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Short Communication

Poloxamer 407 as a general lipase inhibitor: its implications in lipid metabolism and atheroma formation in C57BL/6 mice

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Abstract

Objectives The aims of this investigation were to determine if the activity of endothelial lipase was inhibited *in vitro* by the polymeric surfactant, poloxamer 407 (P-407), and to review the action of P-407 on the biological activity of several other critical lipases involved in lipid metabolism. The overall goal was to suggest a possible explanation for the observed dyslipidaemia and formation of aortic atherosclerotic lesions when present and previous findings, along with key findings proposed by others, were combined and reviewed in the context of the P-407-induced mouse model of atherogenesis.

Key findings Endothelial lipase was expressed using a recombinant adenovirus; subconfluent COS cells were exposed to recombinant adenoviruses and triglyceride lipase activity was determined using standard assay methods. It was demonstrated that P-407 inactivates endothelial lipase *in vitro*. Endothelial lipase was inhibited *in vitro* by P-407, with an IC50 of approximately 11.3 μM. It is suggested that one possible explanation for the eventual formation of aortic atherosclerotic lesions in the P-407-induced mouse model of atherogenesis may be related to the capacity of P-407 to inhibit the activity of several critical lipases involved in lipid metabolism.

Conclusions The following physiological and biochemical processes are all observed in the P-407-induced mouse model of dyslipidaemia and atherosclerosis: inactivation of endothelial lipase (both *in vitro* and *in vivo*), hepatic lipase and lipoprotein lipase by P-407; reduced plasma levels of high-density lipoprotein cholesterol; no activation of either human or mouse peroxisome proliferator-activated receptor α (as well as peroxisome proliferator-activated receptor γ) *in vitro* or *in vivo*; increased plasma concentrations of soluble vascular cell adhesion molecule 1, soluble intercellular adhesion molecule 1 and soluble E-selectin; and aortic atherosclerotic lesion formation following 12–16 weeks of P-407 administration (0.5 g/kg administered every 3 days by intraperitoneal injection). This biochemical-based pathway, or series of events, may contribute, in part, to the dyslipidaemia and eventual formation of aortic atherosclerotic lesions observed in the P-407-induced mouse model of atherogenesis.

Keywords atherosclerosis; cellular adhesion molecules; lipase; poloxamer 407; peroxisome proliferator-activated receptor

Introduction

Based primarily on epidemiologic studies, it has been known for many years that there is a very strong inverse relationship between the plasma concentration of high-density lipoprotein (HDL) cholesterol and cardiovascular events.[1,2] Typically, the atheroprotective benefits of achieving a plasma HDL concentration greater than 60 mg/dl are linked to the role of HDL in reverse cholesterol transport and the efflux of cholesterol from foam cells that reside in aterial plaque. Recently, it has been suggested that HDL may possess anti-inflammatory properties that may also contribute to the inverse relationship between plasma HDL concentrations and the development of atherosclerosis. One of the earliest steps in the atherogenic cascade is adhesion molecule expression and monocyte adhesion to endothelial cells. HDL has been shown to exert an anti-inflammatory effect by inhibiting this process.[3–5] The plasma concentrations of soluble cell adhesion molecules are predictive of future cardiovascular risk, while physiologic concentrations of HDL can inhibit cytokine-induced

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expression of endothelial adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and E-selectin.[4,5]

