limitações que as técnicas apresentam separadamente.

Esta tese pretende descrever as atividades realizadas na dissertação do Mestrado Integrado em Engenharia Biomédica e Biofísica da Faculdade de Ciências da Universidade de Lisboa, no ramo de Sinais e Imagens médicas. Este projecto experimental realizou-se no grupo de Focused Ultrasound, da Universidade de Harvard, em Boston Estados Unidos da América, sobre a orientação do Professor Doutor Nathan McDannold e do Professor Doutor Nuno Matela.

A tese reflecte os métodos, resultados e conclusões de um estudo de experimentação associado a um modelo animal em ratos e ratinhos (com um total de 12 animais) recorrendo a três drogas de teste: *Gadavist®*, *Trypan Blue* e *Dextrans*, de forma a determinar as condições ótimas de design experimental ao utilizar as técnicas de ultra-sons e administração nasal em simultâneo e quais as estruturas biológicas relacionadas com a sua aplicação. Para a verificar o sucesso da aplicação do método foram usadas imagens por ressonância magnética, de forma a verificar de que forma a droga passa através das estruturas nasais (no caso do uso da droga Gadavist®) e ainda para garantir a existência de disrupção da barreira hematoencefálica em locais de interesse para a doença de Parkinson. Recorreu-se ainda a técnicas de imagens de fluorescência e de microscópio para verificar a presença *in situ*, no caso do uso de *Trypan Blue* e *Dextrans*.

Há uma enorme necessidade de verificar a eficácia de sistemas de entrega de drogas no sistema nervoso central, sendo este um dos primeiros estudos feitos combinando ultra-sons de alta frequência focalizados e entrega intranasal para a entrega de drogas modelo sendo que muitos dos resultados foram analisados

empiricamente. Os resultados deste estudo piloto demonstram que relativamente à técnica intranasal, esta envolve as células do epitélio olfativo, sendo que um possível caminho subsequente será a passagem para os bolbos olfactórios. As imagens por ressonância magnética ao longo do tempo demonstram a intervenção de várias estruturas olfativas por meio do aumento do signal devido à presença do contraste Gadavist® nas mesmas. Observou-se ainda que o intervalo temporal mínimo necessário para que a droga modelo chegue a essas mesmas estruturas é de cerca de trinta minutos, sendo que se assume por hipótese que o transporte está essencialmente relacionado com processos de difusão.

Ao utilizar as drogas de teste *Trypan Blue* e *Dextrans* foi ainda possível estabelecer um protocolo de administração por via intranasal, determinando os tempos, quantidades e ainda posição do animal que optimizam o processo. Ao aplicar as técnicas de ultra-som e entrega nasal conjuntamente, existe uma maior quantidade de droga no hemisfério cerebral em que foram aplicados o ultra-sons de alta frequência e a entrega nasal quando comparado com o hemisfério contralateral (controlo), em que apenas se administrou a substância via nasal, sendo este facto comportável por observação de imagens de microscopia de fluorescência e de uma câmera de fluorescência. Recorreu-se a Imagens por Ressonância Magnética de modo a aferir a disrupção da barreira hematoencefálica nos locais de interesse: o corpo estriado e substância negra.

Os diversos resultados apresentados poderão ser indicadores de que o modelo em hipótese foi bem-sucedido, sendo que será necessário aumentar o número de animais testados de forma a reunir uma amostra estatisticamente significativa para confirmação das observações feitas no presente estudo. Foram analisados ainda outros factores de interesse na entrega de drogas no sistema nervoso central usando os dois métodos em conjuntos tais como a posição do animal aquando da entrega, sendo que

fica por estabelecer um paralelismo entre a anatomia do rato e do Homem, sendo este factor importante ao prosseguir para uma fase clínica do estudo.

Estudos posteriores recorrendo a terapia génica serão efetuados pelo grupo, com o objetivo da utilização desta técnica mista aplicada a locais de interesse no cérebro para a doença de Parkinson, usando estes resultados preliminares como referência no estabelecimento do protocolo experimental ao nível de tempos de entrega, volumes, parâmetros dos ultra-sons, entre outros.

