Quantification of the Microvascular Blood Flow of the Ovine Corpus Luteum with Contrast Ultrasound

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Abstract—The ovine corpus luteum is an ideal model for the development of methodologies for angiogenesis monitoring with contrast enhanced ultrasound. We employ three indicator dilution models, namely, the lognormal function, the gamma variate function, and the local density random walk model to fit contrast ultrasound time-intensity curves from ovine corpora lutea at the peak of the estrus cycle. We extract hemodynamic-related parameters such as the mean transit time, the wash-in time, the area under the curve and the peak intensity and measure inter- and intra-animal reproducibility. The mean transit time and the wash-in time are reproducible with acceptable relative dispersions whereas the peak intensity and the area under the curve have large variabilities.

Keywords—angiogenesis anti-angiogenesis; indicator dilution method; mathematical models

I. INTRODUCTION

The corpus luteum (CL) is a microvascular tissue in the ovary that undergoes a natural angiogenic and anti-angiogenic process during the estrus cycle of the ewe [1]. It can also be controlled and monitored endocrinologically, providing a very attractive in-vivo model for the study and the development of microvascular measurement.

Ultrasound imaging is one of the most commonly used diagnostic tools for the pathology of the female reproductive system. It has been shown, however, that changes at capillary level are more important for the physiology of the ovary than those of the larger vessels. Conventional B-mode ultrasound and Doppler techniques are not capable of imaging the microcirculation. On the other hand, contrast enhanced ultrasound (CEUS) at low mechanical index (MI) has been successfully used to detect blood flow at the microcirculation level in cardiology [2] and oncology [3].

CEUS images may be quantified in order to measure microcirculation related parameters. The variation of image intensity as a function of time in a region of interest (called "time-intensity curve") is often formed depicting the passage of contrast microbubbles (wash-in and wash-out) in the microcirculation. Time-intensity curves are usually noisy and altered by the recirculation of the microbubbles. Therefore, one can employ theoretical models based on indicator dilution theory to fit the time-intensity curves in order to suppress the noise and isolate the primary pass of the microbubbles. In addition, the use of indicator dilution models allows under certain conditions for various hemodynamic-related parameters to be calculated in closed form. In this paper we chose three of the most commonly used indicator dilution models, namely, the lognormal function [4], the gamma variate function [5], and the local density random walk (LDRW) model [6] to fit time-intensity curves from images of microvascular flow in the CL after an intravenous bolus injection of contrast microbubbles. The objectives of this work are: 1) select an appropriate model for our specific clinical application, 2) extract hemodynamic-related parameters such as the area under the curve (AUC), peak intensity (I_p), mean transit time (MTT), wash-in time (WIT), and 3) measure intra- and inter-animal reproducibility and variability of the hemodynamic-related parameters.

This paper is organized as follows. In Section II we introduce the three models employed to fit the corpus luteum time-intensity curves. In the same section we also present the animal preparation protocol, the ultrasound imaging protocol, and the data analysis techniques. In Section III we present our data analysis results and in Section IV we discuss the significance of our results and present our conclusions.

II. MATERIALS AND METHODS

A. Indicator Dilution Models

The contrast microbubbles after an intravenous bolus injection traverse a region of interest (ROI) at different times, because they are dispersed through branching vessels, or due to Brownian motion, laminar flow or turbulence. Therefore, a time-intensity curve is interpreted as the probability density function of transit time in a ROI after a bolus of microbubbles is injected intravenously; that is, it specifies the amount of indicator particles traversed through a ROI during every time interval after injection. The three models employed to curve-fit our data are the lognormal function, the gamma variate function and the LDRW model.

The lognormal function [4] is given by

\[ I(t) = \frac{AUC}{\sqrt{2\pi}\sigma(t-t_0)} e^{\frac{(\ln(t-t_0)-\mu)^2}{2\sigma^2}} + I_0, \] (1)
where $\mu$ and $\sigma$ are the mean and standard deviation of the normal distribution from which the logarithmic transformation was obtained. The curve can be scaled horizontally by varying $\mu$ and changed in terms of skewness by varying $\sigma$. The zero time of the distribution is denoted by $t_0$, and $I_0$ is the baseline intensity offset. The inclusion of $I_0$ in (1) applies specifically to ultrasound time-intensity and is not part of the original statistical model. The quantity $[I(t)-I_0]$ is a probability density function when AUC is set to unity. We use the same symbols for $I(t)$, $I_0$ and $t_0$ hereafter for the other models too. The lognormal model is based on bifurcations of vessels with significant number of generations that may also exhibit fractal behavior [7]. The MTT is defined as the first moment of $[I(t)-I_0]$ and the WIT is defined as the time to the peak intensity. In both time measurements we subtract the bolus arrival time $t_0$. These parameters MTT and WIT are given by:

$$MTT = e^{\mu + \sigma^2/2}, \quad WIT = e^{\mu - \sigma^2}.$$  \hspace{1cm} (2)

