



FABACs and Aramchol Status Report

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Summary

Galmed Medical Research develops innovative, proprietary drugs for the treatment of cholesterol and liver diseases. The company has synthesized a series of proprietary fatty acid bile-acid conjugates (**FABACs**) with multiple effects on liver and cholesterol metabolism demonstrated in several species in vivo and in human cells and tissues in vitro. The most advanced compound in the series is **Aramchol** (arachidyl amido cholanoic acid), which is currently in preclinical studies.

FABACs have potential for a variety of therapeutic indications including:

- Fatty liver disease (NAFLD) (curative and preventive)
- Hypercholesterolemia and related disorders (e.g. atherosclerosis)
- Cholesterol gallstones (curative and preventive)

These synthetic new chemical entities were found to have several discreet metabolic effects and to interact at several sites with specific transporters and enzymes related to cholesterol, triglycerides and bile acids. At present the main upstream molecular target of FABACs activity has not yet been fully determined.

Intellectual Property

The first series of patents relates to composition as well as use in cholesterol gallstone disease and atherosclerosis. These patents have been granted in the USA (6,589,946 B2 until 2022) and in Europe (1071702 B1 until 2019) and have been submitted in about 43 countries. The second series PCT/IL02/00303 is in the national phase in the same countries, claiming use for: treatment and prevention of fatty liver, reduction of blood and body cholesterol and treatment and prevention of hyperglycemia and diabetes. This patent application has been granted in Europe.

CMC

Aramchol is synthesized at high yield and purity by conjugating arachidic acid (C20:0) and cholic acid through an amide bond. The conjugation is in the beta-configuration. The resultant powder is sparingly soluble in water. The compound was determined to be at least 98% pure by NMR.

Cholesterol Metabolism & Atherosclerosis

In several series of experiments in various strains of mice or in hamsters in which hypercholesterolemia was induced either by excessive dietary intake or by increased synthesis, oral Aramchol markedly and significantly reduced plasma cholesterol as compared to saline treated controls. In some of these studies the reduction was to levels lower than those found in comparable animals on a regular diet. The effects on plasma cholesterol levels were observed both in animals that have received ARMCHOL together with the high fat diets as well as in animals that were initially primed to have hypercholesterolemia and subsequently treated with Aramchol. These effects of Aramchol in mice were confirmed in 3 series of experiments in hamsters. In these experiments hypercholesterolemia was induced by diet or by stimulation of endogenous synthesis. Aramchol reduced the high cholesterol levels more than simvastatin and similar or more than atorvastatin.

The following demonstrated processes may explain the observed effects on plasma cholesterol:

- 1) Efflux from cells ex vivo via ABCA1. There was a 2-4 fold increase in cholesterol efflux which is the first step in reverse cholesterol transport. Efflux from human macrophages is particularly effective and important in the context of Atherosclerosis while efflux from the liver is particularly important for reduction of body cholesterol stores. Both were shown in human cells. Of interest is to note that in the absence of a functioning ABCA1 transporter, as found in samples from patients with Tangiers Disease, this stimulatory effect is lacking (Biochem. J. 2006, 396:529).
- 2) Moderate reduction (about 50%) of synthesis via HMGC_oA Reductase. Differently from the Statins that directly inhibit the enzymatic activity of HMGC_oA Reductase, Aramchol acts on regulatory pathways that reduce both mRNA and proteins levels of the enzyme, and thereby result in reduced activity.
- 3) Increase in fecal sterol excretion (bile acids + neutral sterols) of about 2 fold. This represents the final step in reverse cholesterol transport, and results in overall reduction of sterol body stores (Biochem. Soc. Trans. 2004:32:131).
- 4) Considerable (2-3 fold) increase in catabolism to bile acids via CYP7A1. These effects, also demonstrated in human cells and tissues in vitro, are mediated through the reduction of the activity and expression of CYP7A1.