Palavras-Chave: Ultrasons, Administração nasal, Barreira Hematoencefálica, Brain Drug Delivery, Parkinson's disease.

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Obrigada.

LIST OF ABBREVIATIONS

AD	Alzheimer Disease
BBB	Blood-Brain Barrier
CNS	Central Nervous System
CSF	Cerebrospinal fluid
EC	Endothelial cells
FUS	Focused Ultrasound
IN	Intranasal
IV	Intravenous
MB	Microbubble
MOB	Main Olfactory Bulb
MOE	Main Olfactory Epithelium
MRI	Magnetic Resonance Imaging
MW	Molecular Weight
P-gp	P-glycoprotein
PD	Parkinson Disease
PZT	Piezoelectric Transducer
TE	Echo Time
TR	Repetition Time

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CHAPTER 1: INTRODUCTION AND MOTIVATION OF THE STUDY

1.1. Introduction

This thesis is inserted in a Focused Ultrasound Foundation Research Award Program funded project entitled *"Focused Ultrasound for Increased Delivery of Intranasal DNA Nanoparticles to Rat Brain"*.

The hypothesis of the project is that Focused Ultrasound (FUS) combined with Intranasal (IN) delivery can result in greater overall delivery of drugs in the central nervous system (CNS), improving tissue penetration, and possibly enrichment of the therapeutic agent (i.e. targeting) in the desired locations in brain. It was a multidisciplinary collaboration project between the Brigham and Women's Hospital, Harvard Medical School and the Northeastern University. The project consisted in a pilot study with the goal of determining the optimal experimental design to use simultaneously FUS combined with IN delivery of drugs in the treatment of CNS diseases in sites of interest.

This chapter is an overview of the project motivation, the aims and objectives.

1.2. Motivation

Brain and mind disorders actually affect every year 1.5 billion people worldwide, and the number is expected to increase [1]. The neurodegenerative CNS diseases, such as Parkinson (PD) and Alzheimer's disease (AD), are among the leading causes of disability and death in the developed world. While the number of deaths due to major disorders such as heart disease, stroke, and prostate cancer has decreased, deaths attributed to brain tumors have not improved and AD and PD deaths are on the rise.

Besides the lost of lives associated to this kind of diseases, there is also a economic cost that is becoming to be a concern in terms of health and research policies (Figure 1).

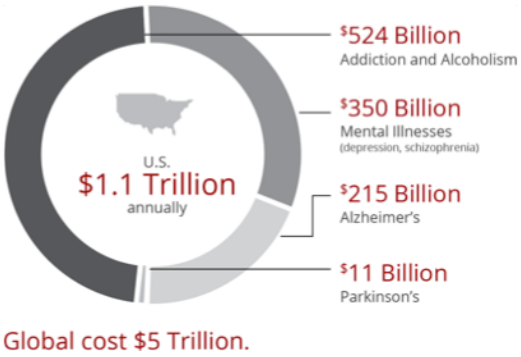

Figure 1 - Economic Cost of Brain diseases. [2]

The development of methods for CNS drug delivery is still a challenge, due to the difficulty to deliver drug molecules across the Blood-Brain barrier (BBB) that adds a further pharmacokinetic hurdle. The BBB prevents the access of 100% of large-molecule drugs and 98% of small-molecule drugs (usually defined as smaller than around 500–600 Da in molecular weight (MW)) [3].

Several brain drug delivery methods have been developed to help assist drugs in circumventing these problems. Methods include direct intracerebral or intracerebroventricular injections, mixing or attaching agents with vasculature-modifying chemicals (i.e., mannitol), and the chemical modification of agents to be delivered through endogenous transport systems. However, many of these methods are either too invasive, limited in spatial specificity, too toxic to be considered or simply do not work for certain drugs. There is the need for the implementation of techniques that solve these constraints.