The second model considered is the gamma variate function [5] given by

$$I(t) = \frac{A}{\beta^{\alpha+1} \Gamma(\alpha+1)} (t-t_0)^\alpha e^{-\frac{(t-t_0)}{\beta}} + I_0.$$ \hspace{1cm} (3)

In the discrete form of the function (not presented here) $\alpha=n-1$, where $n$ is the number of the equal size homogeneous compartments in series, $1/\beta=Q/V$ where $V$ is the volume of each compartment and $Q$ is the constant flow rate. The term $\beta^{\alpha+1} \Gamma(\alpha+1)$ normalizes the gamma variate in the above equation so that it can be a probability distribution that integrates to unity when AUC=1. The MTT and WIT for this model are given by:

$$MTT = \beta(\alpha+1), \quad WIT = \alpha \beta.$$ \hspace{1cm} (4)

The third model considered is the LDRW model [6], which is given by the solution of the one dimensional diffusion with convection partial differential equation in the case of no special boundary conditions at the outlet. The LDRW function is given by

$$I(t) = A \left( \frac{e^{\lambda}}{\mu} \right) \left[ \frac{\mu \lambda}{2 \pi(t-t_0)} \exp \left( \frac{-1}{2 \lambda} \left( \frac{\mu}{t-t_0} + \frac{(t-t_0)}{\mu} \right) \right) \right] + I_0.$$ \hspace{1cm} (5)

The skewness of the curve increases with $1/\lambda$ and $\mu$ is the mean time needed for a microbubble to cover the distance between injection and sampling sites (when $t_0 = 0$). For a straight tube, through which a bolus of microbubbles is flowing, the Preclet ($Pe$) number defined as the ratio between the diffusive time and the convective time is given by $Pe=2\lambda$. $Pe$ gives an estimate of the relative contribution of convection and diffusion in the microbubbles transport. The MTT and WIT for this model are given by:

$$MTT = \mu, \quad WIT = \left( \frac{\mu}{2\lambda} \right) \sqrt{1 + 4\lambda^2} - 1.$$ \hspace{1cm} (6)

An example of a time-intensity curve-fitting is shown in
D. Data Analysis Techniques

For the image analysis and quantification the commercial software QLAB (Philips Healthcare, Andover, MA, USA) was used. The tasks included selection of an appropriate ROI within the CL, and extraction of time-intensity curves for that ROI. The measurement of the mean intensity within the selected ROI for all the frames of a loop as a function of time provides the time-intensity curve. The intention in the ROI selection was to include only the microvascular network of the CL and avoid larger vessels that we know they normally lay in the periphery of the tissue.

An iterative non-linear regression fitting routine based on the minimization of least square errors was employed on the time-intensity curves using a MATLAB function. We provided the algorithm initial guesses and lower and upper bounds for the fitting parameters. The convergence time of the algorithm varied from one to three seconds with small bounds for the fitting parameters. The convergence time of the algorithm varied from one to three seconds with small differences among the various models. In certain cases the wash-out part of time-intensity curves was affected by the recirculation of the contrast microbubbles. To isolate the primary pass of the indicator, these curves were fitted to the models using all the points from the beginning of the curve up to just before the region where the effects of recirculation deteriorated the fit quality. For a quantitative evaluation of the models’ performance on the various data sets we measured for each curve-fitting the coefficient of determination $R^2$.

