APPLICATION OF A DYNAMIC MODEL OF APOE$^{-/-}$ MOUSE ATHEROSCLEROSIS TO ASSESS THE IMPACT OF CIGRETTE SMOKE EXPOSURE ON ENDOTHELIAL CELL FUNCTION

A Berges[1], Y Cheng[2], C Haziza[2], J Hoeng[2], S Lebrun[2], R Lichtner[3], M Lietz[3], W Schlage[3], and M Peitsch[2]

[1]Philip Morris International R&D, Philip Morris Research Laboratories bvba, Leuven, Belgium
[2]Philip Morris International R&D, Philip Morris Products S.A., Neuchâtel, Switzerland
[3]Philip Morris International R&D, Philip Morris Research Laboratories GmbH, Cologne, Germany

Introduction

Atherosclerotic plaque progression is a complex process to which several physiological pathways contribute. Smoking has been shown to accelerate plaque growth [1] by increasing inflammation [2], thrombosis [3], and endothelial cell (EC) dysfunction [4], but the relative contribution of each pathway is not well–understood. Cellular adhesion molecules (CAMs) are preferentially produced by activated ECs and have therefore been proposed as a means to quantify EC dysfunction. Measurements of CAMs in smoke-exposed ApoE$^{-/-}$ mice, however, have produced ambiguous results, with both positive and negative changes relative to control. Modeling the dynamics of EC membrane-bound Inter-Cellular Adhesion Molecule 1 (mICAM-1) will advance our understanding of the impact of cigarette smoke exposure on EC function in the ApoE$^{-/-}$ mouse and allow us to improve the design of future non-clinical experiments.

We licensed the Entelos ApoE$^{-/-}$ Mouse Cardiovascular PhysioLab® platform [5], a comprehensive *in silico* modeling technology enabling dynamic simulation of the biological pathways driving plaque progression. Biological effects of smoking were incorporated into the model and calibrated to histological data on total plaque area and plaque percent macrophage area. The concentration of mICAM-1 was simulated with and without smoking effects to predict the time-dependent ratio of mICAM-1 in smoke-exposed mice and controls. An uncertainty analysis was conducted to generate alternative falsifiable predictions that could be tested with *in vivo* experiments.

Keywords

Endothelial cells (EC): the thin layer of cells that line the interior surface of blood vessels forming an interface between circulating blood in the lumen and the rest of the vessel wall. During atherosclerotic disease progression, ECs become activated and produce more adhesion molecules.

Adhesion molecules: proteins located on the cell surface involved with the binding with other cells or with the extracellular matrix in the cell adhesion process. EC membrane-bound Inter-Cellular Adhesion Molecule 1 (mICAM-1) is one of the well-characterized adhesion molecules involved in the development of atherosclerosis.

Atherosclerosis: a progressive vascular disease characterized by accumulation of cholesterol in the intima of large arteries. The primary drivers affecting rates of atherosclerotic plaque progression include enhanced cholesterol influx to the vessel wall and accumulation and retention of lipid in the intima due to local and systemic inflammation.

ApoE$^{-/-}$ mouse: the mouse devoid of ApoE protein and a well-established animal model to study atherogenesis.

Entelos PhysioLab®: a large-scale mathematical computer model of cardiovascular disease that can simulate the physiology under various experimental protocols to predict the response to smoking and support the design of clinical and non-clinical studies.

Virtual mouse (VM): a set of biological parameters representing the uncertainty in underlying mechanistic diversity of the mouse and the hypothesis for various physiological states resulting in a range of disease phenotypes.

Mechanistic Smoking Effects (MSE): model representation of the key biological processes relevant to atherogenic plaque progression in ApoE$^{-/-}$ mice that are subject to perturbation in response to tobacco exposure.

Methods

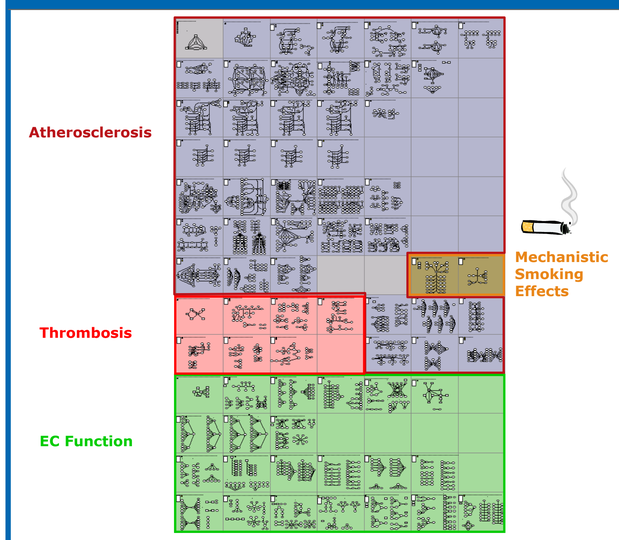

Figure 1. *Screen shot of the PhysioLab platform.* The Rodent Cardiovascular PhysioLab was developed as a means of providing insights gained from mechanistic modeling of translational animal research data. It is intended to allow exploration of hypotheses involving physiological mechanisms of atherosclerosis and smoking effects for which there are limited or no available *in vivo* data from human studies. The platform consists of four submodules describing different aspects of the cardiovascular disease progression: 1) Atherosclerosis, 2) Thrombosis, 3) EC Function, 4) Mechanistic Smoking Effects. The PhysioLab provides a model framework which can be recalibrated to incorporate new experimental data.