To date, the mechanisms responsible for the anti-inflammatory effects of HDL are poorly understood. It has been suggested that HDL may limit the effects of pro-inflammatory mediators by either physical binding or active catalysis. This suggestion has been advanced because of lipid hydroperoxide destruction by paraoxonases present in HDL particles.[6,7] It has been reported that HDL inhibits endothelial cell sphingosine kinase, resulting in decreased nuclear factor-κB activation.[8] Additionally, HDL may also increase nitric oxide levels.[9] However, very little is currently known concerning HDL-dependent pathways in the endothelium that would account for the ability of HDL to regulate transcription and specifically repress adhesion molecule expression. Recently, progress has been made in defining the HDL/endothelial interaction.[10] Ahmed *et al*.[10] recently reported that inhibition of endothelial leukocyte adhesion by HDL was blocked in the presence of the general lipase inhibitor tetrahydrolipstatin. These authors reported evidence for specific mechanisms through which lipoprotein metabolism can regulate distinct transcriptional responses through peroxisome proliferator-activated receptors (PPARs),[11,12] ligand-activated nuclear receptors involved in the transcriptional regulation of metabolism, inflammation and atherosclerosis.[13,14] For example, their research demonstrated that very-low-density lipoprotein (VLDL) hydrolysis by lipoprotein lipase (LPL) can generate peroxisome proliferator-activated receptor α (PPARα) ligands.[11] It has also been shown by others that LPL-treated VLDL can activate PPARδ in macrophages.[15] Findings by Ahmed *et al*.,[10] Ziouzenkova *et al*.,[11,12] Wilson *et al*.,[13] Plutzky,[14] Chawla *et al*.[15] and Marx *et al*.[16] appear to suggest a heretofore underappreciated role for lipoproteins as transcriptional regulators, with effects dictated by the nature of the lipoprotein particle and its uptake, including the biology of specific lipases.[10] As suggested by Ahmed *et al*.,[10] even though synthetic PPAR agonists have been described as decreasing adhesion molecule expression,[16–18] endogenous mechanisms for PPAR activation remain poorly understood despite their potential molecular and clinical implications.

Additionally, Ahmed *et al*.[10] reported that endothelial lipase (EL), a unique member of the triacylglycerol lipase family expressed in the endothelium, limits VCAM-1 expression by activating PPARs through hydrolysis of HDL. These authors established and characterized HDL hydrolysis by EL as a distinct, specific lipolytic pathway in the endothelium that preferentially activates PPARα, regulates VCAM-1 expression and can limit leukocyte adhesion.[10] Thus, our laboratory was extremely interested in how another general lipase inhibitor, poloxamer 407 (P-407), affects EL activity *in vitro*, PPAR activation (using a cell-based transactivation assay), and plasma concentrations of soluble cell adhesion molecules (sVCAM-1, sICAM-1 and sE-selectin), following P-407 administration to mice.

P-407 is a polymeric, nonionic surfactant that has been reported to cause dyslipidaemia in C57BL/6 mice of either sex.[19] The degree of dyslipidaemia that occurs in P-407-treated mice is dose-dependent.[20] Plasma LDL concentrations are increased, in part, by down-regulation in the protein expression of LDL receptors.[21] Long-term treatment (12–16 weeks) of mice with P-407 causes the formation of fibrofatty aortic atherosclerotic lesions that are comparable in size and number with those observed in classic diet-induced mouse models of atherogenesis.[22,23] The P-407-induced mouse model of dyslipidaemia and atherosclerosis is a well-documented and well-characterized mouse model of atherogenesis.[22,23] The introduction of P-407 into the peritoneal cavity of mice is virtually non-toxic, as the P-407 is merely absorbed and slowly cleared by renal elimination and not metabolized. Thus, this chemically induced mouse model of dyslipidaemia has a very favourable safety profile and represents an alternative mouse model of atherogenesis to the cardiovascular research community. Examples of several other popular genetically and nongenetically modified models include, but are not limited to, the apoE- and LDLr-knockout models and diet-induced models,[24,25] respectively.

The aim of the present study was to investigate whether P-407 affects the biological activity of EL *in vitro* in an effort to determine if this hyperlipidaemic agent (P-407) could potentially interfere with EL-mediated hydrolysis of HDL, and, in so doing, play a potential role in atheroma formation observed in the P-407-induced mouse model of atherosclerosis. After determining whether P-407 affected the biological activity of EL *in vitro*, the findings were then combined with previously acquired data to create a brief review of how P-407 affects the activity of critical lipases involved in lipid metabolism, and how this information might serve to provide a more complete and thorough understanding of the mechanisms that contribute to dyslipidaemia in the P-407-induced mouse model of atherogenesis.

