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Phospholipidosis in healthy subjects participating in clinical studies: Ultrastructural findings in white blood cells

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ABSTRACT

Lipid storage disorders and phospholipidosis share similar morphologic characteristics displayed as lamellar bodies at ultrastructural level. More than 50 cationic amphiphilic drugs (CADs), including antidepressants, antianginal, antimalarial, and cholesterol-lowering agents, have been reported to induce phospholipidosis, however, the mechanism by which this occurs has not been extensively studied and is not well understood. Both the Food and Drug Administration (FDA) and the pharmaceutical industry recognized drug-induced phospholipidosis as a significant challenge for drug development. In a randomized, double-blind, placebo-controlled, active-controlled, ascending multiple-dose study to investigate the tolerability, safety, pharmacokinetics, and pharmacodynamics of a new investigational drug (an antihypertensive drug in early drug development) in healthy male subjects, possible drug-induced phospholipidosis was also explored ultrastructurally. Given the presence of these structures both pretreatment and following placebo treatment, it was concluded that the presence of phospholipid-like structures in individual volunteers could be a normal background finding in neutrophilic granulocytes thus emphasizing their role as natural phagocytic cells. Recommendations for the conduct of this type of studies are given.

Introduction

Lipid storage diseases, or lipidoses, are a group of inherited metabolic disorders, e.g., Gaucher-, Niemann–Pick-, Fabry-, Krabbe disease and others in which lipids accumulate in some cells and tissues (Lüllmann-Rauch, 1979). Patients with these disorders either do not produce enough of one of the enzymes needed to metabolize lipids or they produce enzymes that do not work properly. Over time, this excessive storage can cause permanent cellular and tissue damage, particularly in the brain, peripheral nervous system, liver, spleen, and bone marrow. Phospholipids are major components of cellular membranes. Phospholipidosis constitutes a group of lipid storage disorders in which phospholipids accumulate in lysosomes within cells (Sawada et al., 2005), however, the detailed pathogenesis is not completely understood. Lipid storage disorders and phospholipidosis share similar morphologic characteristics displayed as lamellar bodies at ultrastructural level. Under physiological conditions lamellar bodies exist in pneumocytes type 2 containing surfactant, in the gastro-intestinal tract especially in the stomach, in the synovialis of joints, and in the skin (Schmitz and Grandl, 2008). The role of lamellar bodies is summarized as follows: they are lipid storage and secretory organelles having different roles: (1) intracellular lipid storage; (2) secretion of stored lipids; and (3) pathological accumulation of extracellular membranes.

Based on molecular genetic studies the following four processes are hypothetically involved in the induction of phospholipidosis (Sawada et al., 2005):

1. Inhibition of lysosomal phospholipase activity generally regarded as the primary mechanism of induction, as confirmed by the up-regulation of phospholipid degradation-related genes such as N-acylsphingosine amidohydrolase 1 (ASAH1), sphingomyelin phosphodiesterase (SMPDL3A), and hypothetical protein MGC4171 (MGC4171).
2. Inhibition of lysosomal enzyme transport, as demonstrated by the down-regulation of genes involved in lysosomal enzyme transport such as adaptor-related protein complex 1 sigma 1 subunit (AP1S1). AP1S1 is responsible for the transport of newly synthesized lysosomal enzymes between the *trans*-Golgi network and lysosomes (Zhu et al., 1999).
3. Enhanced phospholipid biosynthesis, which is supported by the up-regulation of fatty acid biosynthesis-related genes such as ELOVL family member 6 (ELOVL6) and stearoyl-CoA desaturase (SCD).

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4. Enhanced cholesterol biosynthesis, as shown by the up-regulation of cholesterol biosynthesis-related genes such as 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (HMGCS1), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), squalene epoxidase (SQLE), lanosterol synthase (LSS), and 7-dehydrocholesterol reductase (DHCR7). Inhibition of lysosomal phospholipase activity and lysosomal enzyme transport, coupled with enhanced phospholipid biosynthesis could directly trigger phospholipidosis.

Increased cholesterol biosynthesis is considered to be an indirect trigger for the following two reasons:

1. The accumulation of sphingomyelin occurs concurrently with the increase in cholesterol in visceral tissues (e.g., spleen) in patients with Niemann–Pick type C disease (NPC), which is caused by a genetic defect in the cholesterol trafficking protein NPC1 or, in far fewer patients, the sterol-regulating protein HE1 (Blanchette-Mackie, 2000; Harzer et al., 2003; Vanier, 1983).
2. The induction of lamellar myelin-like bodies and the accumulation of free cholesterol occur in cultured mouse macrophages that have been incubated with acetylated low density lipoprotein or acyl-CoA:cholesterol acyltransferase inhibitor (McGookey and Anderson, 1983; Robenek and Schmitz, 1988). In addition, the up- or down-regulation of transporter genes (e.g., facilitated glucose transporter) and genes that control the cell cycle (e.g., cyclin G2) may also be involved, but modification of the expression of these genes likely reflects secondary changes that occur as a result of an increase in cellular phospholipids.

