AFGIFTEKANTOOR LEUVEN 1
P5 08820
Postbus 1212, 3000 Leuven 1

Lipid Club Letter

Quarterly • July - August - September 2007
Volume 19 - N° 3

Contents

Founding members of the BELGIAN LIPID CLUB

Pr	E. BALASSE	(U.L.B. Bruxelles)
Pr	V. BLATON	(St.-Jan, Brugge)
Pr	J.C. DAUBRESSE	(Hôpital Civil, Charleroi)
Pr	I. DE LEEUW	(U.A. Antwerpen)
Pr	J. DUCOBU	(C.H.U. Tivoli, La Louvière)
Dr	C. HERBAUT	(U.L.B. Bruxelles)
Pr	P. LEFEBVRE	(Univ. de Liège)
Pr	E. MULS	(K.U. Leuven)
Pr	M. ROSSENEU	(R.U. Gent)
Pr	R. ROTTIERS	(R.U. Gent)
Pr	A. SCHEEN	(Univ. de Liège)
Pr	L. VANHAELST	(V.U. Brussel)
Pr	L. VAN GAAL	(U.A. Antwerpen)
Pr	B. VELKENIERS	(V.U. Brussel)

Members of the Bureau of the BELGIAN LIPID CLUB

President:
Prof. G. DE BACKER (R.U. Gent)
Past-President:
Prof. F.R. HELLER (C.H. Jolimont-Lobbes)
Members:
Prof. A. HERMAN (U.A. Antwerpen)
Dr. O. DESCAMPS (C.H. Jolimont)
Prof. Y. CARPENTIER (ULB Bruxelles)
Prof. M. LANGLOIS (St.-Jan, Brugge)

Editorial

In my first editorial to this Lipid Club Letter I want to thank and congratulate the former president Professor F. Heller and the Board of the Belgian Lipid Club (BLC) for what they achieved in the previous period. I will do my best to reach similar standards but I will be relying on each of the members of the BLC to make this happen.

As for 2007, we have already presented the MSD-Schering Plough award to O. Descamps; we are looking forward to an exciting scientific symposium on Lipids and Atherosclerosis on Sept 29th 2007 in La Louvière, organized by J. Ducobu; V. Blaton and M. Langlois are preparing another interesting meeting on October 20th in Bruges related to new insights into the prevention of cardiovascular disease (CVD).
Let me also remind you that the 4th Joint European Task Force on prevention of CVD in clinical practice presented its guidelines at the ESC meeting in Vienna in early September. These guidelines, which are also endorsed by EAS, are published as a supplement to the Eur J CV prevention and rehabilitation 2007.
Finally, let me remind you of the call for proposals related to the 2007-2008 Research Fellowship in Lipidology. We are expecting numerous innovative ideas before December 31st 2007.
In the meantime, enjoy this issue of the Lipid Club Letter and I hope to see you at one of the forthcoming meetings.

Prof. Dr. G. De Backer
President

Responsible editor: Prof. G. DE BACKER (R.U. Gent) Editorial staff: Prof. J. DUCOBU (CHU Tivoli)

MYELOPEROXIDASE: A NEW THERAPEUTIC TARGET FOR THE TREATMENT OF ATHEROSCLEROSIS?

Pierre VAN ANTWERPEN (U.L.B.), Karim Zouaoui BOUDJELTIA (C.H.U. Charleroi), Martine PREVOST (U.L.B.), François DUFRASNE (U.L.B.), Nicole MOGUILEVSKY (F.U.N.P. Namur), Michel VANHAEVERBEEK (C.H.U. Charleroi), Jean DUCOBU (C.H.U. Tivoli).

Myeloperoxidase (EC 1.1.11.7, MPO) is localized in the azurophilic (primary) granules of neutrophils and to a lesser extent in monocytes. The major role of this heme-containing protein is the synthesis of hypochlorous acid (HOCl, an antiseptic) in the presence of hydrogen peroxide (H_2O_2) and chloride: after the phagocytosis of an exogenous structure by neutrophils and the formation of the phago-lysosome, granules are emptied and MPO is released, while the NADPH oxidase system is activated to promote the synthesis of H_2O_2. The consequence is the production of HOCl that rapidly oxidizes the proteins and lipid structures of the targets (bacteria, viruses etc.). This phenomenon facilitates the destruction and the elimination of the pathogenetic organisms. However, in a condition such as 'oxidative stress', MPO is released in the extracellular liquid, in response to the high level of cytokines and the organism has to face a 'circulating' MPO that can promote oxidative damage to host tissue. For this reason, MPO has been carefully examined in major inflammatory syndromes, such as atherosclerosis, and has become a potential therapeutic target. Its inhibition is a challenging, fascinating, but also difficult therapeutic objective. [1]

MPO and atherosclerosis

In 1994, MPO was discovered in atheromatous plaque, pointing to its potential involvement in the development of atheromatous lesions. Furthermore, the presence of MPO in the subendothelial space corroborated the consensus theory of atherosclerosis: low density lipoproteins (LDL) enter the intima and their accumulation promotes their oxidation (e.g. by MPO). The reaction product, called Mox-LDL, is taken in by the macrophages that are present in the intima in the form of foam cells.

