Perfusion imaging in pre-clinics by
*in vivo* Magnetic Resonance Imaging
and Optical Imaging to evaluate an
anti-vascular therapy

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1 Summary

My project started with the implementation of a perfusion model, already developed on CT26 tumor mouse model, into a user-friendly graphical Matlab interface. In order to validate the reliability and robustness of the interface, Magnetic Resonance Images from perfusion experiments were processed. Then, the perfusion method was used to conduct in vivo pre-clinic studies by Optical Imaging. As a result, efficiency of anti-vascular therapies: Combretastatin (CA4P), Flavonoid Acetic Acid (FAA) and UTCBS-X, were assessed by the validity and tested of the processing software.

The interfaces were created to read the MRI and OI images, draw regions of interest over the image, display signal enhancement-curves, fit the enhancement curves by the Nelder-Mead algorithm, and by the Levenberg-Marquardt method, respectively. Also, the deconvolution of the dilution equation were implemented using Singular Value Decomposition.

Regions of interest around the arterial vessels and the tumors were drawn over the MR images, and signal enhancement-time curves were measured for each region. Then, the curves were fitted using Tofts-Kermode model. From plasma concentration fitting, the values $a_1 = 2.39 \pm 0.70$, $a_2 = 4.59 \pm 0.49$, $m_1 = 0.59 \pm 0.15$, $m_2 = 0.041 \pm 0.003$ were obtained. From tumor concentration fitting, the transcapillary transfer constant $k = 0.056 \pm 0.018 \text{ min}^{-1}$ from images of non-treated mice decreased to values between $0.008 < k < 0.050 \text{ min}^{-1}$ after the administration of FAA, CA4P or UTCBS-X anti-vascular treatments. The extracellular volume fraction $v_e = 0.31 \pm 0.11$ from images of non-treated mice increased to values between $0.39 < v_e < 0.53$ after the administration of anti-vascular treatments.

Optical Imaging experiments were performed using the same mouse model as in MRI perfusion experiments. Optical images were processed by the graphical Matlab interface using different mathematical models adapted to the type of contrast agents used, blood pool agent. Regions of interest around the arterial vessels and the tumors were drawn over the images, and signal enhancement-time curves were measured for each region. Arterial vessel curves were fitted using Gamma Variate Function and One Compartment Model. From plasma concentration fitting, the values of vascular circulation half-time $8.2 < t_1 < 11.2 \text{ hours}$, plasma clearance $0.025 < CL < 0.030 \text{ ml/h}$, and volume of distribution $0.19 < V_d < 0.45 \text{ ml}$ were obtained. By deconvolution of the dilution equation, blood volume $BV$, blood flow $BF$ and mean transit time $MTT$ were estimated. A decrease from $BV = 0.69 \pm 0.22$ to $BV = 0.26 \pm 0.10$, and from $BF = 3.22 \pm 1.05$ to $BF = 1.20 \pm 0.47$ were observed after the administration of the CA4P treatment.

2 Overview of the laboratory

The Unit of Chemical and Biological Technologies for Health (abbreviated to UTCBS in French) is a mixed research unit with the National Centre for Scientific Research (CNRS UMR 8258), the National Institute of Health and Medical Research (INSERM U1022), Paris Descartes University and the Ecole Nationale Supérieure de Chimie de Paris (EN-SCP). The research of this Unit is focused on cancer and genetic diseases. Their studies are developed over conventional and genetic therapies of cellular dysfunctions observed in
these pathologies. Projects involve the characterization and synthesis of active molecules and drug vectors which includes the determination of physicochemical properties, biodistribution and biochemical changes and interactions with the selected targets as well as in vitro and in vivo estimations of efficacy.

Three teams are part of the Unit. The first team ‘Synthèse, Electrochimie, Imagerie et Systèmes Analytiques pour le Diagnostic’ (SEISAD), is hosted by the ENSCP-Chimie ParisTech 11, rue Pierre et Marie Curie 75005 PARIS. The team activities focus on three axes: organic synthesis, Magnetic Resonance Imaging (B.T Doan CR) and electrochemistry for biology. Imaging research concern the in vivo methodological development in Magnetic Resonance Spectroscopy and Magnetic Resonance Imaging in small animals. New techniques of in vivo MRS to study the metabolism, and, in vivo MRI methods (new contrasts, MRI perfusion and new multimodal MRI-Optical acquisition method) are developed and validated by applications on the tumoral pathologies and for diagnosis.

The second team ‘Design, synthesis and validation of vectors for therapeutic targeting and Imaging’ is hosted by the faculty of pharmaceutical and biological sciences 4, ave de l’Observatoire 75006 PARIS. This team is specialized in the vectorization and targeting of therapeutic agents and in the development of selective probes for Imaging. The principal research topics are: vectors for therapeutic targeting; vectors for MRI, optical and scintigraphic imaging; imaging studies in small animals. The last topic includes the optimization of imaging techniques for diagnosis.

The third team ‘Innovating therapies for cancer and rare diseases’ has a particular interest concerning to genetic of cancer and rare diseases, and chemical therapy. The team research is focus in the development of in vitro and in vivo innovative therapeutic approaches in animals. This team works in collaboration with the other teams.

3 Introduction

Tumor angiogenesis is a complex multi-step process that involves a wide range of cell types and genetic alterations, however all tumors eventually possess the essential need for blood supply when reaching a certain size [1]. The induction of tumor vasculature is therefore a rate-limiting step in tumor progression and has been intensively addressed in the last three decades by many investigators. The development of new blood vessels, namely, angiogenesis, is a tightly regulated procedure. In pathological angiogenesis, imbalance between angiogenic activators and inhibitors leads to abnormal structural and functional vascular system[2, 3]. Tumor blood vessels are highly disorganized, dilated and leaky due to discontinuous walls and fenestrated endothelial lining [3]. Consequently, this chaotic vascular network generates in some regions poor blood flow, inefficient to deliver nutrients and oxygen. The high vasculature permeability increases water flow into the tissue and induces high interstitial fluid pressure that reduces the driving forces for extravasation and leads to outward convection forces [3]. Hence, the flux of blood-borne molecules from the intravascular compartment to the tissue interstitial space is determined due to hydrostatic pressure and osmotic/oncotic pressure gradients. The extravasated molecules are further transported in the interstitium by diffusion and convection. Thus, both the transcapillary transfer and the transport in the interstitium are affected by concentration and pressure gradients. The irregular architecture and the unique malfunction of tumor vasculature
provide an insight through which tumors can be inspected and diagnosed. Quantification of the various features of the blood vessels and tumor perfusion has the potential to provide diagnostic and prognostic information. Moreover, it is the tool to assess the efficacy of different anti-vascular treatments that target vascular endothelial cells and disrupt the vascular functionality of the tumors.

Imaging has been widely applied for monitoring anti-tumoral therapy. In fact, in vivo studies are used to determine tumor position, define vascular disrupting agents (VDAs) and determine pharmacokinetics constants based on images getting from live mice. The images obtained by Magnetic Resonance Imaging (MRI) and Optical Imaging (OI) have helped for the identification and quantification of tumor growth parameters related to microvascular changes. The quantification of these parameters is possible by using contrast agents during imaging acquisition. In MR imaging, dynamic contrast enhanced (DCE-MRI) is the functional MRI method used for quantification in pre-clinics and clinics. This method consists in the injection of a contrast agent (C.A.) during a dynamic MRI sequence. In optical imaging, the C.A. used is a dye.