Discussion

Clearly, the copolymer surfactant P-407 is a general lipase inhibitor. This has very important implications for the resulting dyslipidaemia and atherosclerotic lesion formation in the P-407-induced mouse model of atherogenesis. Returning to the work of Ahmed *et al*.,[10] the authors hypothesized that EL, a unique member of the triacylglycerol lipase family expressed in the endothelium, limits VCAM-1 expression by activating PPARs through HDL hydrolysis. They presented evidence establishing and characterizing HDL hydrolysis by EL as a distinct, specific lipolytic pathway in the endothelium that preferentially activates PPARα, regulates VCAM-1 expression, and can limit leukocyte adhesion. However, in this review of the effects of P-407 on lipases, we have shown that P-407 not only inhibits the activity of EL, but that it also inhibits the biological activity of a number of other lipases (Figure 1). Lipases evaluated to date include LPL, hepatic lipase (HL) and pancreatic lipase (PL). It is noteworthy that the values of the IC50s of three of the lipases, specifically EL, PL and LPL, were all in fairly close agreement. The values of the IC50s for EL, PL and LPL were 11.3 μM, 15.9 μM and 24.0 μM, respectively,[26,27] and fall within the micromolar concentration range of P-407 and those concentrations observed *in vivo* (from 158 μM in liver to 317 μM in plasma)[28] (Figure 2). Although we have not determined the IC50 for HL, nevertheless the activity of HL was strongly inhibited in post-heparin plasma following P-407 administration to rodents

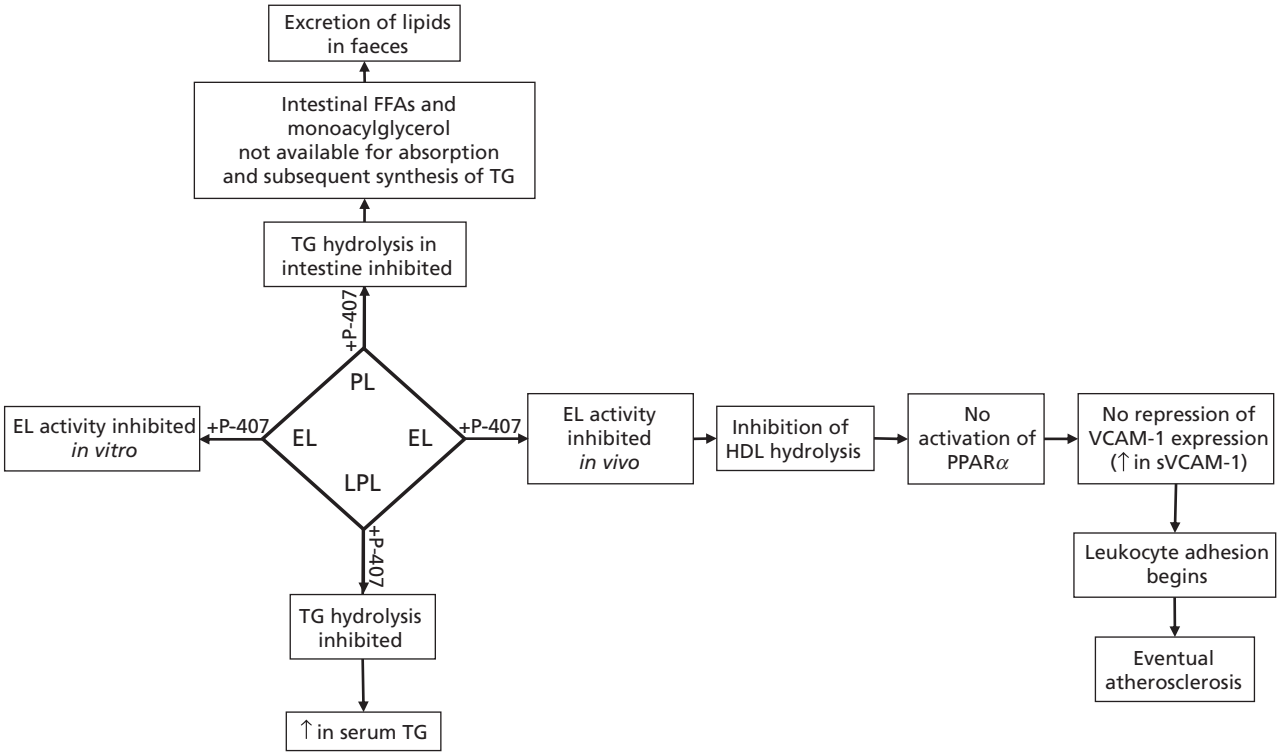

Figure 1 Biochemical cascade of events following incubation of key enzymes involved in lipid metabolism by poloxamer 407. EL, endothelial lipase; FFAs, free fatty acids; HDL, high-density lipoprotein; LPL, lipoprotein lipase; P-407, poloxamer 407; PL, pancreatic lipase; PPARα, peroxisome proliferator-activated receptor α; TG, triglyceride; VCAM-1, vascular cell adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1.