Recent data has brought into perspective the neglected role of 'circulating' MPO. The enzyme is a highly cationic protein (pI > 10) that can be adsorbed on electronegative (negatively charged) macrostructures like the cell surface, high density lipoprotein (HDL), LDL etc. The main consequence is the oxidation of amino acids by HOCl and protein modifications of these structures in the subendothelial space. [2]

MPO and HDL

Two major sites of oxidation have been clearly described for HDL. MPO can be bound on the apolipoprotein A-1 (Apo-A1) and the production of HOCl promotes the oxidation of a methionine (Met112) in sulfoxide and the chlorination of a tyrosine (Tyr192) in 3-Chlorotyrosine. As a consequence, HDLs are unable to ensure cholesterol efflux probably because the oxidation of Apo-A1 impairs cholesterol-dependent ABCA1 transport,which completely modifies the interaction of HDL with the endothelium. [3]

Drugs	Percentage of inhibition at 1 µM
Flufenamic acid	43 %
5-bromoflufenamic acid	51 %
Tryptamine derivatives	66 %

Table 1: Percentages of MPO inhibition at 1 µM measured by the taurine assay

MPO and LDL

While the fixation of MPO on LDL has been clearly demonstrated, its oxidation and the consequence of its modification are not completely elucidated; several residues of the apolipoprotein B-100 (Apo-B100) are proposed as a target. A mouse monoclonal antibody that specifically recognized Mox-LDL was developed to control oxidation and to show that Mox-LDLs are able to induce pro-inflammatory responses by monocytes and endothelial cells that respectively produce TNF-α and IL-8. In addition, the antibody has permitted the localization of specific Mox-LDL in the subendothelial space of human carotid blood vessels. [4]

MPO and endothelium

The direct and indirect effects of MPO on endothelium have been extensively studied but a lot of work remains to be done. For example, MPO decreases the bioavailability of nitric oxide (NO) and this is considered to be a noxious effect. HOCl, produced by MPO, activates metalloproteinases with various physiologic effects on the vascular wall. The oxidations and modifications of lipoproteins (HDL/LDL) are also the source of dysfunctions in the endothelium. Mox-HDLs are unable to scavenge excess cholesterol and may promote the triggering and recruitment of monocytes at the surface of endothelial cells by the concomitant production of TNF-α and IL-8. More interesting is the particular MPO-LDL-endothelium interaction that may initiate the LDL oxidation process.

A recent experiment demonstrated that endothelial cells activated by angiotensin II promote the oxidation of LDL by MPO at their surface. The activation involved NADPH oxidase that synthesizes the superoxide anion (O_2^-), a precursor of hydrogen peroxide. In this way, the production of Mox-LDL at the surface of the endothelial cells may be an additional pro-atherogenic mechanism to subendothelial LDL oxidation. [2]

Strategy in MPO inhibition

The role of MPO in atherosclerosis introduces the possibility of a new treatment for this condition. However, the pharmacological inhibition of this enzyme might be the best protection from its real effect in the pathology. We therefore adopted a rational drug design strategy for selecting or synthesizing inhibitors of the enzyme. This included (i) a virtual computerized docking[1] of the molecule in the catalytic site of MPO, (ii) its eventual synthesis and (iii) finally its assessment based on the three experiments cited below and carried