1.3. Background and Significance

A promising use of FUS in the treatment of CNS disorders is to enhance drug delivery by locally and temporally disrupt the BBB. The ultrasound exposure is combined with intravenously (IV) injected microbubbles (MB), and their mechanical interactions with the vasculature cause the loosening of the tight junctions between vascular endothelial cells (ECs), by stimulating transcytosis or by some as yet undetermined means, causing a transient opening in the BBB. Many previously BBB-impermeable molecules are delivered in the brain by using low acoustic pressures.

Another non-invasive approach for direct delivery to the brain of therapeutic macromolecules is by IN administration. Using this technique, the drugs bypass the BBB structures, entering directly to the CNS utilizing pathways along olfactory and trigeminal nerves innervating the nasal passages. It is a non-invasive method, but only a small fraction of the nasally administered dose reaches the brain, resulting in a low efficient method.

Within this work it is hypothesized that combination of the two methods, FUS and IN delivery, can reduce these disadvantages and result in greater overall delivery, improved tissue penetration, and possibly enrichment of the therapeutic agent (i.e. targeting) in the desired location(s) in brain. Our hypothesis is that disruption of the BBB includes not only changes to the endothelial cells – widening of tight junctions and inducement of active transport – but that it also alters the perivascular channels through which intranasally-delivered agents are thought to flow. This alteration may arise as a physiological response to the mechanical stimulation provided by the oscillating MB in the bloodstream (similar to what may be inducing active transport). It may also be possible that disruption of the BBB results in increased fluid extravasation from the blood vessels, which may aid in transporting substances out and away from the perivascular spaces.

We investigated whether FUS can increase the delivery to the brain of different drugs given by the IN route of administration. This pilot study was designed to test the performance characteristics and capabilities of study designs, measures, procedures, recruitment criteria, and operational strategies that are under consideration for use in a subsequent study after the optimization of the factors referenced.

Therefore, the study was divided in two projects:

- **Gadolinium tracer distribution in the brain after Intranasal delivery** – with the goal of tracing the pathways that a IN delivered drug takes in the brain by using Gadavist® (a Gadolinium based contrast);
- **Focused Ultrasound IN enhanced Delivery** – combining the two techniques using different model drugs, to determine the optimal conditions for the application of this method.

This thesis is structured in 5 chapters: Chapter 2 introduces the concepts related to the physiology of the BBB and to the use of focused ultrasound and the intranasal delivery, Chapter 3 and 4 detail the methods used, the experimental design and the most relevant results. Chapter 5 and 6 are dedicated to the discussion of the outcome of the different subjects and the conclusion summarizing the findings.

CHAPTER 2: REVIEW OF THE LITERATURE AND RELATED RESEARCH: CONCEPTUAL FRAMEWORK

2.1. Target Drug Delivery to the Central Nervous System

2.1.1. Structure and function of the blood–brain barrier

There are several mechanisms to guarantee brain's homeostasis that includes the Blood-Cerebrospinal fluid (CSF) interface formed by epithelial cells of the choroid plexus, the CSF-blood interface where the avascular arachnoid epithelium lies under the dura and encases the brain and one of the most important barriers, the BBB. This interface composes a high-density capillary network, providing blood supply to every neuron.

The BBB is composed of a microvascular endothelium, astrocytes, basement membrane, pericytes and neurons that are in physical proximity to the endothelium, like is represented in Figure 2. This structure regulates the passage of molecules by permiting the entry of nutrients and excluding harmful compounds, protecting the brain. It has selective substance permeability, excluding 98% of small-molecule drugs and approximately 100% of large-molecule neurotherapeutics from the brain parenchyma [5].

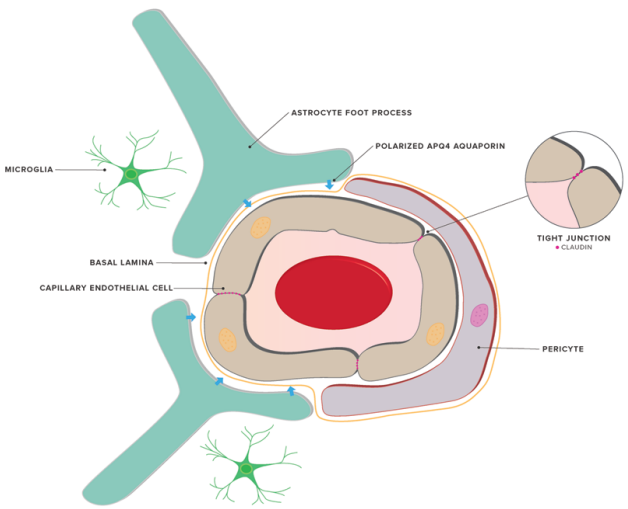

Figure 2 – Normal configuration of the BBB [4].