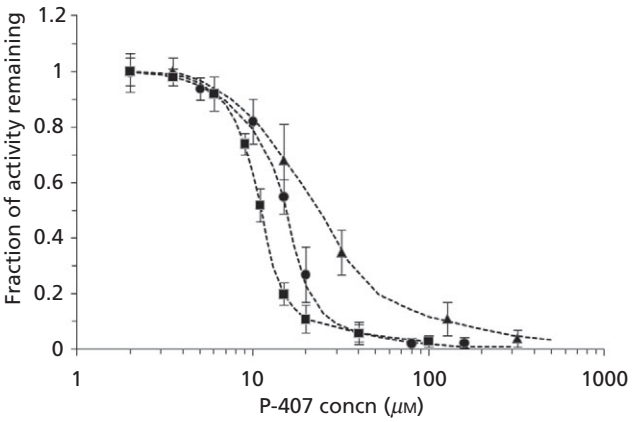

Figure 2 Effect of poloxamer 407 on the biological activity of endothelial lipase, lipoprotein lipase and pancreatic lipase *in vitro*. ■, Endothelial lipase (EL); ▲, lipoprotein lipase (LPL); ●, pancreatic lipase (PL). Data points represent the mean ± SD, $n = 3$. The IC50s of 11.3, 24.0 and 15.9 μM for EL, LPL and PL, respectively, were estimated from the dashed lines drawn through the data points. The dashed lines do not represent a mathematical fit of the actual data points. The methods for the expression of endothelial lipase and the triglyceride lipase assay utilized for EL are described in detail elsewhere.[29] The data for PL inhibition *in vitro* has been reproduced/adapted, with permission, from reference 27. The data for LPL inhibition *in vitro* was reproduced, with permission, from reference 26.

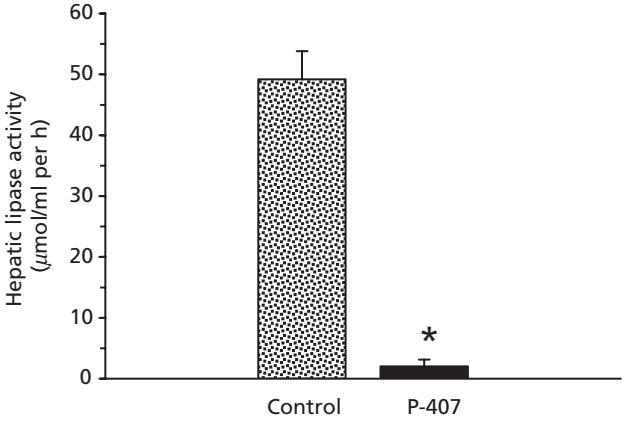

Figure 3 Biological activity of hepatic lipase contained in post-heparin plasma of rats at 24 h after a single 300-mg injection of poloxamer-407 or normal saline. HL, hepatic lipase. Values are the means ± SEM of at least three animals per group. *$P < 0.001$, the mean HL activity for poloxamer-407 treated rats was significantly less than the corresponding mean value of the HL activity determined for the saline treated rats (control). Reproduced, with permission, from reference 30.

(Figure 3).[30] The biological activity of HL in post-heparin plasma 24 h after the administration of P-407 to rats was approximately 4% of the HL activity in post-heparin plasma obtained from saline-treated controls (Figure 3). Additionally, the effect of P-407 on heparin-releasable LPL in plasma