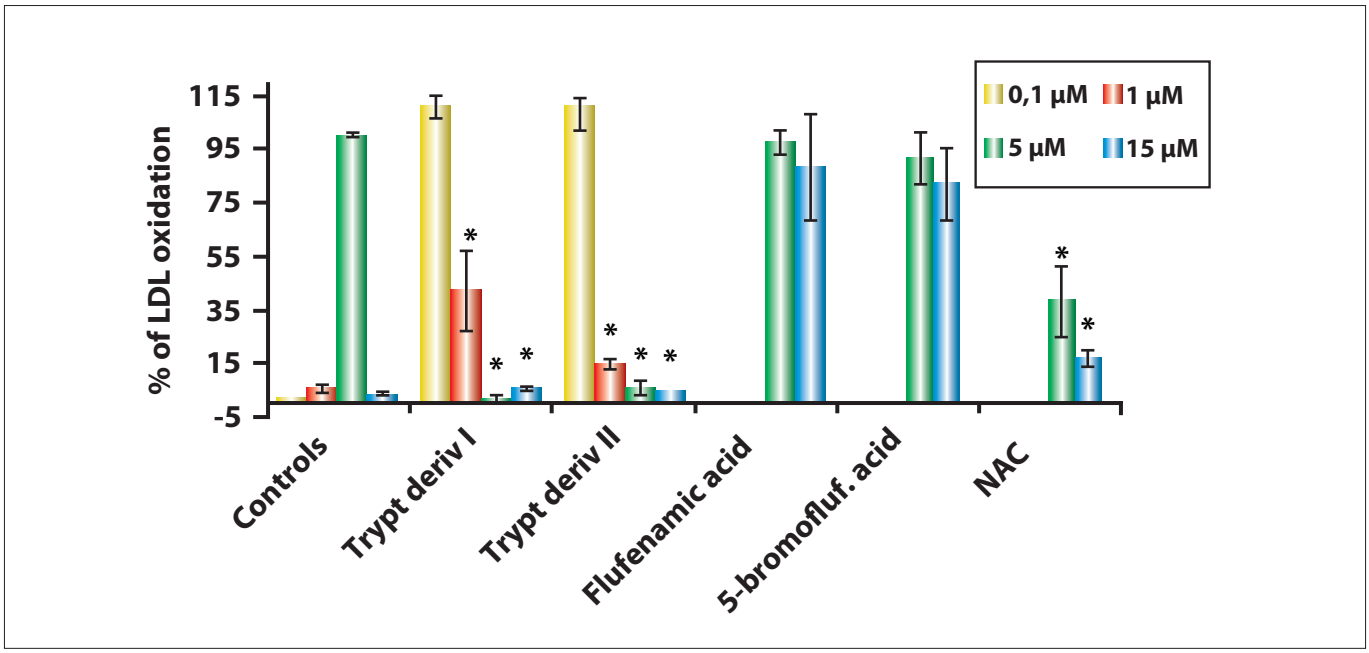

Figure 1: LDL oxidation (in %) in relation to different concentrations of flufenamic acid and derivatives: 5 µM ( ), 15 µM ( ), 30 µM ( ) and 300 µM ( ). Absorbance of LDL (Blank; controls,) and oxidized LDL without drugs (controls,) are respectively considered equal to 0 and 100 %. In absence of H_2O_2, % = 0.5 ± 0.9 % (controls,); in absence of MPO (400 U/ml) (controls,), % = 4 ± 2 %. Results are the mean ± SD for n = 3,
* P< 0.05 vs oxidized LDL.

[1] Docking: The action of virtually placing a molecule in a catalytic site, taking into account rational physical and chemical parameters

out in the presence of several different concentrations of the compound:

- the measurement of MPO activity;
- the measurement of LDL oxidation in the presence of the MPO system using a specific antibody (ELISA);
- finally, the measurement of Mox-LDL production at the surface of the endothelial cells.

The molecules were selected according to previous experiments in which flufenamic acid was shown to have an efficient inhibiting effect ($IC_{50\%}$ = 1.1 ± 0.1 μM). But to give a proper perspective to the efficacy of such a molecule, its activity was initially compared to well-known antioxidants such as thiol-containing molecules (N-acetylcystein (NAC), glutathione, captopril).

Model-dependent results

According to the MPO activity assessment, flufenamic acid was an efficient inhibitor of MPO when compared to thiol-containing molecules. While the antioxidants (NAC, glutathione, captopril) simply scavenge HOCl, produced during the reaction, flufenamic acid was able to inhibit the production of HOCl by the enzyme, improving the inhibiting effect. However, when we compared the inhibiting effect of the molecules in the LDL oxidation, the results were completely different. Flufenamic acid was unable to inhibit LDL oxidation at pharmacological concentrations, while thiol-containing molecules reduced LDL oxidation by 50 % at 5 μM (~1 μg/ml of NAC, Fig. 1). In addition, we synthesised flufenamic acid derivatives by the addition of chemical groups to the initial structure. The presence of voluminous substituents on the structure generally decreased the activity, but some halogenous compounds (5-bromoflufenamic acid) had a higher inhibiting activity (Table 1). However, none of them were able to inhibit LDL oxidation mediated by MPO (Fig. 1).

The main problem that we had to face was the particular interaction between MPO and LDL that probably places the lipoprotein near the entry of the catalytic site of MPO, making access to the site extremely difficult. In this context, flufenamic acid is ill-suited because its structure is more voluminous than that of NAC.

How to cope with MPO-LDL binding?

According to previous results, it would appear that smaller or more planar molecules cope better with MPO-LDL binding (Fig. 2). Planar molecules may be able to slip properly between the MPO and LDL molecules to reduce MPO activity. For this reason, we tested planar molecules, such as tryptamine derivatives (Trypt Deriv I & II), as some authors have recently pointed out the efficient activity of these molecules on MPO. Their inhibiting effect is three times greater than that of flufenamic acid in our model (IC50% = 0.25 ± 0.05 μM vs 1.1 ± 0.1 μM for flufenamic acid). Furthermore, they are able to reduce the production of Mox-LDL to under 50 % at 1.0 μM (~ 0.2 μg/ml) (Fig. 1). However, during the cell test, no inhibition at all was noticed with these tryptamine derivatives or for flufenamic acid (Fig. 3).