Drug-induced phospholipidosis

More than 50 cationic amphiphilic drugs (CADs), including antidepressants, antianginal, antimalarial, and cholesterol-lowering agents, have been reported to induce phospholipidosis (Lüllmann et al., 1978; Halliwell, 1997; Reasor, 1989). While CADs are thought to induce phospholipidosis by inhibiting lysosomal phospholipase activity, the mechanism by which this occurs has not been extensively studied and is not well understood (Hostetler and Matsuzawa, 1981; Joshi et al., 1988; Reasor and Kacew, 2001; Xia et al., 2000). Electron microscopy has been the most reliable method for identifying phospholipidotic cell damage (Drenckhahn et al., 1976).

Both the Food and Drug Administration (FDA) and the pharmaceutical industry recognized drug-induced phospholipidosis as a significant challenge for drug development (Berridge et al., 2007). The FDA's interests in this subject are broad and include, among others, determining the incidence/prevalence of phospholipidosis (Berridge et al., 2007).

Ultrastructural investigations revealed that these intra-cytoplasmic inclusions consist of concentric myelin-like structures (lamellar bodies), the presence of which became the morphological hallmark of phospholipidosis (Dake et al., 1985). Morphologically these inclusions vary in size from 100 to 2400 nm; they are surrounded by a membrane and contain multilamellar lipid membranes. Lamellar bodies may also contain apolipoproteins and lytic enzymes and have an acidic pH, which renders them a lysosomal character. Under normal physiological conditions, the main function of lamellar bodies is the supply of extracellular domains with specialized lipid components related to a specialized function (Schmitz and Müller, 1991). In lysosomal storage disorders, however, the lamellar bodies contain primarily undegraded phospholipids. Essentially, drug-induced phospholipidosis is characterized by phospholipid accumulation in affected tissue of which lung, liver, brain, kidney, cornea and others have been reported (Lüllmann et al. 1975; Anderson and Borlak, 2006).

In a randomized, double-blind, placebo-controlled, active-controlled, ascending multiple-dose study to investigate the tolerability, safety, pharmacokinetics, and pharmacodynamics of a new investigational drug (an antihypertensive drug in early drug development) in healthy male subjects, possible drug-induced phospholipidosis was also explored. Subjects were either treated with the investigational drug, placebo or enalapril, an angiotensin converting enzyme (ACE) inhibitor for comparative pharmacodynamic reasons. Since a literature search did not reveal any morphological (ultrastructural) data on the incidence/prevalence of phospholipidosis in healthy individuals, these findings are reported here.

Material and methods

Study design

The study was designed as a single center, double-blind, parallel, randomized, double-dummy, placebo- and active-controlled, ascending multiple dose, phase I study in healthy male subjects.

A screening evaluation was performed within 31 days before drug administration. Subjects entered the Clinical Research Unit (CRU) at least 48 h prior to first drug administration and were put on a standardized sodium (100 mmol/day) and potassium (60 mmol/day) diet until the end of the in-clinic period. On days 1–7, subjects received the investigational drug (an antihypertensive drug in early drug development) orally at multiple doses of 50, 100, 250, 500, and 1000 mg administered as capsules once daily in the morning. Matching placebo capsules and enalapril 20 mg tablets (active or matching placebo) were administered in the same way as the investigational drug. All treatments were administered using a double-dummy technique followed by an observation period of 120 h after the last administration on day 7. In the evening of days −1 and 6, subjects received a single dose of 40 mg furosemide. The end-of-study (EOS) examination took place 120 h after the last drug administration on day 7. Twenty-eight days following last drug administration a telephone follow-up for serious adverse events (SAEs) took place.

Volunteers: Fifty-nine male volunteers were recruited according to the following criteria at screening:

- Male aged between 18 and 45 years (inclusive).
- Healthy on the basis of medical history and physical examination performed.
- Hematology, clinical chemistry, and urinalysis test results not deviating from the normal range to a clinically relevant extent.

Main exclusion criteria:

- History or clinical evidence of any disease and/or existence of any surgical or medical condition that might interfere with the absorption, distribution, metabolism or excretion of the study drugs.
- Previous treatment with any prescribed or over-the-counter (OTC) medications (including herbal medicines such as St. John's wort) within 2 weeks prior to randomization.

From the total of 59 male volunteers 38 were selected for the electron microscopic evaluation. This selection represented the dose groups 1000, 500, and 250 mg of the investigational drug