The effect of a C.A. in blood is an increase of signal intensity on the images (MR and optical images). The signal intensity for each image is plotted relative to the intensity level of the pre-contrast image, resulting in an enhancement curve (time-intensity curve). The intensity in these images depends on the quantity of C.A. passing throughout the vessels (perfusion), and also, on the concentration of the C.A. in the tissue when there is an increased of vessel permeability. Changes of permeability and perfusion are identified in the enhancement curve by curve fitting. A fitted curve is adapted to a mathematical-biological model to show the concentration of the C.A. at each time point in the blood supply of the tissue of interest.

The evidence of functional changes in tumor vasculature, upon treatment with a VDA, is showed with the variation in the enhancement curves. Changes in the signal intensity produce a posterior changes in the parameters getting from the enhancement curve. Therefore, quantification of the effects during anti-vascular treatments is possible by fitting enhancement curves. The enhancement curves are obtained from the injection of C.A. during imaging acquisition, and the fitting lets a quantitatively estimation of the permeability of tumor microvessels. Thereby, permeability value has become an index of anti-vascular therapeutic efficacy [4].

The objective of this research project was the development of two graphical Matlab interfaces capable of implementing perfusion methods in pre-clinical studies by MRI and Optical imaging, respectively. First, the robustness of the MRI interface was tested on perfusion MRI data. Then, we transferred the perfusion methodology in the field of optical imaging as a new original modality for perfusion studies. In vivo Optical imaging was performed on mice enabling the quantification of pharmacokinetic parameters related to the one obtained by MRI perfusion.

In the part of Magnetic Resonance Imaging, all my activities during my internship were also supervised by Gregory Ramniceanu, UTCBS researcher.
4 Theory

4.1 Biological Concepts

4.1.1 Colorectal tumor model: CT26

Murine tumor models are series of tumoral cells developed for understanding of the molecular pathways responsible for the initiation, progression, and metastasis of cancer cells and to assess properties of anti-cancer drugs. The model lets to measure the variations of the biological and pharmacological properties which represent variations on these properties observed in human tumors [6].

CT26 (Colon Tumor 26) is a colon carcinoma cell line. It is a transplantable murine tumor of colonic origin which is a chemically induced by N-nitroso-N-methylurethane-(NNMU) [5]. This line has the ability to form in vivo tumors that are well vascularized and expresses cellular adhesion to molecules (targeting for anti-vascularity). The CT26 line has been selected because of its high growth rate, the response time between implantation and metastatic spread. The tumor fragments inserted (2-3 mm\(^3\), approximately 9x10\(^5\) tumor cells [19]) where extracted from subcutaneous CT26 tumor in a sacrificed mouse. The ectopic implantation is bilateral at the level of the flanks of the mice. At day 10, tumor growth reaches a size about 800 mm\(^3\) and enters an intermediate angiogenic stage with little necrosis at its center; which is an optimal model for the development of perfusion imaging methods.

4.1.2 Anti-vascular treatments

4.1.2.1 Vascular Disrupting Agents

Vascular Disrupting Agents (VDA) have been developed for treatments in pre-clinical trials. These agents respond to different molecular strategies improving the inhibition of blood vessels growth. These strategies deliver inhibitors, obstruct angiogenic ligands or target tumor vasculature. According to their mechanism of action, there are two groups of VDAs: agents binding to tubulin which anti-vascular action is direct and cytotoxic, and the flavonoids which anti-vascular action is fast, direct and indirect[7].

Combretastatin A4 Phosphate (CA4P) is a tubulin-destabilizing agent. Its target is the endothelial cells of the tumors which are dependent on tubulin cytoskeleton. The binding with the tubulin induces a rapid microtubule depolymerization (disassembly of microtubules). Vascular permeability is increased, then the blood flow is inhibited with a vasoconstriction of the vessels. The vascular shutdown, which is produced in subcutaneous solid tumors, causes tumor necrosis [7, 9].

The flavonoid compounds are related to Flavonoid Acetic Acid. These agents disrupt the vascularization of the tumor by selectively inducing apoptosis (programmed cell death) in vascular endothelial cells. The high mortality of the endothelial cells exposes the basement membrane resulting in the rupture of blood vessels of the tumor. FAA is an agent representative of the flavonoids. The mechanism of action of this agent is independent of the tubulin [8].
4.1.2.2 The molecule UTCBS-X

The molecule UTCBS-X was developed for the collaborated team of Dr. Guy Chabot. The objective of the team is to identify new molecules inhibiting tubulin polymerization that would allow selective destruction of tumor vasculature. This molecule has already been characterized as anti-vascular on CT26 tumors. The studies were developed in the same laboratory (Unit de Technologies Chimiques et Biologiques pour la Santé UPCG-ENSCP) showing that the molecule is very active in mouse colon tumor-bearing (CT26) [8].

4.2 MRI quantitative model

4.2.1 Dynamic contrast Enhancement Method

Dynamic Contrast Enhancement (DCE) is a MRI method based in $T_1$-weighted dynamic MRI sequences, measured during an intravenousous injection of a C.A.. The C.A. increases the signal intensity on these images in order to determine permeability and perfusion changes. When there is an increased of vessel permeability, contrast agent leaks out and concentrate in the tissue. Therefore, DCE links the contrast agent concentration in tissue (model by Tofts-Kermode, section 4.2.2) with MRI signal enhancement (MRI images acquired) to determine the transcapillary transfer constant (permeability surface area product per volume unit of tissue) and the extracellular volume fraction (leakage space per unit volume).

Changes of permeability and perfusion can be identified by the enhancement curve and quantified by curve fitting using mathematical models. The signal enhancement is proportional to the relaxivity which is related with $T_1$-weighted MRI sequences (equation 1) [14].

$$\frac{1}{T_1} = \frac{1}{T_{10}} + r_1 C$$  \hspace{1cm} (1)

The relaxation time $T_1$ is calculated using the relaxivity curve $r_1$. Where $T_{10}$ is the value of $T_1$ before the injection of tracer, $C$ and $r_1$ are the concentration and relaxivity of the C.A.. The $T_{10}$ and $T_1$ values are calculated from $T_1$-weighted images (before and after the C.A. injection, respectively). The concentration of the tracer in function of the time $C_t$ is determined using the equation determined from Tofts-Kermode Model for the estimation of concentration (Equation 9).

FLASH (Fast Low Angle SHot) sequence is a fast spoiled gradient echo sequence which decreases the scan time by having shorter repetition times ($TR$) and flip angles ($\alpha$). This sequence is used in DCE-MRI to produce strong $T_1$-weighted images. The signal intensity in the image obtained from that sequence is given by the equation [16]:

$$S = \rho \sin \alpha \frac{(1 - e^{-\frac{TR}{T_1}}) e^{-\frac{TE}{T_2}}}{1 - \cos \alpha e^{-\frac{TR}{T_1}}}$$  \hspace{1cm} (2)

The C.A. administrated is distributed in the tissue changing the its relaxation rates and showing an effect over $T_1$ and $T_2$ values. Therefore, the signal after C.A. injection can be written as a combination of equations 1 and 2 where signal depends of the C.A. concentration in the tissue.
$$S(C) = \rho \sin \alpha \frac{(1 - e^{-TR(T_{10}^{-1} + R_1 C)}) e^{-TE(T_{20}^{-1} + R_2 C)}}{1 - \cos \alpha e^{-TR(T_{10}^{-1} + R_1 C)}}$$  \[10\]  \[3\]