Conclusions

The particular interaction between LDL and MPO renders the enzyme-inhibition strategy particularly difficult as the results are model-dependent. However, the discrepancy between the results has made it possible to provide indicators in the rational drug design of MPO inhibitors. First, voluminous molecules should be looked at carefully, as the larger the mass the more unfavourable for the inhibition of MPO. Secondly, planar molecules seem to enter the catalytic site of MPO properly when LDLs are present. This could be the result of the presence of LDL near the entry of the catalytic site. Finally, none of the molecules was able to inhibit the oxidation of LDL at the surface of endothelial cells after one hour of incubation. This lack of inhibition in the cellular model will have to be elucidated in further studies.

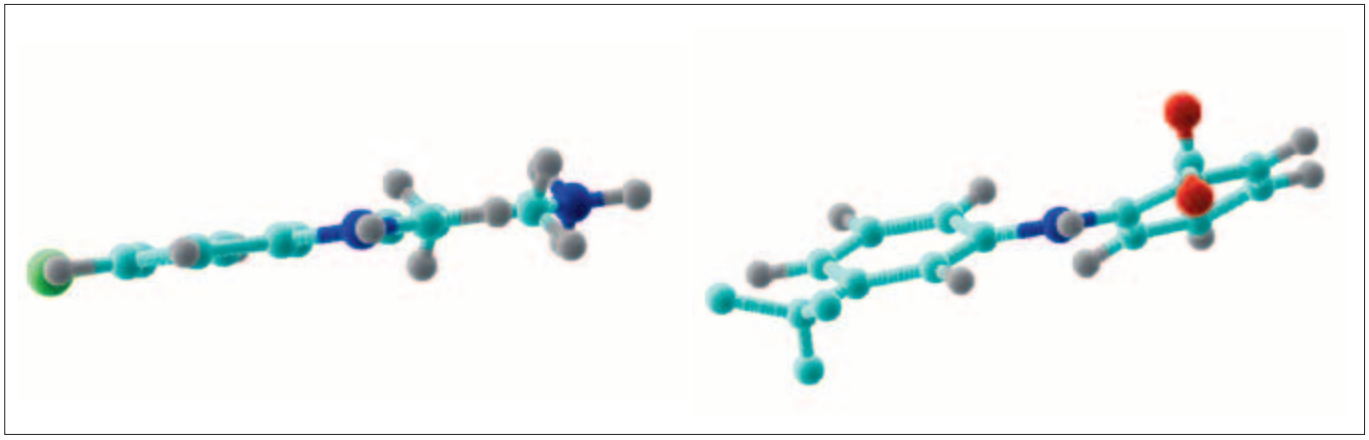

Figure 2 :Comparison of three-dimensional structures of chlorotryptamine (left) and flufenamic acid (right) emphasizing the more planar geometry of the first molecule.