The selectivity is maintained by various transport functions of the otherwise very tight membrane. Efflux transporters also protect the brain from unwanted chemical influence, with P-glycoprotein (P-gp) as the best-known example [6].

Because of this filtration process, most therapeutic agents do not enter the CNS, based in characteristics of the drug like its MW and also by molecular characteristics like charge, structure and degree of protein binding.

2.1.2. BBB targeting strategies and related drug delivery systems

There are several types of methods to deliver drugs through the BBB (Figure 3). The most important factors are the time frame of the treatment application and the need to transpose the BBB and its mechanisms of filtering selectively the compounds.

The most used approaches in the treatment of CNS diseases are to administer drugs intravenously or orally that will be distributed systemically and not only in the area to treat. Different treatments are being implemented, and those methods are to circumvent the BBB, by delivering drugs directly into the brain parenchyma and to disrupt the BBB, by modulating a temporary opening of the barrier. The problem with methods to circumvent, like convection-enhanced delivery (a therapeutic agent is pumped directly into brain tissue through a fine needle or cannula that is inserted into the brain through a small hole in the skull) is that it is an invasive approach.

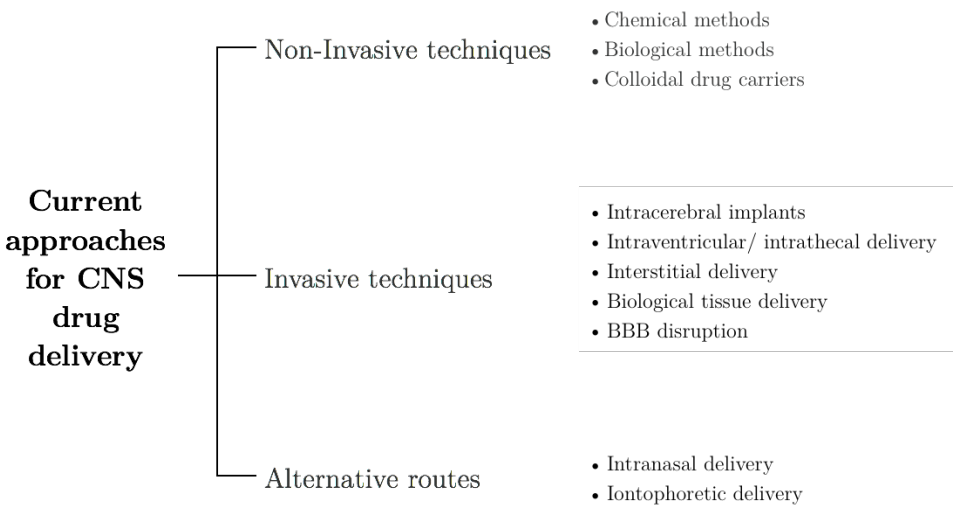

Figure 3 - Current approaches for CNS drug delivery.

Led by Prof. Elisa Konofagou, the Ultrasound Elasticity Imaging Laboratory of Columbia University, New York, has been pursuing methods of targeted drug delivery to the brain by opening the BBB with FUS [7],[8]. They were the first demonstrated that FUS combined with IN technique is potentially useful for treating CNS diseases [3]. In the study, 40-kDa fluorescently labeled dextran as the model drug and FUS was targeted at one region within the caudate putamen of mouse brains.

The results showed that FUS combined with IN enhanced drug delivery within the targeted region compared with that achieved by IN only and that the delivery efficiency of FUS and IN combined was not significantly different from that of FUS and IV. This was a first effort, and more studies are necessary to optimize the FUS and IN treatment parameters for efficient drug delivery.