Signal enhancement ($E$) is relation of the signal after C.A. injection and the signal in absence of C.A. (concentration is equal to zero). Thus, the signal enhancement depends on the agent concentration $C$ and is independent of $T_2^*$ \[10, 17\].

$$E(C) = \frac{S(C)}{S(C=0)} - 1 = \frac{(1 - e^{-TR(T_{10}^{-1} + R_1 C)})}{(1 - e^{-TR T_{10}^{-1}})} \frac{(1 - e^{-TR T_{10}^{-1} \cos \alpha})}{(1 - e^{-TR (T_{10}^{-1} + R_1 C) \cos \alpha})} - 1$$  \[4\]

### 4.2.2 Tofts-Kermode Model

Tofts-Kermode model could theoretically be applied to any tissue with leaking capillaries \[12\]. Microvaculature in tumor tissue has higher permeability to macromolecular tracers (ex. contrast molecules) than normal tissues \[11\]. Thereby, Toft-Kermode model can be applied into the quantification of perfusion parameters of cancer tissue. In fact, this model have been widely proved to estimate the kinetic parameters related to microvascular changes in tissues \[10, 12\].

Tofts-Kermode model is a multi-compartment exchange model. The compartments are normal extracellular space, plasma (intravascular), tumor extracellular space. Some assumptions are that the tracer concentration in each compartment is uniform (well-mixed), a linear inter-compartment flux (difference of concentrations), time-invariant parameters, and the relaxation rate is proportional to tracer concentration \[14\].

The transport of the contrast agent molecules through a capillary wall (from plasma to extracellular space) is modeled by diffusion theory, where flow rate is proportional to the concentration gradient. Thus, permeability is the flow of tracer per concentration and per area. The flux of the tracer throughout the semipermeable membrane from the plasma into abnormal extracellular space can be described by equation 5. Also, from the compartment model the concentration of the contrast agent in the extracellular space ($C_e$) is given as a function of the plasma ($C_p$) and the tissue concentration ($C_t$) (equation 6) \[12\]. In Both models, bolus and slow infusion, the C.A. is distributed in the plasma volume rather than in the whole blood volume; therefore, the equations should be corrected by $C_p(1ht)Ca$, where $h_t=0.42$ (hematocrit value).

$$v_e V_t \frac{dC_e}{dt} = (P_{in} C_p - P_{out} C_e) SM_t$$  \[5\]

$$v_e C_e = C_t - v_p C_p$$  \[6\]

### 4.2.2.1 Contrast agent concentration during bolus injection

The contrast agent can be administrated by an intravenous bolus injection or by an intravenous slow infusion injection. A bolus injection can be described as an ‘instantaneous’ administration or given a dose over a short amount of time (large amount at once). Thus,
after a bolus dose \( D \) (mmol/kg), given at time \( t=0 \), the plasma concentration decays bi-exponentially [12]:

\[
C_p = D \sum_{i=1}^{2} a_i e^{-m_i t} = D \left( a_1 e^{-m_1 t} + a_2 e^{-m_2 t} \right) \tag{7}
\]

The constants \( a_i \) can be determined from the initial conditions \( (C_p|_{t=0}=\frac{D}{V_p}, C_e|_{t=0}=0) \) taking into account that the coupling between plasma-extracellular compartments (represented by \( K_1 \)) is stronger than the coupling between plasma-kidneys (represented by \( K_2 \) (Table 1). The components \( a_1 \) and \( m_1 \) are denominated as fast components and describes the ‘equilibration between plasma and extracellular space (mixing phase)’; in contrast, the components \( a_2 \) and \( m_2 \) are denominated as slower ones and describes the ‘emptying of the plasma and extracellular space compartments to the kidneys (renal excretion phase)’ [10, 12, 13].

\[
\frac{dC_t}{dt} - v_p \frac{dC_p}{dt} = k_{in}^{PS\rho} C_p - k_{out}^{PS\rho} C_t - v_p C_p \tag{8}
\]

A combination of the equations 5, 6, 7 and 8 determined the amount of C.A. in the tissue as function of the time [14]:

\[
C_t = D k_{in}^{PS\rho} \sum_{i=1}^{2} a_i \frac{e^{\frac{k_{out}^{PS\rho}}{v_e} t} - e^{-m_i t}}{m_i - \frac{k_{out}^{PS\rho}}{v_e}} + v_p D \sum_{i=1}^{2} a_i e^{-m_i t} \tag{9}
\]

Taking account that the permeability is equal \( (k_{out}^{PS\rho} = k_{in}^{PS\rho} = k) \) and the plasma volume is negligible \( (v_p = 0) \), as well as \( m_3 = \frac{k}{v_e}, b_1 = \frac{ka_1}{m_3-m_1}, b_2 = \frac{ka_2}{m_3-m_2}, b_3 = -(b_1 + b_2) \), thus equation 9 can be simplify by [12]:

\[
C_t(t) = D \left( b_1 e^{-m_1 t} + b_2 e^{-m_2 t} + b_3 e^{-m_3 t} \right) \tag{10}
\]

As explained in [18], the equation 10 represents the concentration of the agent in tissue \( (C_t) \) (tumor tissue for this project) because of the diffusion of C.A. from the blood vessels into the extracellular space of the tumor tissue. The maximum \( C_t \) will produce when the plasma concentration \( (C_p) \) is equal to the extracellular concentration \( (C_e) \). The posterior decrease of C.A. in the tumor tissue is due to the agent will back to the plasma and excreted via the kidney.

The coefficients \( a_i \) and \( b_i \) can be estimated by the values of signal enhancement (equation 4) which are then injected as input in the fitting process for determining the concentration of C.A. in the tumor tissue giving the transcapillary transfer constant \( (k) \) and the extracellular extravascular volume fraction \( (v_e) \).

4.2.2.2 Contrast agent concentration during slow infusion injection

Slow infusion injection is the administration of a set of small doses over a period of time. It means, that the same amount of dose is administrated as a series of small doses (doselets) in a lapse of time. Thus, after a slow infusion dose \( D^{inf} \) (mmol/kgmin\(^{-1}\)) (starting at time
Table 1: Tofts-Kermode Model: Standard Set of Symbols. [12, 14]

\[ t = 0 \) the total plasma concentration (sum of the contributions from each doselet) at time \( t \) is given by [13]:

\[
C_{p}^{\text{inf}} = D^{\text{inf}} \sum_{i=1}^{2} a_i \left( 1 - e^{-m_i t} \right) / m_i
\]  

(11)

Using equation 9, the tissue concentration at time \( t \) is the sum of contributions from each doselet [13]:

\[
C_{t}^{\text{inf}} = D^{\text{inf}} k \sum_{i=1}^{2} \frac{a_i}{m_i - m_3} \left( \frac{1 - e^{-m_3 t}}{m_3} - \frac{1 - e^{-m_i t}}{m_i} \right)
\]  

(12)

Similar than in the bolus injection, equation 12 represents the C.A. concentration in the tumor tissue at time \( t \) during a slow infusion injection.
4.3 Optical Imaging quantitative models

Using optical contrast agents, the quantification of measurements from perfusion techniques are not widely spread. Even though optical imaging lets a visualization of the microvascular network when a contrast agent is injected, there are some limitations in the ability of quantifying perfusion parameters from the signal enhancement of the tissue of interest. The limitations include the inability to provide adequate spatial and temporal resolutions, the poor tissue penetration, high light scattering, and the autofluorescence of living organism [15, 25]. All of these problems joined to the lack of theoretical models well adapted to quantify perfusion parameters.