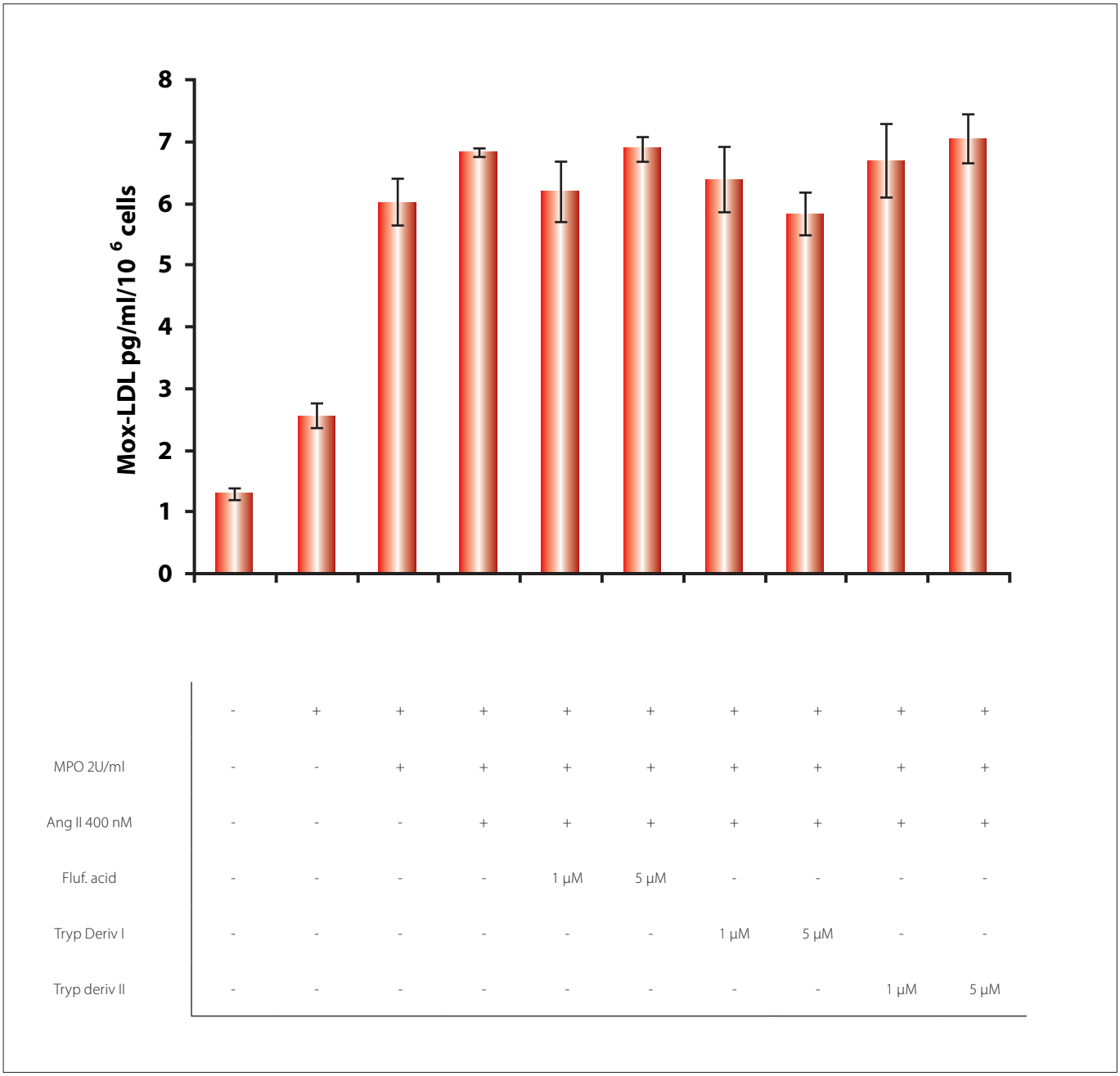

Figure 3: LDL oxidation (in pg/ml/106 cells) in relation to different concentrations of flufenamic acid and derivatives (1 and 5 µM).

References

[1] Klebanoff, S. J. (2005) Myeloperoxidase: friend and foe. J. Leuk. Biol. 77, pp 598-625.

[2] Lau, D. and Baldus, R. M. (2006) Myeloperoxidase and its contributory role in inflammatory vascular disease. Pharmacol. Therapeut. 11, pp ,16-26

[3] Zheng, L., Settle, M., Brubaker, G., Schmitt, D., Hazen, S. L., Smith, J. D., Kinter, M. (2005) Localization of nitration and chlorination site on apolipoprotein A-I catalyzed by myeloperoxidase in human atheroma and associated oxidative impairment in ABCA1-dependent cholesterol efflux from macrophages. J. Biol. Chem. 280, pp 38-47.

[4] Zouaoui Boudjeltia, K., Legssyer, I., Van Antwerpen, P., Lema Kisoka, R., Babar, S., Moguilevsky, N., Delree, P., Ducobu, J., Remacle, C., Vanhaeverbeek, M., Brohee, D. Triggering of inflammatory response by myeloperoxidase-oxidized LDL. Biochem Cell Biol. 84, pp 805-812.

DISTINCTION BETWEEN HIGH SYNTHESIZERS AND HIGH ABSORBERS OF CHOLESTEROL IN OPTIMIZING CHOLESTEROL-LOWERING THERAPY

Regis Radermecker, André Scheen
Department of Diabetes, Nutrition and Metabolic disorders
C.H.U. Liege, University of Liege, Belgium

Introduction

Hypercholesterolaemia represents a major risk factor in cardiovascular disease (CVD). There is a linear relationship between LDL cholesterol (LDL-c) levels and the incidence of coronary artery disease. The lower the LDL-c concentration, the better the cardiovascular protection of the patient. However, sometimes reaching target LDL-c levels may be a problem, even if statins are remarkably powerful in decreasing LDL-c by inhibiting hydroxymethyl glutaryl-CoA (HMG CoA) reductase, a key enzyme in hepatic cholesterol synthesis. The failure to reach LDL-c target values may result from insufficient titration of the statin dosage and/or from poor compliance with the drug therapy, but also may be due to a partial resistance to such an approach, which focuses on the inhibition of cholesterol synthesis.

Cholesterol absorption/synthesis and serum markers of absorption/synthesis

Cholesterol absorption and synthesis contribute to maintaining cholesterol homeostasis, and thus cholesterol levels in the blood. Exogenous cholesterol is absorbed within the small intestine via a transporter called Niemann-Pick C1-like protein or NPC1L1, whereas endogenous cholesterol is produced in both hepatic and peripheral tissues [1].

