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L'ACIDE LIPOÏQUE MOLECULE ANTIRADICALAIRE A EFFET PROTECTEUR DE LA FONCTION ENDOTHELIALE.

Les cellules lisses, dénommées les cellules endothéliales, qui tapissent les parois internes de nos veines et de nos artères permettent au sang de circuler sans entraves. Le sang glisse à l'intérieur de notre système circulatoire.

On constate que les risques d'altération de cette magnifique rhéologie sont en augmentation depuis quelques années.

Les causes des pathologies dites cardiovasculaires sont de mieux en mieux connues et parmi les facteurs impliqués on peut sans erreur citer les diabètes de type I et II (il est connu que l'insulinorésistance accroît le risque de maladie vasculaire), l'excès de cholestérol, les hyperlipémies, les dyslipidémies, l'obésité (la relaxation à l'acétylcholine est altérée chez l'obèse), les radicaux libres de l'oxygène et leur pouvoir oxydant, l'oxydation de la lipoprotéine LDL, l'hypertension, les métaux lourds et le tabagisme.

Tous ces facteurs sont à la base de la sclérose de nos vaisseaux sanguins ou si vous préférez, ils sont la source de l'athérosclérose avec ses conséquences que l'on connaît à savoir : l'infarctus du myocarde, les thromboses veineuses et artérielles, la thrombose rétinienne, les accidents vasculaires cérébraux, l'artérite oblitérante et souvent la mort.

L'oxydation de la LDL provoque sa précipitation sur les parois vasculaires et la formation des cellules spumeuses par les macrophages qui réduisent le diamètre des vaisseaux. Les lésions de la couche de cellules endothéliales mettent à nu le collagène du subendothélium qui devient substrat de l'adhésion des plaquettes.
Le rétrécissement des anneaux aortiques dont le diamètre est normalement de l'ordre de 25 mm est une des causes de l'infarctus et de l'hypertrophie cardiaque.

Un article récent publié dans le British Journal of Pharmacology **153,** 1587-1588 de mai 2008 décrit l'action protectrice de l'acide lipoïque sur les cellules endothéliales vasculaires.

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L'article décrit les modes d'action et les effets bénéfiques de l'acide lipoïque sur les anneaux aortiques de rats et la production de glutathion réduit in situ.

L'acide lipoïque est un anti-inflammatoire et un anti-radicalaire puissant qui induit un effet vasorelaxant par augmentation de la production de NO par les cellules endothéliales.
L'article cité montre que non seulement cette molécule protectrice active la phosphorylation de la NO-synthase mais qu'elle réduit aussi le taux de triglycérides.

Nous pensons que l'association d'acide lipoïque et de L-arginine, connu pour son action vasorelaxante, ne peut qu'être bénéfique dans le combat mené contre les risques de maladie vasculaire.

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COMMENTARY

Lipoic acid supplementation and endothelial function

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Endothelial dysfunction is caused by all the recognized cardiovascular risk factors and has been implicated in the complex processes leading to the initiation and progression of atherosclerosis. Short-term treatment with lipoic acid is shown in the current issue of the *British Journal of Pharmacology* to improve endothelial function of aortic rings of old rats. The age-related decrease in phosphorylation of nitric oxide synthase and Akt was improved by lipoic acid supplementation. The improved phosphorylation status may have been due to reduced activity of the phosphatase PPA2, associated with decreased levels of endothelial ceramide induced by lipoic acid. Neutral sphingomyelinase activity was also reduced by lipoic acid, which was due, at least in part, to increased glutathione levels in endothelial cells. The favourable antioxidant, anti-inflammatory, metabolic and endothelial effects of lipoic acid shown in rodents, in this and other recently published studies, warrant further assessment of its potential role for prevention and treatment of cardiovascular diseases.
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Keywords: lipoic acid; antioxidants; endothelial function; nitric oxide; glutathione; ceramide; sphingomyelinase; Akt phosphorylation; phosphatase activity

Endothelial dysfunction predisposes to the initiation and progression of atherosclerosis, and its multiple devastating clinical consequences, including myocardial infarction, stroke and death. All the recognized cardiovascular risk factors, smoking, dyslipidemia, diabetes and hypertension, have been shown to cause endothelial dysfunction. Classes of medications that provide major clinical cardiovascular benefits, such as statins and angiotensin-converting enzyme inhibitors, have also been shown to improve endothelium-dependent vasorelaxation. The residual cardiovascular risk of patients with coronary artery disease, despite chronic treatment with these highly useful drugs, entirely justifies the search for additional therapeutic approaches to be administered in addition to standard care. Intensely investigated pharmacological targets include oxidative stress and inflammatory pathways (Tardif, 2006; Moubayed *et al*., 2007).