2.2. Focused Ultrasound

2.2.1. Physical Principles

Acoustic waves are organized and mechanical vibrations of molecules or atoms that can travel through media (gases, liquids, and solids). Ultrasound is an example of an acoustic wave which oscillations are generated by a transducer and transmitted sinusoidally within the medium, alternating local regions of compression (positive pressure) and rarefaction (negative pressure along the direction of propagation). This pressure wave oscillates at a frequency above 20 kHz or 20000 cycles per second and for medical applications, these frequencies are usually above 1 MHz and can be used to predict the way which ultrasound propagates and interacts with the tissue by knowing of the wave properties such as velocity, absorption, attenuation or reflection.

The wavelength (λ) of the ultrasound is determined by the frequency (f) and the velocity (v) for a given medium according to the following mathematical expression:

$$\lambda = \frac{v}{f} \qquad (1)$$

A transducer with a spherical segment can focus ultrasonic waves, making them converge by transferring the energy to a focal region, providing high gains. The configuration of the transducer is represented in Figure 4.

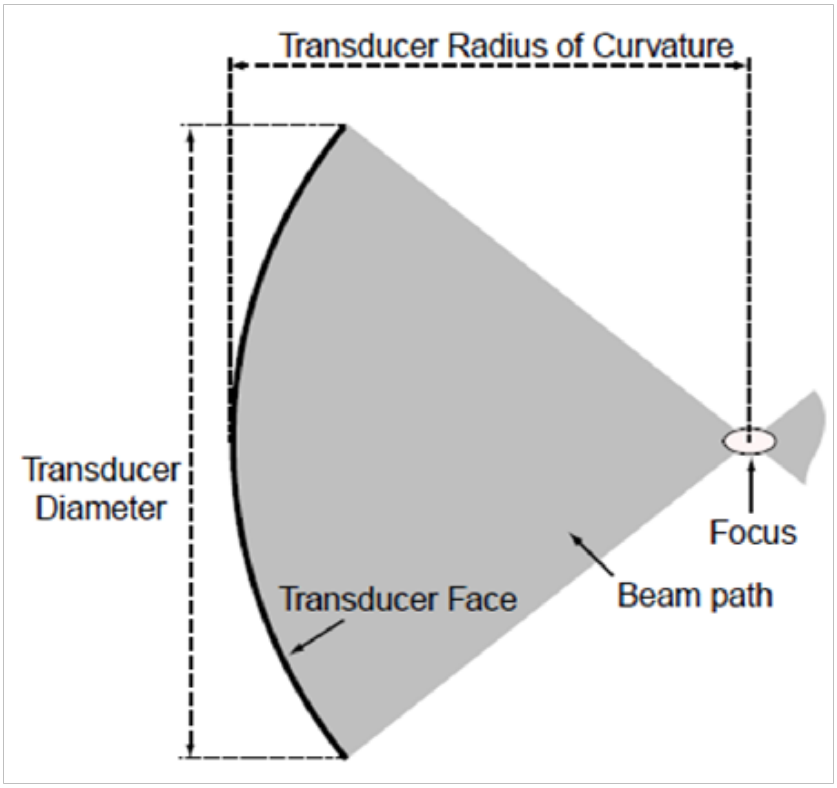

Figure 4 - Diagram of a focused ultrasound transducer. [9]

2.2.2. FUS for BBB disruption

2.2.2.1. Mechanism – Ultrasound and Microbubbles

The use of focused ultrasound with microbubbles is a noninvasive approach to deliver drugs by inducing targeted BBB disruption for a certain period of time (few hours). This method uses the mechanical interactions (scattering, absorption of ultrasound and bubble oscillation) created by the ultrasound field between the microbubbles oscillating and the vasculature, leading to a transient disassembly of tight junction complexes and the induction of active transport [10].

In 2000, Professor Nathan McDannold and his laboratory at Brigham and Women's Hospital found that if short ultrasound bursts are preceded by an intravenous injection of microbubbles contrast agent, the BBB could be consistently opened without the production of lesions or apparent neuronal damage. The BBB opening occurs using sonications applied as short (~1–20 milliseconds) bursts and at a low duty cycle (1–5%) for 0.5–1 minutes [5].