NPC1L1 is localized to the brush border of the enterocyte, acting as the gate-keeper for absorption of cholesterol and plant sterols from the lumen of the small intestine. This protein is the target of ezetimibe, a well-known inhibitor of cholesterol absorption. Cholesterol and plant sterols that have entered the enterocyte can be resecreted into the intestinal lumen via the ABCG5/G8 heterodimer. The majority of plant sterols (more than 95%) are immediately resecreted by this route whereas a large part (about 50 %) of the cholesterol remains in the organism and exerts its physiological (and sometimes pathophysiological) role [2]. Absorbed cholesterol contributes to the free cholesterol pool, as does the cholesterol from the endogenous biosynthetic pathway for which HMG CoA reductase is the rate-limiting factor.

Of the noncholesterol sterols in human sera, cholestenol, desmosterol, and lathosterol levels, especially when divided by total serum cholesterol concentration and expressed as ratios to cholesterol, reflect cholesterol synthesis. In contrast, cholestanol (a metabolite of cholesterol) and plant sterols, campesterol and sitosterol, are surrogate markers of cholesterol absorption, especially when they are expressed as ratios to cholesterol. Homeostasis of cholesterol metabolism implies a balance between synthesis and absorption so that the high absorption of cholesterol suppresses its synthesis, whereas the blocking of cholesterol synthesis leads to increased intestinal absorption of the sterols (both cholesterol and phytosterols: see below). Therefore, the ratios of the surrogate synthesis and absorption markers should also relate negatively to each other. Thus, synthesis of cholesterol is positively related to the ratios of the synthesis markers and negatively to those of the absorption markers [3]. Certain physiological factors, such as genetics, circadian rhythm, body weight, diabetes status, have been shown to affect either cholesterol absorption or synthesis [1].

High phytosterol serum levels as markers of CVD

Plant sterol esters or stanols act as lipid-lowering agents, by competing with cholesterol for incorporation into micellae, thereby decreasing the intestinal absorption of cholesterol [4]. However, the pathophysiological significance of plant sterols or plant stanols is currently under intense discussion, as there are some indications that plant sterols, once absorbed, may contribute to atherosclerosis. Evidence in support of this hypothesis comes from experimental studies in rats [5]. An important clinical observation is that patients with the mutations in ABCG5 or ABCG8 associated with sitosterolaemia suffer from premature coronary artery disease [6]. Many papers have shown that increased plasma plant sterol levels have been associated with a higher incidence of coronary heart disease, with a positive family history for such a condition and with deposition of those sterols in atheromatous tissues [7-11]. It has been suggested that statins, by inhibiting cholesterol synthesis, may not only enhance cholesterol intestinal absorption (see below), but also the absorption of various phytosterols, which might be deleterious for the arterial wall [12].

Inhibition of cholesterol absorption enhances cholesterol synthesis and vice versa

In addition to the physiological mediators of cholesterol metabolism, certain therapeutic factors have also been

shown to modulate cholesterol homeostasis. These factors include dietary components, mainly plant sterol and stanol supplementation, and drugs such as ezetimibe and statins. Many data have shown that inhibition of cholesterol absorption enhances cholesterol synthesis [1]. This is the main reason why ezetimibe, a specific inhibitor of cholesterol absorption, has a rather poor cholesterol-lowering effect as monotherapy. The mechanism by which a compensatory elevation in cholesterol synthesis occurs when absorption is inhibited may be explained through closer examination of the pathway of plasma cholesterol production. In this pathway, the rate-limiting enzyme, HMG CoA reductase, is regulated by the intracellular cholesterol concentration through negative feedback. More specifically, intracellular cholesterol regulates the HMG CoA reductase concentration and activity, through its ability to influence transcription, mRNA translation, and degradation of the enzyme. Suppression in cholesterol absorption by plant sterols (or ezetimibe) results in lower circulatory and cellular cholesterol concentrations. As a result, negative feedback of cholesterol on HMG CoA reductase is inhibited, leading to an increase in cholesterol synthesis.

Statins lower circulating levels of cholesterol by inhibiting the action of HMGCoA reductase in the hepatocytes. This leads to an increased expression of LDL receptors, which bind circulating cholesterol, a phenomenon that mainly explains the LDL-lowering effect of statins. As a result of this inhibition of the synthesis, statin therapy has been shown significantly to decrease cholesterol concentrations in a dose-dependent manner in many clinical trials. This means that even if a compensatory elevation in cholesterol absorption occurs in case of statin therapy, it is not equivalent to the decrease in cholesterol synthesis caused by the statin and so the overall effect remains largely favourable. Nevertheless, the more potent the inhibition of cholesterol synthesis, the higher the partial compensatory effect via increased cholesterol intestinal absorption. This has been confirmed in a double-blind trial comparing treatment with atorvastatin and simvastatin [12, 13]. After 1 year of treatment, those who were taking atorvastatin showed significantly lower cholesterol-synthesis rates than those who were taking simvastatin. As a result of this decrease in synthesis, a reciprocal increase in cholesterol absorption markers was found which was greater with atorvastatin than

