Initial Data on the Effects of Long-Term Cigarette Mainstream Smoke Exposure and High Fat Diet in Apolipoprotein E-Deficient Mice

S. Lebrun, W. Stinn, H. Weiler, P. Kuhl, B. Friedrichs, H.-J. Urban, K. von Holt, T. Wallerath, R. Schleef; PHILIP MORRIS Research Laboratories GmbH, Fuggerstraße 3, 51149 Cologne, Germany

Raymond.Schleef@pmintl.com

Introduction

Progression of coronary plaques to stenotic coronary artery disease represents the pathologic basis for the most common of all heart diseases. Epidemiologic studies have demonstrated dose-response associations between smoking and the presence and progression of subclinical atherosclerosis (1).

The apolipoprotein E-deficient (Apo E-/-) mouse has been established as a model of atherosclerotic lesion development. The brachiocephalic artery and the aortic arch are sites of predilection for the development of atherosclerotic plaques (2). Short-term experimental studies with Apo E-/- mice have revealed that (i) cigarette sidestream smoke increases atherosclerosis (3, 4, 5) and (ii) cigarette mainstream smoke (MS) increases intimal thickening after arterial cuffing (6).

Objective

This study was performed to investigate the influence of MS and high fat diet on the development of atherosclerotic plaques. The amount of plaques in the aortic tree and the existence of calcium deposits in plaques in the brachiocephalic artery were determined in Apo E-/- mice.

Methods

Male Apo E-/- mice were whole-body-exposed for 12 months (6 hours/day, 5 days/week) to diluted cigarette mainstream smoke at total particulate matter (TPM) concentrations of 100 µg/l (low MS) and 200 µg/l (high MS) or to filtered fresh air (sham).The mice were fed one of three different diets (ad libitum) differing in cholesterol/fat content:
(i) normal chow (0.02%/4.5%),
(ii) milk-fat-enriched (0.17%/21%), and
(iii) beef-fat-enriched (0.14%/21%).
Three additional sham groups were diet-restricted to match the body weight in the high MS groups.
Carboxyhemoglobin was measured as a biomarker of exposure in blood samples collected from the retro-bulbar venous plexus of halothane-anesthetized mice.
To determine the amount of plaques that covered the intimal area of the aorta, the aortic arch was removed (**Figure 1A**), cut longitudinally, and opened out with acupuncture needles. One digital image was taken and the size of the aortic arch and the plaques were measured with Diskus software. The intimal area covered by plaques was calculated in percent of total aortic intimal area (**Figure 2**).
To identify calcium deposition, the van Kossa stain was used; to identify plaque size, elastin staining was done. Five consecutive cross sections of paraffin-embedded brachiocephalic artery were prepared and stained (**Figure 1B**). One digital image of each section was taken and the images were analyzed with Diskus software as described elsewhere (7) (**Figure 3**).
Free cholesterol in the aortic arch was determined by HPLC.
For statistical analysis, one-way ANOVA and linear regression were performed. Data presented as means ± SE. Asterisks represent statistical significance (*: p ≤0.05; ***: p ≤0.001).

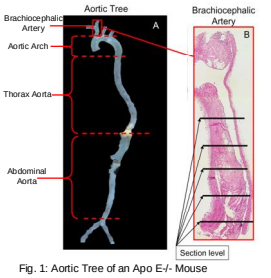

Fig. 1: Aortic Tree of an Apo E-/- Mouse
A whole tree with sections labeled
B hematoxylin + eosin-stained longitudinal section of the brachiocephalic artery

Planimetry
Aortic Arch

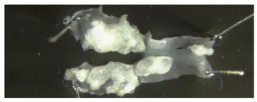

Fig. 2: Opened Aortic Arch with Atherosclerotic Plaques (6 months)

Normal diet Sham (6 months) | Normal diet low MS (6 months)

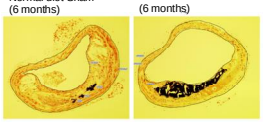

Fig. 3: Van Kossa-Stained Cross Sections of the Brachiocephalic Artery
Calcium deposition appears as black spots.

Acknowledgements
The authors are grateful to C. Bajraktari, A. Daniels, S. Gaschina, M. Grabow-Caspari, T. Grün, R. Janssens, O. Jörke, S. Jurgeit, K. Landwehr, and M. Lorbeer for technical assistance.

