Scientists Identify Protein Essential for Cholesterol Absorption

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Media contacts:

Emma Keeling

t: +44 (0)20 7413 3157 ekeeling@hillandknowlton.com

Abby Webster

t: +44 (0)20 7973 4462 abby.webster@hillandknowlton.com

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Schering-Plough Scientists Identify Protein Essential for Cholesterol Absorption from Intestine

Findings in Science Advance Understanding of Intestinal Cholesterol Pathway and Action of EZETROL® (Ezetimibe), a Cholesterol Absorption Inhibitor Complementary to Statin Therapy

KENILWORTH, New Jersey, U.S.A., February 19, 2004 -- In a major advance in understanding the intestinal pathway for cholesterol absorption and the mechanism of action of EZETROL® (ezetimibe), marketed by a partnership between Schering-Plough and Merck, scientists at Schering-Plough Research Institute have identified and characterized a long-sought protein critical to intestinal cholesterol absorption. In an article published in the February 20 issue of the journal Science, Schering-Plough scientists report on the identification of the protein, named NPC1L1, as playing an essential role in the ezetimibe-sensitive cholesterol absorption pathway.

Cholesterol levels in the blood are largely controlled through two sources in the body: the liver, which synthesizes (produces) cholesterol, and the intestine, where cholesterol is absorbed into the blood stream.

“By demonstrating the function of the NPC1L1 protein, scientists at SPRI have made a significant advance into deciphering the cholesterol-absorption pathway in the intestine, which has been elusive to scientists for some time,” said Cecil B. Pickett, Ph.D., president, SPRI.

“This discovery reflects the successful integration of new technologies, including genomics and bioinformatics, into discovery research and scientific excellence by a team of SPRI scientists representing a variety of disciplines.”

“While research in the past few decades has contributed much to our understanding about the production of cholesterol in the liver, this finding represents an important new discovery which helps explain how the body regulates cholesterol absorption in the second critical pathway – the intestine,” said Christie Ballantyne, M.D. FACC, FACP, director of the Center for Cardiovascular Disease Prevention and professor of medicine at Baylor College of Medicine/The Methodist DeBakey Heart Center in Houston.

Bioinformatics and genomics tools supported finding

Led by Michael Graziano, Ph.D., senior director, Cardiovascular/Metabolic Discovery Research, and senior author on the research paper, Schering-Plough scientists identified NPC1L1 after years of studying specific intestinal cells, called enterocytes, which are known to absorb cholesterol. These cells comprise a small percentage of the total number of cells in the intestine and have not previously been studied in great detail.

Scientists began by compiling two “libraries” of more than 16,000 segments of nucleotide sequences (ESTs, or expressed sequence tags) of the genes that are present in enterocytes. The bioinformatics team, led by Nicholas Murgolo, Ph.D., Senior Principal Scientist, Discovery Technologies and Bioinformatics, SPRI, used proprietary capabilities to explore publicly available genomics databases. They focused their search on genes encoding proteins whose predicted structures suggested cell surface expression and potential interaction with cholesterol – two key characteristics a protein involved in cholesterol-absorption would likely possess. The scientists characterized and determined the function of a previously identified gene known as Niemann-Pick C1-Like 1 (NPC1L1) gene, whose name derives from its similarity to another gene that is mutated in individuals with the rare disorder Niemann-Pick disease. NPC1L1 has no role in Niemann-Pick disease.

Linking NPC1L1 to cholesterol absorption

Schering-Plough molecular biologists led by Associate Principal Scientist Scott Altmann, Ph.D., cloned the NPC1L1 gene and characterized its expression. Employing immunohistochemistry they showed that the NPC1L1 protein is specifically located on the brush border membranes of jejunal enterocytes, the sides of the cell that come in direct contact with the contents of the small intestine. The jejunum is the specific region of the small intestine where the majority of cholesterol absorption occurs.

To confirm the role of the newly identified protein in cholesterol absorption, scientists led by coauthor Harry “Chip” Davis, Ph.D., Distinguished Research Fellow, Cardiovascular/Metabolic Discovery Research, studied “gene knockout” mice that had been genetically engineered to lack the NPC1L1 protein. Davis and his colleagues found that the knockout mice absorbed 70 percent less cholesterol from their diets than did normal mice, demonstrating that NPC1L1 is a critical component in the cholesterol absorption pathway. Administration of EZETROL had no effect in the knockout mice, suggesting that the compound works by blocking NPC1L1. In addition, when EZETROL was administered to mice that were not

NPC1L1 deficient, the percentage reduction in cholesterol absorption was similar to the NPC1L1 deficient mice. While further research is needed, these findings suggest that EZETROL interacts with NPC1L1 to reduce cholesterol absorption. Scientists are beginning to identify other proteins in the pathway that orchestrate cholesterol absorption from the intestine. They are also conducting biochemical assays to prove that ezetimibe binds directly to NPC1L1, thereby preventing it from ferrying cholesterol into enterocytes lining the jejunum.

Hyperlipidaemia is an important cardiovascular risk factor and is estimated to cause about 4.4 million deaths, about 7.9% of the global total.1

About EZETROL

EZETROL (ezetimibe), a cholesterol absorption inhibitor, has been developed and is being marketed by Merck & Co., Inc. (NYSE:MRK) and Schering-Plough Corporation (NYSE:SGP) in connection with a partnership formed by both companies to develop and market worldwide (excluding Japan) new prescription medicines in cholesterol management. Ezetimibe was approved for marketing by the United States Food and Drug Administration on 25 October 2002, and is marketed in the United States as ZETIA™. Following the successful completion of the European Union Mutual Recognition Procedure, EZETROL has now been launched in six European countries -- Germany, the United Kingdom, Switzerland, Sweden, Ireland, and Holland.

About Merck

Merck & Co., Inc., which operates in many countries as Merck Sharp & Dohme, is a global research-driven pharmaceutical products and services company. Merck discovers, develops, manufactures and markets a broad range of innovative products to improve human and animal health, directly or through its joint ventures.

About Schering-Plough

Schering-Plough Corporation is a research-based company engaged in the discovery, development, manufacturing and marketing of pharmaceutical products worldwide.

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1. http://www.who.int/hpr/NPH/docs/gs_chronic_disease.pdf

Company contacts:

Joe Azzinaro
Merck, Sharp & Dohme
+1 908 423 4263

Investor contact:

Mark Stejbach
Merck, Sharp & Dohme
+1 908 423 5185

Chris Loder
Merck, Sharp & Dohme
+ 1 908 423 3786

Denise Foy
Schering-Plough Corp.
+1 908 298 7616