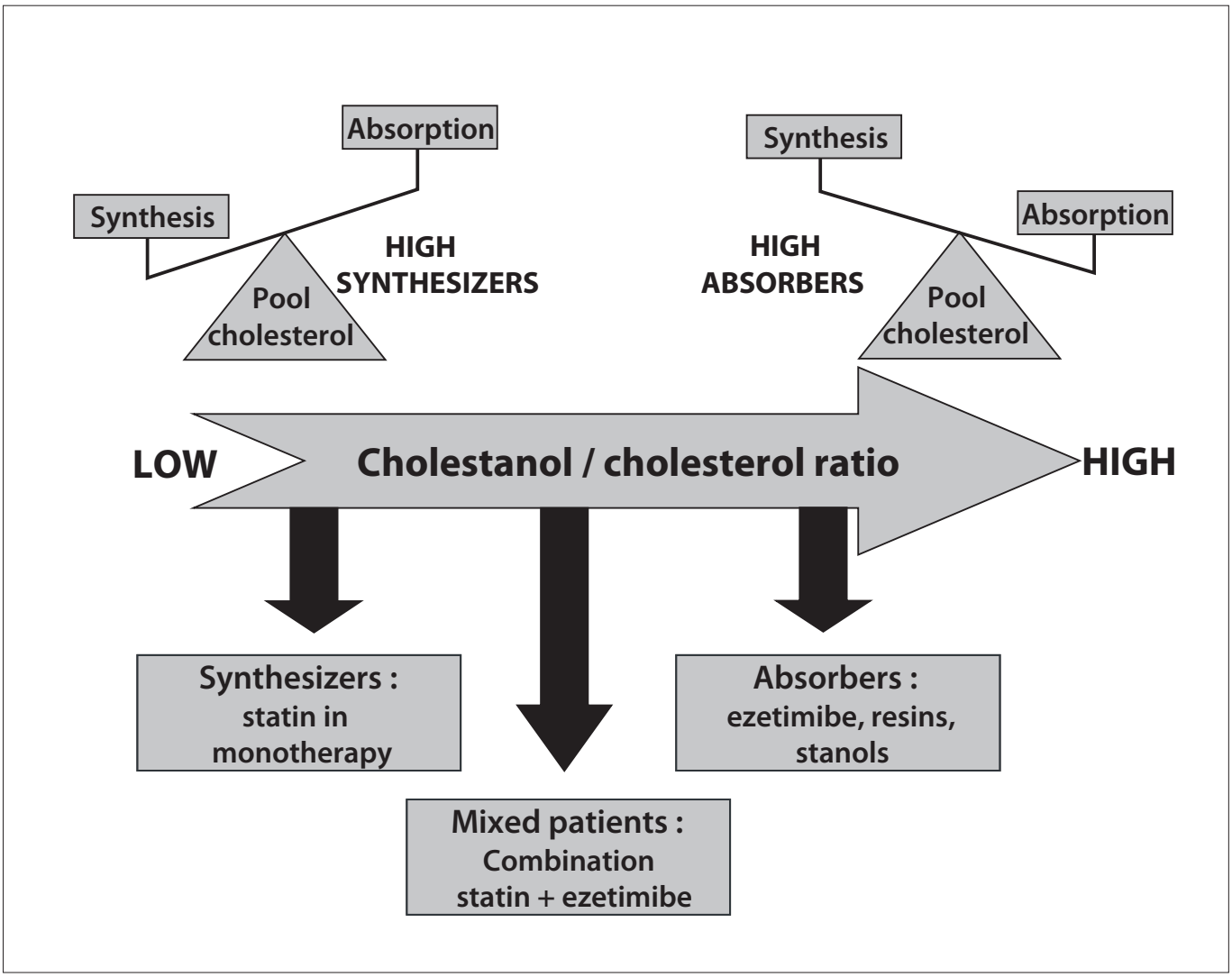

Figure 1 : Cholesterol lowering approaches according to the synthesizer/absorber status of the patients (adapted from references 15 and 16).

with simvastatin.