In this project, a quantification of different perfusion parameters were done by three mathematical models. The aim was the quantification of the C.A. concentration in the plasma and in the tumor tissue. The two first models, designated as, Model 1 and Model 2 are mathematical equations used to estimate the arterial concentration (linked to the plasma concentration). Thus, it is possible to quantify the elimination constant ($K_e$), the vascular circulation half-time ($t_{1/2}$), the plasma clearance ($CL$) and the volume of distribution ($V_d$). The last model (Model 3) uses the deconvolution method to estimate the blood volume ($BV$), the blood flow ($BF$) and the mean transit time ($MTT$).

4.3.1 Model 1 : Gamma variate function

The first pass of the arterial concentration-time curve can be modeled by a gamma variate function where the recirculation is eliminated from the signal [20, 31, 33]. The function is expressed as:

$$f(t) = K \cdot (t - t_o)^\alpha e^{-\frac{(t-t_o)}{\beta}},$$  \hspace{1cm} (13)

where $K$ is the scaling factor, $\alpha$ and $\beta$ are shape parameters, and $t_o$ is the bolus arrival time ($t > t_o$). A simplified equation to determine the arterial concentration when $t_o = 0$ can be derived of that gamma variate function:

$$C_a(t) \approx D K \cdot t^\alpha e^{-\frac{t}{\beta}},$$  \hspace{1cm} (14)

where $D$ is the contrast agent dose, $\alpha$ is a parameter which depends on the architecture of tissue vasculature and $\beta$ is related to the blood flow [20]. Because, the C.A. is distributed in the plasma volume rather than in the whole blood volume, the equation 14 should be corrected by $C_p = (1 - h_t) * C_a$, where $h_t = 0.42$ (hematocrit value) and $C_p$ is the plasma concentration [32].

$$C_p(t) \approx D K \cdot (1 - h_t) \cdot t^\alpha e^{-\frac{t}{\beta}},$$  \hspace{1cm} (15)

The estimation of the $\beta$ and the area under the plasma concentration-time curve ($AUC$) lets an approximative determination of the elimination constant ($K_e$), the vascular circulation half-time ($t_{1/2}$), the plasma clearance ($CL$) and the volume of distribution ($V_d$).

$$K_e = \frac{1}{\beta},$$  \hspace{1cm} (16)

$$t_{1/2} = ln(2) \cdot \beta$$  \hspace{1cm} (17)
Mathematically, it is possible determine the maximum value of time $t_{max}$ at which the function reaches its maximum. After taking the first derivative, it easily gets that $t_{max} = \alpha \beta$.

In order to estimate the C.A. concentration, it is essential to determine its relation to the signal intensity of the optical images. Zheng et al [23] have determined a relation between signal enhancement measured and the plasma concentration of a specifically designed blood pool C.A.. The correlation is based on plasma concentration measurements. This equation was adapted for this project as:

$$E(C_p) = \frac{S(C_p)}{S(C_p = 0)} - 1 = a \ (1 - e^{-bC_p}), \quad (20)$$

where $E$ is the signal enhancement, $a$ and $b$ are coefficients that will be determined after the adjust of the signal values with the Gamma Variate function.

### 4.3.2 Model 2 : One compartment model

Different authors have estimated the arterial concentration of an optical agent using the compartment model method [23, 24]. As was explained in the Tofts-Kermode model (section 4.2.2), a compartment model describes the motion of a tracer through biological regions described as ‘compartments’. The compartment defined here is the blood plasma. However, the measurements of signal intensity are taken in blood vessels, and the tracer is distributed in the plasma volume rather than in the whole blood volume (blood pool tracer). So, it is necessary to apply a correction given by : $C_p = (1 - h_t) \ast C_a$, where $h = 0.42$ [32].

For a single dose, which is administrated at time $t=0$, the model for an intravenous bolus injection is given for the equation 21, where $D$ is the contrast agent dose, $K$ is a scaling factor, $\beta = K_e$ which is known as the elimination constant [26].

$$C_p = D \ (1 - h_t) \ K \ e^{-\beta \ t} \quad (21)$$

The estimation of $K_e$ and $AUC$ lets to determine other pharmacokinetic parameters: the vascular circulation half-time ($t_{1/2}$), the plasma clearance ($CL$) and the volume of distribution ($V_d$) [23].

$$t_{1/2} = \frac{\ln(2)}{\beta} \quad (22)$$

$$CL = \frac{Dose}{AUC} \quad (23)$$

$$V_d = \frac{CL}{\beta} \quad (24)$$

Equation 20 also will be considered to determine the plasma concentration from signal intensity measurements.
Table 2: Pharmacokinetic parameters: Standard Set of Symbols [23, 32].

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>mL/h</td>
<td>clearance of elimination</td>
</tr>
<tr>
<td>D</td>
<td>mg</td>
<td>Dose of contrast agent</td>
</tr>
<tr>
<td>Ke</td>
<td>min(^{-1})</td>
<td>elimination constant</td>
</tr>
<tr>
<td>V(_d)</td>
<td>mL</td>
<td>volume of distribution</td>
</tr>
<tr>
<td>t(_{1/2})</td>
<td>h</td>
<td>vascular circulation half-time</td>
</tr>
</tbody>
</table>

4.3.3 Model 3: Dilution theory

After the venous injection of a contrast agent, the agent arrives and concentrates in tissues through perfusion. Thus, the concentration inside an interest tissue is a function of time and factors that depend on the blood flow. Blood flow (\(BF\)) and the mean transit time (\(MTT\)) of the tracer in the tissue are two parameters widely determined [21, 27, 28]. A way to quantify these parameters involves the use of model-independent methods (deconvolution).

Based on the dilution theory, the total volume of blood (\(BV\)) in a given region of the tissue is defined as the ratio between the areas of the concentration-time curves in the tissue (\(C_t(t)\)) and in the arterial (\(C_a(t)\))[21]:

\[
BV = \frac{\int_{-\infty}^{\infty} C_t(\tau) d\tau}{\int_{-\infty}^{\infty} C_a(\tau) d\tau}
\] (25)

For an infinitely short injection, the tissue concentration (\(C_t\)) depends on the blood flow (\(BF\)), the arterial concentration (\(C_a\)) and a residue function (\(R(t)\)), transforming the equation 25 in:

\[
C_t = BF \int_0^t C_a(\tau) R(t - \tau) ;
\] (26)

where the blood flow (\(BF\)) is the volume of blood moving through an interest region per unit time, representing the capillary flow in the tissue (in milliliters of blood per 100 gram of tissue per minute). \(R\) represents the fraction of the injected tracer still present in the vasculature at time taking into account \(R|_{t=0} = 1\) and \(R|_{t=\infty} = 0\).