In a physiological context, modeling the MBs behavior is dependable on the assumptions made, which are related to the acoustic pressure.

At low acoustic pressures, stable cavitation causes MBs to oscillate linearly with the incident amplitude of the applied ultrasound pulse, resulting in the generation of not only nonlinear harmonic ultrasound waves of the transmitted fundamental frequency (i.e., half the fundamental frequency - subharmonics - 2-fold of the fundamental frequency - second harmonics - etc.), but also causes shear stresses around the MBs (e.g., microstream)[9][11]. The equation of bubble motion can be modeled as:

$$m\frac{(d^2x)}{(dt^2)}+\beta\frac{dx}{dt}+Sx=F_{driv} \qquad (2)$$

where m is the mass of the bubble–liquid system, b is the mechanical resistance related to the dissipation, S is the stiffness of the system, $Fdriv(t)$ is the driving force, and $x(t)$ is the radial displacement of the bubble wall relative to the initial radius R_0, according to:

$$x(t) = R(t) - R_0 \qquad (3)$$

Equation 2 was formulated considering the following assumptions [9]:

a. A bubble is considered spherical and surrounded by a liquid of infinite extent and a constant viscosity. The bubble volume is defined by a single variable, the radius and the motion of bubble is spherically symmetric;
b. The wavelength of the ultrasound field is much larger than the bubble diameter, and the motion of the bubble surface is only of interest. The vapor pressure remains constant during the compression and expansion phase and that there is no rectified diffusion during the short period of exposure to ultrasound;
c. The gas inside the bubble is assumed to be ideal and compression and expansion takes place according to the gas law;
d. At small excitation levels, the displacement of the bubble wall can be compared to the displacement of a simple one-dimensional mass spring oscillator. The oscillator is defined by its mass, restoring force, damping, and applied force.

At higher acoustic pressures, a more sophisticated model is needed once the MBs become instable and grow rapidly during the rarefaction phase of ultrasound and violently collapse due to the inertia of the fluid. This type of cavitation is referred to as inertial cavitation. The collapse of MBs splits them into many smaller MBs and even fragmentations, producing wideband signals that are usually recognized as a signature of inertial cavitation.

$$\varrho_1\left(R\frac{(d^2R)}{dt}+\frac{3}{2}\left(\frac{dR}{dt}\right)^2\right)=\left(\varrho_0+\frac{2\sigma}{R0}\right)\left(\frac{R}{R0}\right)^{(-3\varkappa)}\left(1-\frac{3\varkappa}{c}\left(\frac{dR}{dt}\right)\right)-\frac{2\sigma}{R}-\frac{4\mu}{R}\left(\frac{dR}{dt}\right)-\varrho_0-P_{ac}(t) \quad (4)$$

where R, dR/dt, dR^2/dt^2 represent the radius, velocity and acceleration of the bubble wall, r is the density of the liquid, ϱ_0 is the ambient pressure, s is the surface tension, k is the polytropic gas exponent, m is the viscosity of the surrounding water, c is the speed of sound, and $P_{ac}(t)$ is the applied acoustic field.

For the nonlinear Bubble Vibration following the next assumptions were used [9]:

a. The bubble is spherical and surrounded by an incompressible liquid with constant viscosity to infinite extent;
b. The gas in the bubble is compressed and expanded according to the gas law with the polytropic exponent remaining constant during the vibration;
c. Pressure at the bubble wall is at equilibrium. Combining the Rayleigh–Plesset equation and the polytropic gas law with the boundary condition, Nico-de Jong.

2.2.2.2. Correlation of Ultrasound Parameters on BBB Disruption

The use of ultrasound and microbubbles can produce BBB disruption, but there is still a concern about the bio effects produced by inertial cavitation, that can cause damage to cellular structures, producing undesired situations like intracerebral hemorrhage, per example.