Heterogeneity of the population and potential influence of the absorber/synthesizer status

The characteristics of the absorber vs synthesizer status has been observed in several randomised clinical trials using statin therapy. For instance, Miettinen *et al.* studied a subset of the Scandinavian Simvastatin Survival Study (4S) population, a long-term trial evaluating the effect of simvastatin on cardiovascular complications in secondary prevention [14, 15]. Eight hundred and sixty-seven patients, randomized to receive either placebo or simvastatin (20 to 40 mg/day) for five years, were divided into four quartiles based on cholestanol-to-cholesterol ratios (indicator of baseline cholesterol absorption). Results revealed that lower baseline cholesterol absorption was associated with higher baseline synthesis precursor concentrations, indicating a high heterogeneity in the status of the patients [14]. Even more interesting, whereas the patients with the lower cholestanol/cholesterol ratio (and thus low absorbers) showed a significant 38 % reduction in coronary events with simvastatin, those with the higher ratio (corresponding to high absorbers) showed no protection with simvastatin (in contrast a 15 % increase in coronary events was observed). The risk of recurrence of coronary events increased 2.2-fold on statin therapy, from the lowest to the highest quartile of the cholestanol/cholesterol ratio [15]. The possible role of increased phytosterol absorption in the latter subgroup, combining high absorber profile and poor outcome on statins, remains to be investigated.

Therapeutic consequences

This reciprocal effect of a compensatory increase in cholesterol absorption or synthesis, when respectively a synthesis or an absorption inhibitor is given, seems to be an important concept in the management of dyslipidaemia. It could be interesting to screen patients for absorber and synthesizer qualities in order to introduce the most appropriate drug. In the case of a mixed profile and insufficient response to monotherapy, a combined therapy associating a synthesis inhibitor (statin) and an absorption inhibitor (ezetimibe) should be considered (Figure 1) [16, 17].

Conclusion

It might be interesting in future research to assess the synthesizer/absorber status of patients in order to optimize cholesterol-lowering therapy. Because of the compensatory mechanism between cholesterol synthesis and cholesterol absorption, the potential protection of a dual treatment with a synthesis inhibitor and an absorption inhibitor is conceptually attractive although it has still to be proven in a large-scale randomised clinical trial with definite clinical endpoints. Finally, we need routine dosages of markers of cholesterol absorption and synthesis to give a better assessment of the individual profile of each patient before changing our clinical approach.

References

1. Santosa S et al. Physiological and therapeutic factors affecting cholesterol metabolism: does reciprocal relationship between cholesterol absorption an synthesis really exist? Life Sci, 2007; 80: 505-514
2. Huff MW et al. NPC1L1: evolution from pharmacological target to physiological sterol transporter. Arterioscl Thromb Vasc Biol, 2006; 26: 2433-2438
3. Miettinen TA et al. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. Am J Epidemiol, 1990; 131: 20-31.
4. Gylling H et al. The effect of plant stanol- and sterol-enriched foods on lipid metabolism, serum lipids and coronary heart disease. Ann Clin Biochem, 2005; 42: 254-63
5. Huang MZ et al. Unusual effects of some vegetable oils on the survival time of stroke-prone spontaneously hypertensive rats. Lipids, 1997; 32: 747-751
6. Shulmann RS et al. Betasitosterolemia and xantomatosis. N Engl J Med, 1976; 294: 482-483
7. Glueck CJ et al. Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol, and premature coronary heart disease in hyperphytosterolemic probands and their first-degree relatives. Metabolism, 1991; 40: 842-8
8. Rajaratnam RA et al. Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. J Am Coll Cardiol, 2000; 35: 1185-91
9. Sudhop T et al. Serum plant sterols as a potential risk factor for coronary heart disease. Metabolism, 2002; 51: 1519-21
10. Assmann G et al. Plasma sitosterol elevations are associated with an increased incidence of coronary events in men : results of a nested case-control analysis of the Prospective Cardiovascular Munster (PROCAM) study. Nutr Metab Cardiovasc Dis, 2006; 16: 13-21
11. Miettinen TA et al. Plant sterols in serum and in atherosclerotic plaques of patients undergoing carotid endarterectomy. J Am Coll Cardiol, 2005; 45: 1794-801
12. Miettinen TA et al. Serum noncholesterol sterols during inhibition of cholesterol synthesis by statins. J Lab Clin Med, 2003; 141: 131-7
13. Miettinen TA et al. Synthesis and absorption markers of cholesterol in serum and lipoproteins during a large dose statin treatment. Eur J Clin Med, 2003; 33: 976-82.
14. Miettinen TA et al. , for the Finnish Investigators of the Scandinavian Simvastatin Survival Study Group. Noncholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients. Relation to basal serum cholestanol. Arterioscler Thromb Vasc Biol, 2000; 20: 1340-6
15. Miettinen TA et al., for the Finnish 4S Investigators. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian simvastatin survival study. BMJ, 1998; 316: 1127-30
16. Hoenig MR, Rolfe BE, Campbell JH. Cholestanol : a serum marker to guide LDL cholesterol-lowering therapy. Atherosclerosis, 2006; 184: 247-54
17. Radermecker RP, Scheen AJ. Distinction entre patients bons synthétiseurs et bons absorbeurs pour optimiser le traitement hypocholestérolémiant. Rev Med Suisse, 2006; 2: 1910-5