In this issue of the journal, Smith *et al*. (2008) report the effects of the antioxidant lipoic acid on endothelial function in Fischer 344 × Brown Norway rats that are typically used in aging studies. Lipoic acid is shown in this study to improve, but not completely normalize, acetylcholine-induced vasorelaxation of aortic rings of old rats. Smith *et al*. also show that lipoic acid supplementation improves age-related decrease in phosphorylation of nitric oxide synthase and Akt. The improved phosphorylation status may have been due to reduced activity of the phosphatase PPA2, associated with decreased levels of endothelial ceramide induced by lipoic acid. Although there may be more than one pharmacological mechanism underlying reduced ceramide levels (such as potential changes in *de novo* synthesis), lipoic acid also reduced neutral sphingomyelinase activity in old rats. While the activity of endothelial ceramidase is reported by the authors not to be altered by age or lipoic acid, the enzyme ceramide synthase responsible for *de novo* synthesis of ceramide was not evaluated in this study. The lipoic acid-induced reduction in neutral sphingomyelinase activity by 30% was probably due, at least in part, to increased glutathione levels in endothelial cells, as supplementation with glutathione monoethylester also reduced this activity by 25%. Old rats treated with lipoic acid had higher levels of reduced glutathione and a trend for a higher glutathione redox ratio compared with untreated animals of similar age. As observed with lipoic acid, administration of glutathione monoethylester also restored partially the age-related loss in phosphorylation of nitric oxide synthase and Akt.

The short duration of treatment is a limitation of the current study, as acknowledged by the authors. Nevertheless, the improved endothelial function induced by 24 h of therapy may in part explain the anti-atherosclerotic effects of lipoic acid after more prolonged supplementation in genetically modified mice models (Zhang *et al*., 2008). An important question not entirely resolved by the current study is the mechanism of action of lipoic acid, and particularly to what degree its antioxidant properties

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mediated the beneficial effect on endothelial function. The assessment of oxidative stress was indeed very limited in this study. Furthermore, lipoic acid has been shown in other animal studies to have anti-inflammatory effects such as the ability to reduce adhesion molecules and chemokines, to lower serum triglycerides and to activate the phosphoinositide 3-kinase/Akt-signalling pathway leading to reduced activation of nuclear factor-kappa B, a key proinflammatory transcription factor (Zhang and Frei, 2001; Zhang *et al*., 2007). Lipoic acid has also been reported to have 'anti-obesity' effects in genetically modified mice (Zhang *et al*., 2008), but weight changes were not reported in the current study probably due to the short duration of treatment.

The most important question, however, is what these recent findings with lipoic acid in preclinical studies can mean ultimately for primary and secondary prevention of cardiovascular diseases in the clinical setting. Although oxidative stress and inflammation are involved in the atherosclerotic process, much remains to be learned about the clinical effects of medications with antioxidant and/or anti-inflammatory properties in patients with coronary heart disease. Atherosclerosis is now indeed understood to be a chronic inflammatory disease characterized by excess accumulation of monocyte-derived macrophages within the arterial wall (Ross, 1999). However, the protective cardiovascular effects of medications primarily targeting inflammatory pathways remain to be demonstrated in patients (Moubayed *et al*., 2007). Compelling evidence also points to oxidative stress as an important trigger in the complex chain of events leading to the initiation and progression of atherosclerosis (Kunsch and Medford, 1999). While prospective epidemiological studies have supported a protective role for antioxidant vitamins in cardiovascular diseases, results of randomized clinical trials have been disappointing (Tardif, 2006). There are however potentially important problems associated with the use of these vitamins, which include their potential pro-oxidant effects (Bowry *et al*., 1992). This may explain the worsening of endothelium-dependent vasodilation with high-dose α-tocopherol (Keaney *et al*., 1994), and the negative results of the vitamin arms of several clinical trials. Observations made with antioxidant vitamins cannot however be directly extrapolated to lipoic acid supplementation.

Clinical evaluation of other chain-breaking antioxidants demonstrates the complex process that lipoic acid should undergo before being used clinically for cardiovascular protection. The synthetic antioxidant probucol has been shown to reduce post-angioplasty re-stenosis (Tardif *et al*., 1997), but its effects on carotid and femoral atherosclerosis have been conflicting ((Tardif, 2006). The antioxidant succinobucol (AGI-1067), a probucol derivative (Tardif *et al*., 2003), was recently shown to reduce the composite of hard atherosclerosis-related outcomes (cardiovascular death, myocardial infarction and stroke) in a clinical trial (ARISE) of more than 6000 patients with a recent acute coronary syndrome, but the finding for this pre-specified secondary endpoint will require confirmation because the antioxidant did not alter the incidence of the combined primary endpoint that also included unstable angina and coronary revascularization (Tardif *et al*., unpublished data). The path to full clinical validation of the favourable results obtained experimentally with lipoic acid will be long and include determination of the minimal dosage required to induce vascular, metabolic and/or anti-inflammatory effects in patients, as well as demonstration of safety and tolerability of pharmacological doses.