As a consequence of reduction of plasma cholesterol levels, it is believed that several of the cholesterol dependent pathologies will be alleviated. In this respect, we have demonstrated that Aramchol reduces experimental atherosclerosis in mice following a period of atherogenic diet (Pathobiology 2003; 70:215.).

Fatty Liver (NAFLD)

Fatty liver affects over 20% of the general population in most westernized countries and its prevalence is rising rapidly in parallel with the prevalence of obesity. Some 25% of patients with fatty liver progress to inflammation (NASH) and cirrhosis. FABAC treatment reduces mostly triglycerides in the liver. Neither fatty liver nor gallstones have currently an accepted medical treatment.

Aramchol, given orally, was demonstrated to prevent diet induced fatty liver in several animal species, using different diets (Hepatology 2003; 38:346). More recently we have also shown that they also reduce preexisting diet induced fatty liver; i.e. they are effective in a therapeutic setting (unpublished). The rapidity and the magnitude of the therapeutic effect (weeks or months) are inversely proportional to the fat concentration in the maintenance diet during the treatment period.

Two mechanisms were shown to be involved in this effect.

1. Inhibition of Stearoyl CoA Desaturase activity.
2. Reduction of fatty acid synthesis

Cholesterol Gallstones

Gallstones affect about 15% of the general population in industrialized countries. Close to 80% of them are cholesterol gallstones. Some 20-25% of patients with gallstones develop pain and complications and are referred to surgery.

Aramchol and other FABACs were shown to both prevent and dissolve preformed gallstones in numerous in vivo experiments (Gut 2001; 48:75, Lipids 2001; 36:1135, Hepatology 2002; 35:597). The extent of the dissolution was dose-dependent and in the majority of experiments, a full elimination of gallstones was obtained.

Our current analysis indicates that dissolution using oral Aramchol will cost only a fraction of the price of surgery. It eliminates the small mortality and moderate morbidity associated with surgery (laparoscopic or open). Previous experience and current surveys indicate that most patients and general physicians favor a trial of medical dissolution prior to surgery. Intermittent maintenance therapy may or may not be needed following dissolution.

Mechanisms of action

A large number of experiments were carried out to date to assess the processes and mechanisms of action of FABACs and Aramchol on cholesterol and triglyceride metabolism. While several key enzymes and transporters were shown to be modulated, the upstream, central, control target(s) are still being studied. In relation to reduction of liver triglycerides, inhibition of Stearoyl CoA desaturase can explain all the observed effects. In relation to the multiple effects on cholesterol metabolism, a partial agonistic effect on LXR is now being tested.



ADME

Aramchol is rapidly absorbed in its unchanged form and exercises its biologic effects upon oral administration. The observed Cmax in the rat is at 3-4 hours and the T1/2 of elimination is estimated to be 3-5 hours. Recent studies with modified formulations resulted in a significant enhancement of absorption.

Toxicity

On acute oral LD₅₀ assessment, there was no mortality and no evidence of toxicity at doses of 2000 mg/kg in mice and rats. This indicates a particularly large therapeutic window with no evidence of toxicity at more than 100-fold the therapeutic dose. No toxicity was noted in chronic experiments lasting over 4 months. Biochemical and histological studies were negative (Europ. J. Gastroent. Hepatol. 2003; 15:1). Formal chronic toxicity studies are currently ongoing.

FABAC Effects shown in HUMAN cells and fluids

The following effects were demonstrated in cells from human origin:

- Cholesterol efflux (Reverse Cholesterol Transport) from human fibroblasts.
- Effects on the ABCA1 transporter were elucidated in these cells;
- Efflux from macrophages was demonstrated in human cells, this is particularly relevant to treatment of Atherosclerosis
- Efflux from the liver was shown in a human liver cell line.
- Increase in Cholesterol Catabolism to bile acids was demonstrated in fresh human liver cells.
- Inhibition of fatty acid synthesis and reduction of hepatocyte triglyceride content were shown in a Human Liver cell line
- Inhibition of cholesterol crystallization and dissolution of formed cholesterol crystals were demonstrated in human bile.