Results

Body Weight Development

- Body weight was decreased up to 10% in the smoke-exposed groups.

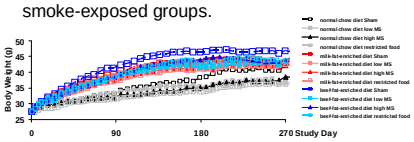

Fig. 4: Body Weight Development of Apo E-/- Mice

Carboxyhemoglobin

- Carboxyhemoglobin levels were 10% ± 0.3 in the low MS groups and 20% ± 0.6 in the high MS groups.

Plaque Size in the Aorta

- Both high fat diets increased aortic plaque content compared to the normal chow diet (p ≤0.001).
- Smoke exposure significantly increased the amount of plaques only in the normal chow diet group (high MS vs restricted) over the 9-month time course.

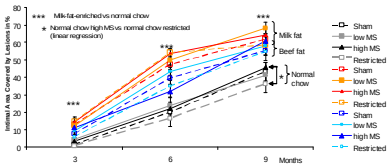

Fig. 5: Amount of Plaques in the Aortic Arch of Apo E-/- Mice

Calcium Deposition in the Brachiocephalic Artery

- The fractional area of the blood vessel covered by calcium deposition was significantly increased only in the low MS normal chow diet group compared to normal chow sham group at 6 months (p ≤0.05).

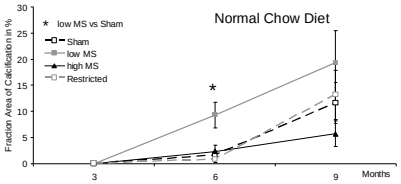

Fig. 6: Calcium Deposition in the Brachiocephalic Artery of Apo E-/- Mice

Cholesterol Content in the Aorta

- Both high fat diets increased aortic cholesterol content compared to the normal chow diet (p ≤0.001).
- Smoke exposure significantly increased the amount of cholesterol only in the milk-fat-enriched diet group (high MS vs sham) over the 9-month time course (p ≤0.05).

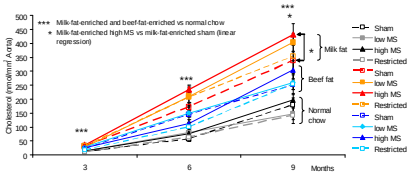

Fig. 7: Amount of Free Cholesterol in the Aortic Arch of Apo E-/- Mice, Individual Groups

- When data were combined with each exposure condition (sham, low MS, high MS, and restricted) irrespective of diet type, the cholesterol content was also significantly elevated in the high MS group compared to sham at 9 months (p ≤0.05).

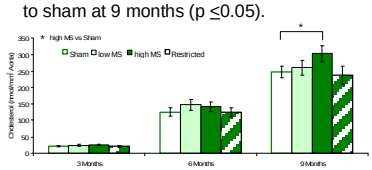

Fig. 8: Amount of Free Cholesterol in the Aortic Arch of Apo E-/- Mice, Combined Groups

Plaque Size in the Brachiocephalic Artery

- Smoke exposure significantly increased the fraction area of plaque size
  - in the normal chow diet group (high MS group vs sham) over the 9-month time course (p ≤0.05);
  - in the milk-fat-enriched diet group (high MS vs sham) over the 9-month time course (p ≤0.05) and at 6 months (p ≤0.05).

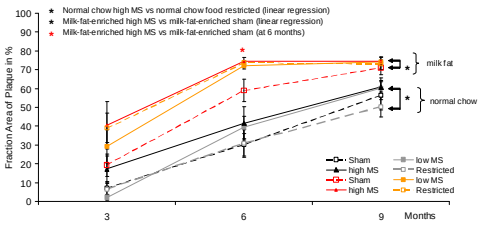

Fig. 9: Fraction Area of Plaque Size as a Percentage of the Brachiocephalic Artery

Conclusion

Our initial data suggest that exposure to cigarette mainstream smoke affects the composition of plaques in the apolipoprotein E-deficient mouse model in a complex manner.

References
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This work was funded by Philip Morris U.S.A.
The International Society on Thrombosis and Haemostasis XXth Congress, 6-12 August 2005, Sydney, Australia