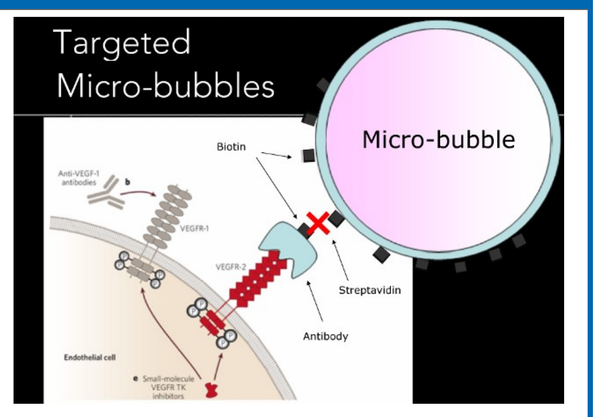

Figure 2. *Contrast-enhanced ultrasound and microbubble visualization*. High-frequency ultrasound Vero®770 provided by VisualSonics [6] offers instantaneous, non-invasive, and real-time *in vivo* imaging of vascular morphology and performance in small laboratory animals such as mice and rats [7]. Contrast-enhanced ultrasound assesses vascular structures and dynamics at the molecular level. These methods rely on ultrasound detection of micro-bubble contrast agents that are targeted to endothelial cell adhesion molecules, by conjugation of specific ligands (such as anti-ICAM-1 antibodies) to the surface. A destructive pulse disrupts all the micro-bubbles in the imaging plane. Before this pulse, the frames contain bound and circulating micro-bubbles, while the frames acquired after the destructive pulse contain only the rapidly re-appearing circulating micro-bubbles. Therefore, difference before and after the destruction pulse is indicative of the amount of membrane-bound micro-bubbles and thus of the concentration of mICAM-1.

Results

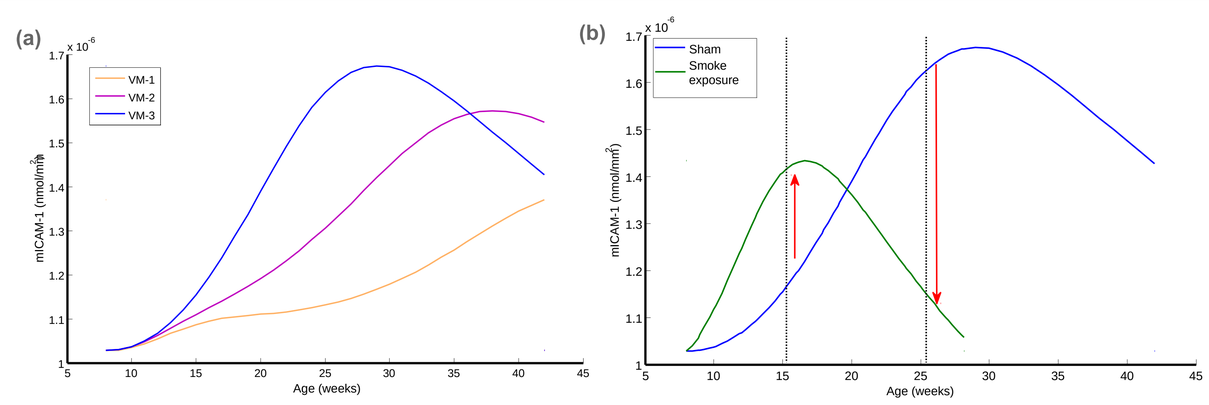

Figure 3. *PhysioLab simulation results of the mICAM-1*. Zibara et. Al. [8] reported that ApeE$^{-/-}$ mice fed with a chow or a cholesterol-rich diet showed first an increase (at 6 weeks) and then a reduction (at 16 weeks) in the mean concentration of mICAM-1. Such modulation of the mean expression of adhesion molecules was not observed in wild-type mice. In that paper, the results were also confirmed by Northern blots performed on the aortic arch of ApoE$^{-/-}$ chow-fed mice over a period of 20 weeks. The PhysioLab platform was calibrated based on this finding and was able to simulate the "bell-shape" of mICAM-1. Three virtual mice (VM-1, VM-2, VM-3) were created to represent different potential rates of atherosclerosis progression. Figure 3(a) shows the dynamics of mICAM-1 in the three chow-fed virtual mice and Figure 3(b) demonstrates the altered progression of mICAM-1 due to cigarette smoke exposure. Figure 3(b) also suggests that the effect of smoke exposure may appear to be either positive (early) or negative (late) depending upon the time of the comparison. By modeling the physiological effects of smoke exposure, we hypothesis that the mICAM-1 maximum occurs earlier resulting in an increased rate of atherosclerosis progression.