A number of sonication parameters can be varied in ultrasonic BBB disruption. Each parameter variation may impact the threshold pressure amplitude needed to disrupt the BBB, along with the magnitude of its disruption, and the resultant drug quantity delivered to the brain parenchyma. For a fixed set of parameters, the magnitude of the BBB disruption is proportional to the increase of the pressure amplitude and to the sonication duration. Other parameters that influence the BBB opening threshold are the ultrasound frequency and the burst length. Several experiments [12][13][14] showed that it is possible to achieve effectively BBB disruption, by using a reliable group of settings [5], even without knowing the exact mechanism by which microbubble-enhanced FUS induces BBB disruption.

2.3. Intranasal drug delivery

2.3.1. Physiological principles - Nasal Pathways

The intranasal route of administration it is a non-invasive method by which therapeutic biomolecules can be delivered directly to the CNS, bypassing the BBB. The mechanisms underlying nose-to-brain transport have been widely investigated, but still unknown. Briefly, nasally administered substances follow the trajectory of the olfactory and trigeminal nerve pathways, by transcellular and extracellular processes. Transcellular transport involves neuronal uptake and retrograde transport within axons, a slow process that can take several days. However, transport of intranasally administered substances into the brain can occur within minutes.

Both nerves originate in the nasal cavity, having a first contact with the nasal mucosa. The olfactory pathway begins in the upper nasal passages, where olfactory receptor neurons extend from the mucus layer of the olfactory epithelium, through the *lamina propri*a and cribiform plate, and into the CSF-filled subarachnoid space of the cranial cavity. Once substances reach the CSF, they can travel in perivascular spaces formed between the outer membrane of microvessels and basement membrane of neighboring tissue, such as the glial basement membrane, which is comprised of astrocyte endfeet. These perivascular channels are thought to be the conduits through which intranasally delivered substances are distributed throughout the brain. Bulk flow mechanisms and arterial pulsations create a driving force for this 'perivascular pump'.

The trigeminal nerve, on the other hand, originates in the respiratory region of the nasal passages and enters the CNS at two points: i) the anterior lacerated foramen located near the pons; and ii) the cribiform plate near the olfactory bulbs. Thus, intranasally administered substances can reach both caudal and rostral brain regions by following the trajectory of the trigeminal nerve. The blood supply to the respiratory epithelium is relatively greater compared to the olfactory epithelium, making it an ideal site for systemic absorption of nasally applied drugs. Figure 5 represents the a

anatomical areas related to the nasal delivery.