III. RESULTS

A typical enhancement pattern after a SonoVue bolus injection into a mature CL can be seen in Fig. 2. All fully developed CLs exhibited significant enhancement after injection of the contrast agent. The contrast agent arrives from larger feeding vessels (seen to the top right of the drawn ROI in Fig. 2a). The ROI in the CL has subsequently a rapid increase in average intensity (Fig. 2b and c). Finally the contrast intensity within the ROI starts to decrease after several seconds during the wash-out phase of the bolus (Fig. 2d) and eventually ends in the original background noise levels (Fig. 2e). Fig. 2f shows the time-intensity curve which is the contrast intensity over a CL as a function of time after injection. In some animals a small area with no enhancement is observed in the center of the CL, as can be seen at the center of the ROIs in Fig. 2a-e. This is in agreement with histology analysis of the microvasculature and larger blood vessels in each of the CLs which were collected at the end of the scanning session in each ewe.

Forty eight image loops were collected from ten animals all at the peak (day 8 to 12) of the estrus cycle. The three indicator dilution models presented in Section II.A, namely the lognormal function, the gamma variate function and the LDRW model were employed to fit the time-intensity curves. In Fig. 3 we show a typical example of a CL time-intensity curve together with the fitting functions from the lognormal model, the gamma variate model and the LDRW model. The data were fitted for the whole time range, because there are no signs of recirculation on the wash-out tail. The values of the coefficient of determination for the three models are: $R^2=0.997$ for the lognormal, $R^2=0.995$ for the gamma variate, and $R^2=0.996$ for the LDRW. As seen in Fig. 3 the gamma variate function produced a fit which is a little steeper than the other models in a small region near the origin of the time-intensity curve causing an increase in the predicted value of $t_0$ and a decrease in the values of AUC, MTT, and WIT.

It should be noted that the not significantly different fit quality of the gamma variate model shows that the region near the origin has a negligible impact on the overall fit performance of the model, because the width of this region is very small. This trend of the gamma variate was also observed in the other time-intensity curves analyzed for this work. The average values of $R^2$ together from all the data with their standard deviations for each model are: 0.978±0.002 for the lognormal, 0.974±0.002 for the gamma variate and 0.976±0.002 for the LDRW. From curve-fits to the forty-eight image loops we also extracted the hemodynamic-related parameters MTT, WIT, AUC, and $I_p$. Both the LDRW model and the lognormal function produced very good fits. We have chosen the LDRW to use for this analysis. The results for both the inter- and intra-animal relative dispersions defined as the standard deviations divided by the mean values, for all the parameters are presented in Table I. The values of relative dispersions for MTT and WIT are small, but they are larger than 100% for $I_p$ and AUC.

Figure 2. Images from a contrast loop of a fully developed CL (day 9 of estrous cycle) over the transit of the injected bolus (a) – (e). The corresponding time-intensity curve is shown in (f).

Figure 3. CL time-intensity image data fitted with the lognormal function, the LDRW model, and the gamma variate function.
IV. DISCUSSION AND CONCLUSIONS

The ovine CL is proposed as an ideal tissue-model to allow investigation into angiogenesis changes with the aid of contrast ultrasound. Three indicator dilution models, namely the lognormal function, the gamma variate function, and the LDRW model were employed to fit time-intensity curves from the CLs of ten animals at the peak (8-12 days) of the estrus cycle. All three models produced good quality fits. By visual inspection, the gamma variate function had a lower performance near the origin of the curves. This can be explained by the fact that this model is based on unidirectional motion of the indicator particles [8], implying that it does not properly take into account the indicator dispersion, which would otherwise smooth sharp changes in the gradient. As a result, the gamma variate tends to underestimate the values of MTT, WIT, and AUC. Both the LDRW model and the lognormal function have the appropriate physical and physiological basis as they take into consideration the diffusive architecture of the CL microvascular bed and thus produced good fits to the experimental time-intensity curves. No real distinction can be made between these two models based on our in-vivo data.

Our intra- and inter-animal analysis showed that the MTT and WIT are reproducible with acceptable relative dispersions, and proposed to be used as imaging biomarkers to monitor the CL angiogenesis changes. These parameters do not depend on ultrasound scanner settings, because they are time parameters. The PI and AUC are more dependent on scanner parameters and user settings (depth, attenuation, frequency) and thus are less reproducible.

REFERENCES


| TABLE I. INTER- AND INTRA-ANIMAL RELATIVE DISPERSIONS FOR MTT, WIT, I_p, AND AUC PRODUCED WITH THE LDRW MODEL |
|-----------------|-----|-----|-----|-----|
|                 | MTT | WIT | I_p | AUC |
| Inter-animal     | 11% | 12% | 110%| 134%|
| Intra-animal     | 23% | 25% | 137%| 137%|