The product \(BF R(t)\) is called as the tissue impulse response function, which is the tissue concentration as a result of the infinitely short \(C_a\). In equation 26, \(C_a\) will not arrive to be as infinitely short, thus \(C_a\) needs an interval of time. If we take the smallest time interval for \(C_a\), the tissue concentration will become the convolution of the arterial concentration and the residue function [20, 21, 22, 27]:

\[
C_t = BF C_a(t) \otimes R(t)
\] (27)

As in the last models, the hematocrit value should be incorporated as a correction factor linking the arterial concentration and the plasma concentration \((C_p)\) by \(C_p(t) = (1 - h_t) C_a(t)\) [27, 32]. Thus, the tissue concentration is:

\[
C_t = \frac{1}{(1 - h_t)} BF C_p(t) \otimes R(t)
\] (28)
4.3.3.1 Linear Algebraic approach

The model is based on a reformulation of the equation of convolution (Equation 28). It was assumed that $C_t$ and $C_p$ are measured at $N$ discrete time points $t_1, t_2, ..., t_N$ at a constant interval $\Delta t$ [22], so:

$$C_t(t_j) = \frac{B F}{(1 - h_t)} \int_0^{t_j} C_p(\tau) R(t - d\tau) \approx \Delta t \frac{B F}{(1 - h_t)} \sum_{i=1}^{j} C_p(t_i) R(t_j - t_i),$$

(29)

where $C_t(t_j)$ is the tissue concentration at time $t_j$. The equation can be rewritten into a matrix equation as:

$$\begin{pmatrix}
C_t(t_1) \\
C_t(t_2) \\
\vdots \\
C_t(t_N)
\end{pmatrix} = \frac{B F}{(1 - h_t)} \Delta t \begin{pmatrix}
C_p(t_1) & 0 & \cdots & 0 \\
C_p(t_2) & C_p(t_1) & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
C_p(t_N) & 0 & \cdots & 0
\end{pmatrix} \begin{pmatrix}
R_t(t_1) \\
R_t(t_2) \\
\vdots \\
R_t(t_N)
\end{pmatrix},$$

(30)

$$C = B F \cdot A \cdot R$$

(31)

This is a standard matrix equation that can theoretically be inverted to get $B F R(t)$ values. However, the technique described above assumes that arterial and tissue concentrations are constant between measurements. In contrast, both concentrations are expected to show little variation within the temporal resolution of the measurements in dynamic imaging. Therefore, a modification of the $C_p$ matrix was introduced where $C_p$ and $R$ both vary linearly with time[21]. The elements of the $C_p$ matrix become:

$$a_{i,j} = \begin{cases} 
\frac{\Delta t}{1 - h_t} (C_p(t_{i-j-1}) + 4 C_p(t_{i-j}) + C_p(t_{i-j+1}))/6, & 0 < j < i \\
0, & \text{else}
\end{cases}$$

A stable solution of the equation 31 is given by applying a singular value decomposition (SVD) method. SVD is a factorization method which is used widely in linear inverse problems. It was used to calculate a pseudo-inverse matrix of $A$ to help in the estimation of $B F R(t)$ from the equation 30.

$$A = U \cdot W \cdot V^T$$

(32)

$$B F \cdot R = V \left[ \text{diag} \left( \frac{1}{w} \right) \right] (U^T \cdot C)$$

(33)

To increment the stability in that solution a regularization method known as Standard form Tikhonov Regularization (SFTR) is apply to perform a smooth truncation of the singular values [28]. The method suggests to replace $\text{diag}(1/w) = \text{diag}(w_j/(w_j^2 + P_{SVD}^2))$. Thus:

$$B F \cdot R = V \left[ \text{diag} \left( \frac{w_j}{w_j^2 + P_{SVD}^2} \right) \right] (U^T \cdot C),$$

(34)

18
where $P_{SVD}$ is the truncation parameter of a truncated singular value decomposition (tSVD). In a tSVD method, all values below the truncation parameter are zeros. This method reduce the effects of noise in data [28].

### 4.3.3.2 Blood Flow and Mean Transit Time estimations

Using deconvolution, blood flow $BF$ should be determined when $R|_{t=0} = 1$, however this is only valid without the presence of delay and dispersion. Therefore in several articles the maximum of $BF R(t)$ (equation 34) is chosen as blood flow value [20, 21, 29]. This will be the value used in this report as well.

$$\overline{BF} = \max(BF \cdot R)$$ (35)

The mean transit time ($MTT$) is a measure of the mean time for blood to perfuse a region of interest within a tissue (Table 3). $MTT$ is related to $BF$ and $BV$ [21, 20] by:

$$MTT = \frac{BV}{BF} \approx \Delta t \sum_{i=0}^{N} BF R(t_i) \max(BF \cdot R)$$ (36)

### 5 Materials and Methods

#### 5.1 Magnetic Resonance Imaging: Data from *in vivo* studies

##### 5.1.1 Contrast Agent and Image Acquisition

Gd-DOTA (Dotarem) was used as a contrast agent in all MRI experiments. This is a paramagnetic agent with low molecular weight (0.44 kDa). Dotarem was injected at a dose of $0.25 mmol/kg$ in the bolus injection experiments and at $0.011 mmol/kg min^{-1}$ in the slow infusion injection experiments.

The experiments were carried out on a vertical imaging spectrometer fitted with an ultra shielded refrigerated 300WB magnet at 7 T (Bruker, Avance II, Wissembourg, France) equipped with a whole-body 40mm inner diameter birdcage RF coil (Bruker) for mouse and Paravision5.1 as acquisition software.

##### 5.1.2 Perfusion Method

DCE method was performed in BALB/c female mice with ectopic CT26 bilateral tumor to get $T_1$-weighted images from dynamic MRI sequences which were measured during an intravenous injection of Dotarem. Between 3 to 4 axial slices were acquired with a temporal resolution of 12.8 seconds for the bolus experiments and 50 seconds for the slow infusion.
experiments. The Dotarem was injected 1 min after the starting of the first sequence (Figure 1).

![Figure 1: Sequence of MRI images after Dotarem injection at t=0min. The images present an axial slice in different time points. The green line shows the contour of a right tumor and the red line the contour of a left tumor.](image)

The mean signal intensity was determined over Regions of Interest (ROIs) in all of images from all experiments. These ROIs were drawn over two different regions: blood vessels and tumor tissue. The ROIs over blood vessels were used to determine the arterial concentration, and the ROIs around the tumor tissue were used to determine the concentration of the Dotarem in tumor tissue.

The signal enhancement-time curves were obtained using the mean signal intensity. Enhancement-time curves were fitted using the Nelder-Mead algorithm to estimate the concentrations of the Dotarem in the ROIs. The estimated values of concentration were used to estimate the transcapillary transfer constant $k$ and the extracellular volume fraction $v_e$ (Tofts-Kermode model, section 4.2.2).

After the characterization of perfusion reference curves, in vivo perfusion studies were done with the anti-vascular treatments: CA4P (100mg/kg mouse), FAA (170mg/kg mouse), UTCBS-X (100mg/kg mouse). At day 10, all animals were monitored as the control group. Then, treatments were administrated followed by another monitoring 24 hours later.
5.1.3 Data Acquisition

Data from 20 perfusion experiments were acquired from the Bruker spectrometer. The experiments were done on mice with CT26 bilateral tumor implantations. Each mouse was monitored before (reference) and after VDA treatments. In twelve of experiments, the Dotarem was injected by bolus injection (fast injection). In the other eight experiments, the Dotarem was injected by slow infusion injection (slow injection).

A Matlab interface was created as a user-friendly tool that can process this MRI data. The interface was developed and implemented for perfusion experiments using both bolus and slow infusion injections. The goal of the interface was the quantification of the perfusion parameters: $k$ and $v_e$. This graphical interface is explained in more detail in Appendix A.

5.2 Magnetic Resonance Imaging: Data Analysis

The data analysis can be divided in: the determination of the Dotarem concentration in plasma and the determination of the Dotarem concentration in tumor tissue.