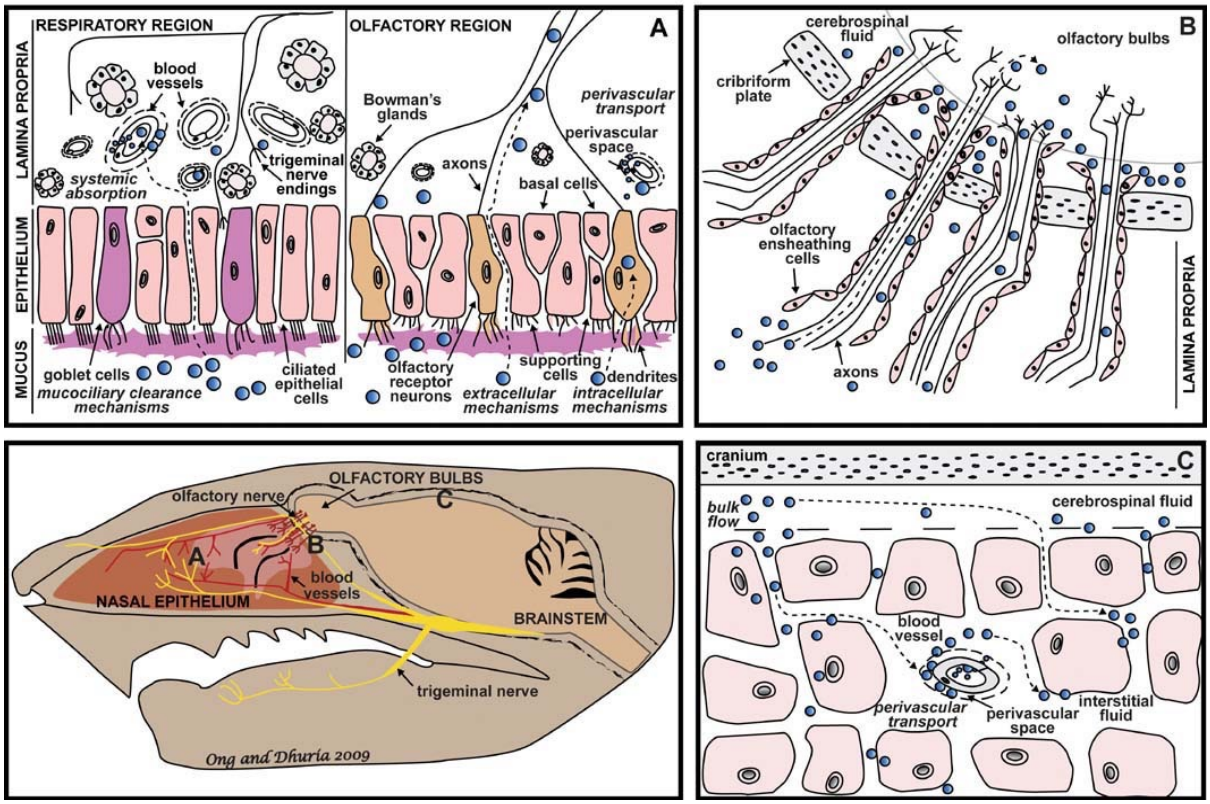

Figure 5 - Nasal Pathways. [15] (A) Dashed lines surrounding blood vessels represent the entering of the drugs in the perivascular channels and the dashed arrows pathways involving the olfactory and trigeminal nerves; (B) After reaching the lamina propria, they can access the cerebrospinal fluid (CSF) and olfactory bulbs (dashed arrows).

In accordance to the studies of Professor Barbara Waszczak [16], although nose-to-brain transport is rapid, only a small percentage of the total intranasally administered dose successfully reaches the brain, that is, delivery to brain was estimated to be about 1% of the administered dose in animal studies.

Animal models have been tested, and it is possible to notice the differences of the characteristics of the nasal cavity between Humans and rats (Table 1). This can be a factor to take in consideration in the results, once there are some anatomical differences between the animal model used for the intranasal delivery and Humans and it will be always necessary clinical trials to prove that the mechanisms involved are the same and the conclusions can be extrapolated.

Table 1 - Characteristics of nasal cavity between Human and rat[17].

Nasal Cavity	Human	Rat
Length (cm)	7.5	2.3
Volume (cm^3)	20.0	0.4
Surface area (cm^2)	150.0	14.0
Olfactory area (cm^2)	10.0	7.0

2.3.2. Factors that can influence the delivery by the IN route

There are several important factors to have in consideration in order to drugs to be delivered to the CNS via a nasal route. To achieve an efficient and rapid deposition of the drug, it needs to transverse the nasal mucosa, which is influenced by many factors such as mode of administration, particle size of the formulation, velocity of the delivered particles and angle [15] 18] [19].

In the choice of a model drug, it is important to have in consideration the physicochemical properties of the formulation, which are critical in terms of the regulation of its absorption when intranasally administrated. From the literature, it is known that for compounds with a MW up to 300 Da, the absorption of the drug decreases with the increase of the MW and the inverse can be noticed for compounds with a MW greater than 1000 Da [17].

Particle size and shape is another critical parameter in the design of nasal drug delivery systems, as particles with a diameter greater than 10 μm remain in the nasal cavity and small particles in general tend to go all the way through the nasal passage to the throat and lungs. Particles with diameters of less than 5 μm were deposited in the alveoli region, those with diameters between 5 and 10 μm were deposited into the bronchi, and those with diameters of $>$ 10 μm were localized and distributed within the nasal cavities [17]. This can be an important fact in the choice of the model drug, once we are interested in the nasal pathways that go to the CNS, instead of the deposition of the substance in different locations of the respiratory system.

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