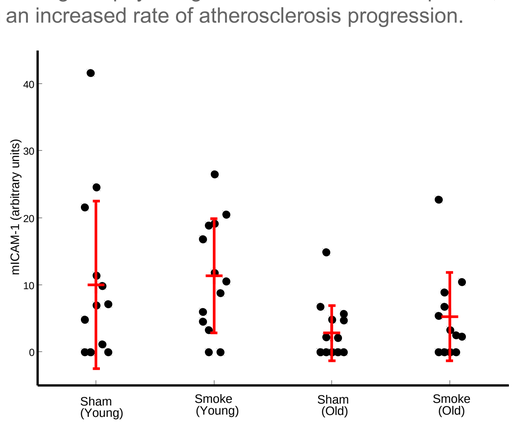

Figure 4. *In vivo contrast-enhanced ultrasound experiment data.* Two groups of ApoE$^{-/-}$ mice (young group with age 7~9 weeks and old group with age 15~16 weeks) were whole-body exposed for 90 days to 4 x 1 h of diluted mainstream smoke (MS) at a total particulate matter (TPM) concentration of 600 µg/l or to filtered fresh air (sham). Afterwards, visualization and quantification of mICAM-1 were performed using contrast-enhanced ultrasound and micro-bubbles. Three observations could be made from the study: 1) cigarette smoke exposure is likely to increase the mICAM-1 concentration compared with Sham exposure at 90 days; 2) mICAM-1 concentration appears to be reduced at age 28 weeks compared with 20 weeks regardless of smoke exposure; 3) the large variation in the data may indicate the underlying complexity. These two observations can be further investigated with improved experimental design based on the simulations.

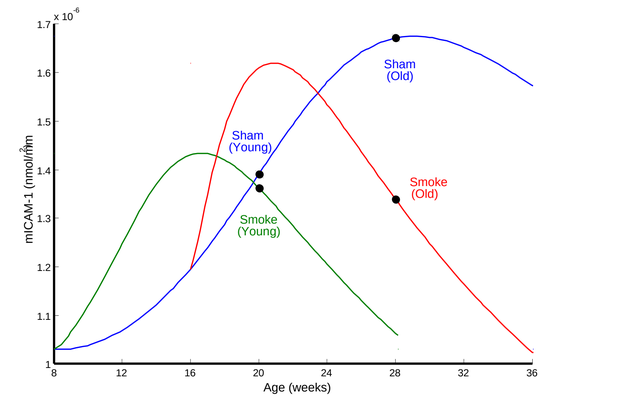

Figure 5. *Naive simulations of the experimental protocol shown in Figure 4.* PhysioLab *in silico* experiment for VM-3 have been setup to reproduce the *in vivo* experimental protocol without model recalibration. The four points in the figure correspond to the four measurements shown in Figure 4. The complex dynamics can be seen in the simulation and the measurements at different time points could lead to different conclusions about the mICAM-1 concentration for Sham or Smoke exposure and age at initial exposure. The simulation suggests: 1) more data are required to recalibrate the model for better prediction of the peak time of mICAM-1; 2) the future design of the experiment should include a reference time point prior to smoke exposure and additional points after a short period of smoke exposure; 3) given limited resources, it would be more informative to measure multiple time points for a single exposure initiation age than a single time point across multiple exposure initiation ages.

Conclusions

An analysis of the simulation of smoke-exposed mice highlighted the understanding of the dynamics of mICAM-1 during disease progression. The large variability in the observed mICAM-1 concentration in this and other studies may be partly explained by real plaque variation and/or measurement difficulties; however, an understanding of the dynamics of mICAM-1 may be used to improve experimental protocol and reduce uncertainty. I*n vivo* measurements at multiple time points may be used to refine the calibration of the PhysioLab model and the hypothesis of the mechanistic smoking effects. Specifically, multiple time points for a single exposure initiation age provide more information than a single time point across multiple exposure initiation ages. Understanding the dynamics of mICAM-1 could provide insight into the impact of smoke exposure on endothelial cell function and its role on atherosclerotic disease progression.

References

1. Howard G et al, JAMA 1998; 279(2):119-124
2. Helmersson J et al, Atherosclerosis 2006; 181(1):201-7
3. Nowak J et al, Circulation 1987; 76(1):6-14
4. Celermajer DS et al, Lancet 1992; 340(8828):1111-15
5. Shoda L et al, Clin Exp Immunol 2010, 161(2):250-67
6. www.visualsonics.com
7. Lindner JR, Nat Rev Drug Discov. 2004; 3:527-32
8. Zibara K. et al., Arterioscler Thromb Vasc Biol 2000, 20:2288-96

PMI RESEARCH & DEVELOPMENT

Philip Morris International Research & Development, Quai Jeanrenaud 5,
2000 Neuchâtel, Switzerland
T: +41 58 242 21 11, **F**: +41 58 242 28 11, **W**:
www.philipmorrisinternational.com