5.2.1 Plasma concentration Measurements

The Dotarem plasma concentration was determined in four steps (Figure 2). First, some ROIs were drawn over the image in a region that shows blood vessels. Second, the signal enhancement-time curve was obtained using the signal intensity before and after the Dotarem injection (Equation 4). The signal enhancement-time curves were obtained using the mean signal intensity data. There was a curve per ROI. Third, as the signal enhancement is related to the agent concentration in the tissue, enhancement-time curves were fitted to estimate the agent concentrations in the ROI. The concentration of Dotarem were derived using its relaxivity rate ($3.8mM^{-1} s^{-1}$), $T_{10} = 200ms$ and hematocrit level 0.42. For bolus injection, a combination of equation 4 (signal enhancement) and equation 7 (plasma concentration) from Tofts-Kermode Model was used. Slow infusion injection were fitted using equation 11. Finally, as a result of the fitting the components $a_1$, $a_2$, $m_1$, $m_2$ were obtained.

5.2.2 Tumor concentration Measurements

The Dotarem concentration in tumor was determined in four steps (Figure 3). First, a ROI was drawn around the tumor tissue regions. Second, the signal enhancement-time curve

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1 All MRI experiments were conducted by Gregory Rannniceanu, UTCBS researcher.
was obtained using the signal intensity before and after the Dotarem injection (Equation 4). Third, enhancement-time curves were fitted by equation 10 (bolus injection), and by equation 12 (slow infusion injection). Also, the estimated components of the plasma equation: \( a_1, a_2, m_1, m_2 \) were considered in the fitting. The obtained concentrations were used to estimate the transcapillary transfer constant \( k \) and the extracellular volume fraction \( v_e \) (Tofts-Kermode model, section 4.2.2).

![Figure 3: Sequence to estimate the agent concentration in tumor. First, a ROI is drawn (tumor regions). Second, the signal enhancement-time curve is obtained. Third, enhancement curve is fitted using the estimated parameters in plasma concentration. Fourth, transcapillary transfer constant \( k \) and the extracellular volume fraction \( v_e \) are gotten.](image)

5.3 Optical Imaging: *In vivo* studies

5.3.1 Animal Preparation

Perfusion assays were done using CT26 mouse tumor model at 10 days after the implantation of tumor fragments. 8 to 10 weeks old female Balb/c mice with body weights ranging from 19g-21g. Experiments were carried out under general anesthesia. This was performed by an intra-peritoneal injection of ketamine 80mg/kg and xylazine 10mg/kg.

Combretastatin A4 Phosphate (CA4P) was used as the anti-vascular treatment. CA4P (MW:440.29, lot:TB422.1) was administered by an intra-peritoneal injection. A solution of 100mg/kg mouse was injected 24 hours before the perfusion session.

For optical imaging purposes, mice were shaved in the thoraco-abdominal region in order to show clearly the tumoral region. Also, this procedure suppresses the autofluorescence and artifacts from the hair. All animal studies were performed in agreement with the French guidelines for animal care and in compliance with procedures approved by the appropriate institutional ethics committee.

5.3.2 Contrast agent injection and Image Acquisition

A solution of a confidential macromolecule-Cy5 (OPTx, 55kD) at 2.7mg/ml was used as an optical blood pool agent (long blood circulation time). The solution was injected intravenously into the tail vein of each mouse. Perfusion images were acquired during the bolus injection (fast injection in less than 5s) of the contrast agent. This C.A. was injected one minute later of the first acquisition of images.

Apogee Alta U47 CCD camera and the Micro-Manager software were used to acquired the optical images. Two filters were used: \( \lambda_{ex} = 680 \text{ nm} \pm 5 \text{ nm} \) (band pass excitation
filter), \( \lambda_{em} = 700 \text{ nm} \pm \) (High pass emission filter). 210 images (1024x1024x2,16bits) were collected each 12.8 seconds in each experiment (Figure 4). The images were acquired in three sequences of 70 images each one (approximately 15min per sequence). The exposure time was 100ms and a multi-dimensional acquisition mode was used.

5.4 Optical Imaging: Data analysis

5.4.1 Plasma concentration Measurements

In the Model 1 and Model 2, the OPTx concentration in plasma was determined in four steps (Figure 5). First, some small ROIs were drawn in the blood vessels regions. Second, the mean intensity was measured in each ROI. The signal enhancement-time curve was obtained using the signal intensity before and after the OPTx injection (Equation 20). Third, enhancement-time curves were fitted to estimate the agent concentrations along the time (equation 15 and equation 21, respectively). As result of the fitting, the elimination constant \((K_e)\), the vascular circulation half-time \((t_{1/2})\), the plasma clearance \((CL)\) and the volume of distribution \((V_d)\) were obtained (section 4.3).

5.4.2 Tumor concentration Measurements

In the Model 3, the pharmacokinetic parameters were determined in four steps (Figure 6). First, ROIs were drawn over each image. ROIs were: blood vessels, left and right tumors. Second, the mean intensity was measured in each ROI. The signal enhancement-time curves were obtained using the signal intensity before and after the C.A. injection \((E(C) = \frac{S(C) - S(C=0)}{S(C=0)} - 1)\). Third, the enhancement curves from blood vessels were used as plasma concentration, and the enhancement curves from tumor tissue were used as tumor concentrations (equation 30). These values were entered in the standard matrix equation. The matrix was inverted to get \(BFR(t)\) values. Finally, the blood volume \(BV\), blood flow \(BF\) and mean transit time \(MTT\) were obtained from the \(BFR(t)\) values (section 4.3.3).

6 Results

6.1 Magnetic Resonance Imaging

6.1.1 Parameters from plasma concentration

The components from plasma concentration were determined after the analysis of the fitted curves. The enhancement-time curves for each ROI were analyzed picking the most representative curves (Figure 7). The enhancement-curves come from reference experiments (non-treated mice) with bolus and slow infusion injection. Mean values of the plasma components were determined after the analysis (Tabla 4).

6.1.2 Perfusion parameters from tumor tissue concentration

The values from Table 4 were used to estimate \(k\) and \(v\) values from reference curves and from VDA curves. At least two ROI were taken per slice (left tumor and right tumor). The
enhancement curves from tumor tissue regions were analyzed picking the most representative curves. The curves were from bolus and slow infusion experiments.

In bolus experiments, enhancement curves were obtained from: non-treated mice (reference), treated with CA4P, treated with FAA, treated with the molecule UTCBS-X. Figure 8 shows the behavior of enhancement curves from different experiments within each group. The red curve is the average representative curve from those set of curves. If we compare reference curves with VDA curves, we will see the changes in the curves behavior. For example, figure 9 illustrates the decrease of the enhancement curve after the mice were treated with CA4P in a bolus injection (figure 9.a) and slow infusion (figure 9.b).

Figure 10 summarized the mean $k$ results from the whole tumor, before and after treatments. Measurements of transcapillary transfer constant ($k$) in tumors decrease after treatment with the molecule UTCBS-X (10.1%), FAA(83.4%), and CA4P (85.6%). However, the reduction in the $k$ value does not have significance when the mice where treated with the molecule UTCBS-X. Moreover, mean values of the extracellular volume fraction $v_e$ show a significant rise after treatment with FAA(69.6%) and CA4P (60.9%).

Figure 11 summarized the mean $k$ results from the whole tumor, before and after treatments. Measurements of transcapillary transfer constant ($k$) in tumors decrease significantly after treatment with the molecule UTCBS-X (40.5%), and CA4P (81.6%). Moreover, mean values of the extracellular volume fraction $v_e$ show a significant rise after treatment with the molecule UTCBS-X (44.6%) and CA4P (76.1%).