In summary, the favourable antioxidant, anti-inflammatory, metabolic and endothelial effects of lipoic acid in rodents warrant further assessment of its potential role for prevention and treatment of cardiovascular diseases.

References

Bowry VW, Ingold KU, Stocker R (1992). Vitamin E in human low-density lipoprotein: when and how this antioxidant becomes a pro-oxidant. *Biochem J* **288**: 341–344.

Keaney Jr JF, Gaziano JM, Xu A, Frei B, Curran-Celentano J, Shwaery GT *et al*. (1994). Low-dose alpha-tocopherol improves and high-dose alpha-tocopherol worsens endothelial vasodilator function in cholesterol-fed rabbits. *J Clin Invest* **93**: 844–851.

Kunsch C, Medford RM (1999). Oxidative stress as a regulator of gene expression in the vasculature. *Circ Res* **85**: 753–766.

Moubayed SP, Heinonen T, Tardif JC (2007). Anti-inflammatory drugs and atherosclerosis. *Curr Opin Lipidol* **18**: 638–644.

Ross R (1999). Atherosclerosis—an inflammatory disease. *N Engl J Med* **340**: 115–126.

Smith AR, Visioli F, Frei B, Hagen TM (2008). Lipoic acid significantly restores, in rats, the age-related decline in vasomotion. *Br J Pharmacol* **153**: 1615–1622 (this issue).

Tardif JC (2006). Antioxidants: the good, the bad and the ugly. *Can J Cardiol* **22** (Suppl B): 61B–65B.

Tardif JC, Côté G, Lespérance J, Bourassa M, Lambert J, Doucet S *et al.* (1997). Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. *N Engl J Med* **337**: 365–372.

Tardif JC, Gregoire J, Schwartz L, Title L, Laramée L, Reeves F, *et al.*, Canadian Antioxidant Restenosis Trial (CART-1) Investigators (2003). Effects of AGI-1067 and probucol after percutaneous coronary interventions. *Circulation* **107**: 552–558.

Zhang WJ, Bird KE, McMillen TS, LeBoeuf RC, Hagen TM, Frei B (2008). Dietary alpha-lipoic acid supplementation inhibits atherosclerotic lesion development in apolipoprotein E-deficient and apolipoprotein E/low-density lipoprotein receptor-deficient mice. *Circulation* **117**: 421–428.

Zhang WJ, Frei B (2001). Alpha-lipoic acid inhibits TNF-induced and NF-kB activation and adhesion molecule expression in human aortic endothelial cells. *FASEB J* **15**: 2423–2432.

Zhang WJ, Wei H, Hagen T, Frei B (2007). Alpha-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. *Proc Natl Acad Sci USA* **144**: 4077–4082.

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Lipoic acid significantly restores, in rats, the age-related decline in vasomotion

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Abstract

Background and purpose: The age-related decline in vasorelaxation is largely due to ceramide-induced induction of phosphatase 2A (PP2A), which limits nitric oxide synthase (eNOS) phosphorylation at stimulatory sites. We hypothesized that ceramide accumulation was from an age-related loss of endothelial glutathione (GSH) and subsequent activation of neutral sphingomyelinase (nSMase), an enzyme whose activity increases when GSH is limited.

Experimental approach: Old (30-32 mo) F344xBN rats were given (*R*)-α-lipoic acid (LA), an agent known to induce GSH synthesis. Vasorelaxation was measured in aortic rings; GSH and ceramide levels, activity of nSMase and eNOS phosphorylation (by Western blot) was measured in aortic endothelial cells, isolated from the same aortas.

Key results: In old animals, endothelium-dependent relaxation in aortic rings was decreased, GSH levels and its redox state in aortic endothelia were over 30% lower and nSMase activity and endothelial ceramide levels were three-fold increased, relative to young (2-4 mo) rats. LA treatment of old animals improved relaxation in aortic rings, reversed the changes in endothelial GSH, in nSMase activities and in ceramide levels. Similar effects on GSH levels and nSMase activity in old rats were also induced by treatment with GSH monoethylester. Activation (by phosphorylation) of eNOS was decreased by about 50% in old rats and this age-related decrease was partially reversed by LA treatment.

Conclusions and implications: Decreased endothelial GSH was partly responsible for the age-related loss of vascular endothelial function and LA might be therapeutically evaluated to treat endothelial dysfunction.

Abbreviations:

eNOS, endothelial nitric oxide synthase; GSH, glutathione; GSSG, oxidized glutathione; GE, glutathione monoethylester; PP2A, protein phosphatase 2A; SMase, sphingomyelinase; LA, (*R*)-α-lipoic acid