### 6.2 Optical Imaging

#### 6.2.1 Perfusion parameters from plasma concentration

The pharmacokinetic parameters were obtained by the plasma concentration model (Model 1 and Model 2). Enhancement-time curves were calculated and analyzed picking the most representative curves (Figure 12). From the selected curves, the mean values of the pharmacokinetic parameters were determined per each model.

The figure 13 illustrates the pharmacokinetic parameters obtained from the enhancement curves. The three parameters show a non significant difference between the results of both models (Mann-Whitney test). Also using the Gamma Variate Function, it was possible determine an interval at which the peak of the function could be. The maximum time is $t_{max} = 3.9 \pm 1.7$ hours (n=6).

The values of the signal-concentration equation, $a$ and $b$ (Table 5) do not show a great difference. In fact, it will be feasible consider as constants $b$ for the next experiments.

#### 6.2.2 Perfusion parameters from tumor tissue concentration

The blood flow, the blood volume and the mean transit time were calculated using Dilution Theory and deconvolution methods (Figure 15). The blood volume $BV$ and blood flow $BF$ decrease in 62.4% and 62.8%, respectively, after CA4P treatment. The mean transit time $MTT$ has a slight increase of 8%.
Additionally, the areas under the signal enhancement curves were measured. The quantification shows the reduction of 62.7% of the area after the administration of CA4P (Figure 16). Areas were calculated at 35min after OPTx bolus injection.

7 Discussion

In this study, perfusion experiments have been done on mice bearing CT26 tumors to assess CA4P, FAA and UTCBS-X as VDA treatments. DCE-MRI and DCE-OI have been utilized to monitor the effects of vascular disrupting agents on the tumor vasculature. The effects in tumor has been demonstrated by the quantification of pharmacokinetic parameters as: transcapillary transfer, extracellular volume fraction, vascular circulation half-time, clearance, volume of distribution, blood volume, blood flow and mean transit time.

The use of Magnetic Resonance Imaging and also Optical Imaging techniques has let to compare the treatment effectiveness. Thus, when we compare the $k$ values with $BF$ and $AUC$ values after CA4P treatment, all results show a significant decrease by more than 60%. Nonetheless, the biological interpretation of these values diverges. The decrease in $k$ values means a reduction in the capillary permeability (flux of C.A. trough the vessels), on the other hand, the $BF$ reduction is due to the contraction of vessel wall (flux of C.A. along the vessels).

In Magnetic Resonance results, two injection methods were used to equate the $k$ and $v_e$ values. Bolus and slow infusion injection are different experimental methods with distinct way to quantify (distinct equations), so the evaluation of data from both methods is a mean to confirm the results. It is possible to check similarities in: values and tendency. The decrease of $k$ value is clear and statistically significant, except for the mice treated with the molecule UTCBS-X where the lack of significance could be because of the few data. The rise in the $v_e$ value can be explained by an increase in the extracellular volume, and a decrease in the tumor tissue which is an effect of the anti-vascular treatment. The rapid destruction of the vessels induced a leakage of the blood and C.A. in the extracellular extravascular space, and more death of cells produce more free extracellular space ($v_e$ increases). The destroyed vessels 24 hours after VDA treatments limit the transcapillary transfer ($k$ decreased) [36]. Finally, all $k$ and $v_e$ values are in the ranges given in the literature for tumor tissues [13].

In Optical Imaging results, the evaluation of the plasma concentration were done using two models: Gamma Variate Function and One Compartment Model. All the results obtained have a non-significant dependence of the model. It can be interpreted as a models similarity. However, Gamma Variate Function offers some advantages over One Compartment. For example, the possibility to calculate the maximum time (peak of the curve) and the better curve fitting (smalls RMSD values).

The estimated half-life times are in agreement with the pharmacokinetic studies where we saw a complete elimination of the OPTx 72 hours after venous injection. Besides, comparing with studies where C.A. has similar characteristics, the total elimination of that agents is produced approximately seven times the $t_{1/2}$ [23, 24]. The clearances and the volume of distribution are according the literature values.
Blood flow and blood volume values are lower than the published results [29, 30]. However, it will be considered that the size of the OPTx is higher than the sizes of C.A. of these articles. Also, as well as Schor-Bardach et al. [30], the $BF$ decreases in more than 50% after treatment, and the mean transit time shows a severely decreased perfusion. It means that the pass of the contrast agent through the tumor region is slower.

8 General conclusion

Contrast enhancement methods have been used to estimate pharmacokinetic parameters to quantify the effects of anti-vascular treatments. Magnetic Resonance Imaging and Optical Imaging techniques were utilized to perform dynamic sequence images during the injection of the Dotarem and OPTx, respectively.

The pharmacokinetic parameters depend on the technique and the mathematical model used. In MRI perfusion images, we demonstrated that the transcapillary transfer constant $k$ decreases, and the extracellular volume fraction $v_e$ increases in the tumor region after the administration of anti-vascular treatments. The variation of these parameters has a direct relation with the effects of the anti-vascular in the tumor tissue.

In Optical perfusion images, the vascular circulation half-time $t_{1/2}$, the plasma clearance $CL$ and the volume of distribution $V_d$ were calculated. These are parameters related to the concentration of the OPTx in the plasma, and they quantify the elimination of the contrast agent from the plasma. Moreover, the blood flow $BF$, the blood volume $BV$ and the mean transit time $MTT$ were estimated in tumor regions before and after the administration of CA4P treatment. The blood flow and the blood volume presented a significant decrease.

Two Graphical User interfaces were created and success tested to the processing of MRI and OI perfusion data, respectively. The interfaces are strong tools that let the easy handle of images, the display of enhancement-time curves and the automatic quantification of perfusion parameters.

In perspective, similar sizes of MR and optical contrast agents will be used to further corroborate the perfusion data and to check the feasibility of perfusion imaging validated by MRI perfusion.
References


Appendix A  Graphical User Interface for MRI images

The interface was developed in Matlab 8.1.0 (R2013a). The graphical interface lets to run MRI data (2dseq and RECO files) to reconstruct images from 7T imaging spectrometer (Bruker, Avance II, Wissembourg), draw regions of interest over the images, display signal enhancement-time curves, fit the enhancement curves by Tofts-Kermode model, determine perfusion parameters, and export results to excel sheets.

The main window of the MRI interface shows four general sections: the general information section, the display DCE-MRI images, the display anatomic images, the quantification of perfusion parameters (Figure A17). At the top of the window, the first section shows the information of the project, the address of the images file, the slices number. In the slice section, it is possible to charge a new slice or back to a previous one. In the second part, the images from MRI perfusion sequence are displayed. On the right, the set of buttons are used for the selection, drawing, saving and showing ROI regions over the current image. These buttons are linked with some sub-windows. In the third section, anatomic images are displayed. These images come from a set of axial cuts that were registered to acquire images of whole tumors. At the bottom section, the two buttons will display sub-windows suitable for quantification of the parameters related to plasma and tissue concentrations (Figure A18).

There are two windows for quantification of perfusion parameters (Figure A18). The first window is for plasma concentration parameters. This window is divided in four sections: type of injection, initial parameters, the display of the enhancement curve and results. The C.A. injection could be: bolus or slow infusion. When a option is chosen the equation used is automatically displayed (section 4.2). In the second section, the first panel shows the ROI selected; the middle panel lets the user-entering initial parameters for the coefficients of the plasma equation; and the final panel lets the entering of biological and experimental parameters. In the third section, the signal enhancement-time curve is displayed after press the button “RUN” located at the middle right of the window. In the four section, the results of the curve fitting: a1, a2, m1, m2 are displayed (section 5.2.1). The $R^2$ value was calculated using the values of the enhancement curve and the fitted curve by the equation:

$$R^2 = 1 - \frac{\sum (\text{Enhancement} - \text{FittedEnhancement})^2}{\sum (\text{Enhancement} - \text{averagedEnhancement})^2}$$ [17, 18].

For fitting, the interface uses the Nelder-Mead simplex algorithm [35]. It is an unconstrained nonlinear optimization to find the minimum of a scalar function of several variables. The implementation of the algorithm utilizes as maximum number of iterations 200. Besides, the constraints defined are: $a1 > 1$, $a2 > 4$, $m1 > 0.15$, $m2 > 0.007$ \textsuperscript{2}.

The second window has a similar structure than the first window (Figure A18.b). It was developed for tissue concentration parameters. This window also is divided in four sections: type of injection, initial parameters, the display of the enhancement curve and results. As well that in the plasma concentration window, the type of injection (bolus or slow infusion) should be selected. The tissue concentration equation is automatically displayed. In the second section, the first panel shows the ROI selected; the second panel lets the user-entering previous calculated plasma parameters; the third panel lets the entering of

\textsuperscript{2}The constraints and the minimizing algorithm have been already used in similar projects. They were defined by Gregory Ramniceanu, UTCBS researcher.
initial $k$ and $v_e$ values; and the final panel lets the entering of biological and experimental parameters. In the third section, the signal enhancement-time curve is displayed after press the button “RUN” located at the middle right of the window. In the four section, the results of the curve fitting: $k$, $v_e$ are displayed.

**Appendix B  Graphical User Interface for OI images**

The interface was developed in Matlab 8.1.0 (R2013a). The graphical interface lets to open images with extension ’.tiff’ from Apogee Alta U47 CCD camera, draw regions of interest over the images, display signal enhancement-time curves, fit the enhancement curves by Model 1, Model 2 and Model 3.

The main window of the OI interface shows three sections: the general information section, the display DCE-OI images, the quantification of perfusion parameters (Figure B19). At the top of the window, the button ’Perfusion Quantification’ will display sub-windows suitable for quantification of the parameters related to plasma ant tissue concentrations. In the middle part, the images from OI perfusion sequence are displayed. On the right, the set of buttons are used for the selection, drawing, saving and showing ROI regions over the current image. These buttons are linked with some sub-windows.

The window for quantification of plasma concentration parameters is divided in four sections: type of model, initial parameters, the display of the enhancement curve and results. The models are: Gamma Variate Function and One Compartment model. Gamma variate function and the One compartment model are fitted to the arterial concentration-time curves by the algorithm of Levenberg-marquardt. When a option is chosen the equation used is automatically displayed (section 4.3). In the second section, the first panel shows the ROI selected; the middle panel lets the user-entering initial parameters for the coefficients of the plasma equation; and the final panel lets the entering of biological and experimental parameters. In the third section, the signal enhancement-time curve is displayed after press the button “RUN”. In the four section, the results of the curve fitting: $t_{1/2}$, $CL$, $Vd$ are displayed. The $R^2$ value was calculated using the values of the enhancement curve and the fitted [17, 18].
Figure 4: Sequence of OI images after OPTx injection at t=0min. The green line shows the contour of a right tumor and the red line the contour of a left tumor. Images show an accumulation of the OPTx in the tumor tissues.
Figure 5: Sequence to estimate the agent concentration in plasma. First, a ROI is drawn. Second, the signal enhancement-time curve is obtained. Third, enhancement curve is fitted by Model 1 (Gamma variate function) and by Model 2 (One Compartment model). Fourth, plasma concentration parameters are gotten from curve fitting.

Figure 6: Sequence to estimate \( BV \), \( BF \) and \( MTT \) in tumor tissue. First, a ROIs are drawn. Second, the signal enhancement-time curve is obtained for each ROI. Third, enhancement values are used as concentrations in Model 3 (Dilution Theory). Fourth, blood volume \( (BV) \), blood flow \( (BF) \) and mean transit time \( (MTT) \) are gotten from the deconvolution method.

Figure 7: Signal Enhancement-time curves from arterial vessels regions. The plasma concentration curve was calculated using these curves. In red, representative fitted curve of the set.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean value</th>
<th>S.D.</th>
</tr>
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<tbody>
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<td>( a_2 )</td>
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<td>( m_1 )</td>
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<tr>
<td>( m_2 )</td>
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</table>

Table 4: Parameters of plasma concentration.
Figure 8: Signal Enhancement-time curves from bolus experiments. In red, representative fitted curve of the set.
Figure 9: Non-treated and treated Signal Enhancement-time curves. In red, representative reference curve. It was calculated in non-treated mice. In yellow, representative CA4P curve. It was calculated in treated mice with CA4P.
Figure 10: $k$ and $v_e$ in CT26 tumors following UTCBS-X, FAA and CA4P treatment. Bolus injection method. All values showed $R^2$ values higher than 0.7. ns: $p > 0.05$, **: $p < 0.01$, ***: $p < 0.001$ (two-tailed Mann-Whitney Test).
Figure 11: $k$ and $v_e$ in CT26 tumors following UTCBS-X, FAA and CA4P treatment. Slow infusion method. All values showed $R^2$ values higher than 0.7. *: $p < 0.05$, ****: $p < 0.0001$ (two-tailed Mann-Whitney Test).

Figure 12: Signal Enhancement-time curves from vessels regions. The ROIs were selected in arterial vessel regions. A representative fitted curve of the set is showed in red: (Left) using Gamma Variate Function. RMSD lower than 0.001. (Right) using One Compartment Model. RMSD lower than 0.9.

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RMSD: Root-mean-square deviation
Figure 13: Pharmacokinetic values from plasma concentration. Left: calculated values from fitted curve using Gamma Variate Function (Model 1). Right: calculated values using One Compartment Model (Model 2). All values showed $R^2$ values higher than 0.8. $ns$: $p > 0.05$ (two-tailed Mann-Whitney Test)

Figure 14: Pharmacokinetic values from plasma concentration. $ns$: $p > 0.05$ (two-tailed Mann-Whitney Test)

Table 5: Resulted Coefficients of the plasma concentration using Model 1 and Model 2. $ns$: $p > 0.05$, $**$: $p < 0.01$ (Mann-Whitney Test)
Figure 15: Pharmacokinetic values from tumor tissue concentration. Values were calculated using Model 3. *: $p < 0.05$, **: $p < 0.001$ (Mann-Whitney Test).
Figure 16: Quantification of the area under the reference enhancement-time curve and CA4P enhancement-time curve. Values were calculated using Model 3. ***: $p < 0.001$ (Mann-Whitney Test).
Figure A17: Main window of MRI-Perfusion interface. A: General information. B: DCE-MRI images. C: anatomic images. D: Quantification of perfusion parameters.
Figure A18: Windows for quantification of perfusion parameters in MRI interface. A: Type of injection: Bolus or Slow Infusion. B: Initial Parameters. C: Enhancement-time curve. D: Results.
Figure B19: Windows for quantification of perfusion parameters in OI Graphical interface. A: Type of model: Gamma variate function or One Compartment Model. B: Initial Parameters. C: Enhancement-time curve. D: Results.