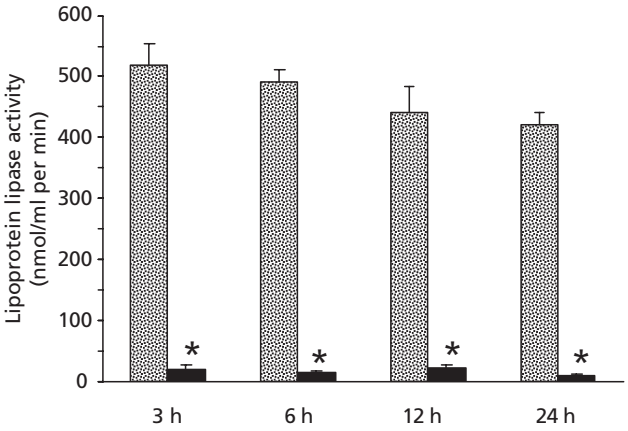

Figure 4 Biological activity of lipoprotein lipase contained in post-heparin plasma of rats at various times following a single 300-mg injection of poloxamer-407 or normal saline. Solid bars, poloxamer-407; dotted bars, normal saline. Values are the means ± SEM of at least five animals per group. All mean lipoprotein lipase activity values were significantly ($P < 0.001$) less than the corresponding mean values of the activities determined for saline treated controls. Reproduced, with permission, from reference 26.

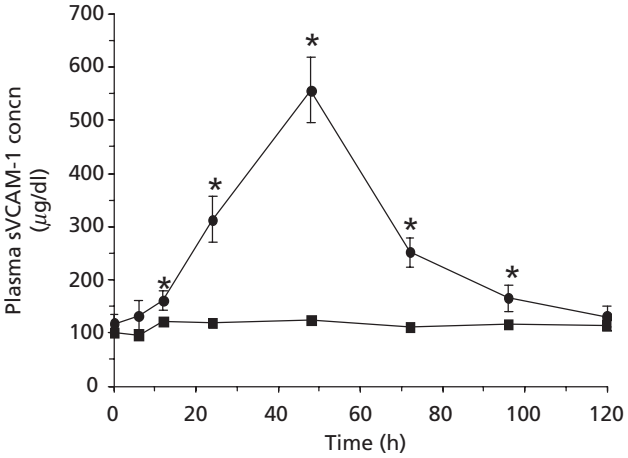

Figure 5 Effect of poloxamer-407 on plasma soluble vascular cell adhesion molecule 1 concentrations following administration of either saline or poloxamer-407 to C57BL/6 mice. sVCAM-1, soluble vascular cell adhesion molecule 1. ■, Saline; ●, 0.5 g/kg poloxamer-407. Data represent the mean ± SD. *$P < 0.05$, significant increase in the mean plasma sVCAM-1 concentration when individually compared with the corresponding mean plasma concentrations of sVCAM-1 in saline-injected (control) mice at each time point. Reproduced/adapted, with permission, from reference 34.

demonstrated that LPL activity was inhibited more than 90% compared with controls for 24 h following a single dose of P-407 to rats (Figure 4).[26]

The implications that inhibition of these lipases have with regard to atherosclerotic lesion formation in this model will now be discussed in the context of the hypothesis advanced by Ahmed *et al*.[10] If P-407 inhibits the enzymatic activity of EL similar to what was observed with tetrahydrolipstatin, then EL cannot hydrolyse HDL, as suggested by Ahmed *et al*.[10] They went on to demonstrate that because EL activity was inhibited by tetrahydrolipstatin, it lost its capacity to hydrolyse HDL. In so doing, HDL could not act as a potent activator of PPARα and repress the expression of VCAM-1. Therefore, leukocyte adhesion to tumour necrosis factor α-stimulated human endothelial cells occurred.[10] Perhaps if they had chronically administered tetrahydrolipstatin to wild-type mice, similar to what we previously performed with P-407, atheroma formation might have occurred, although this is mere speculation.

Using the copolymer P-407 in the present investigation, we have shown that the activity of EL is inhibited *in vitro*. Therefore, similar to the effects of tetrahydrolipstatin, P-407 presumably renders EL unable to hydrolyse HDL *in vitro*. Very recently, we have also shown that P-407 administration to both C57BL/6 mice and EL-deficient knockout ($EL^{-/-}$) mice results in inactivation of EL *in vivo* as well.[31] In that work, we speculated that the presence of EL protein in P-407-treated wild-type mice, even if catalytically inactive, may, through its bridging function, promote the clearance of HDL from the plasma, and partly contribute to the low HDL cholesterol levels in wild-type mice reported in this mouse model of dyslipidaemia.[31] The EL gene transfer data in our previous work[31] lends credence to this assumption. When wild-type EL was overexpressed in P-407-treated mice, HDL cholesterol levels diminished to less than 3.9 mg/dl (0.1 mmol/l), suggesting that P-407 treatment did not completely inhibit exogenously produced excess EL activity.[31]

We have previously shown that P-407 is unable to activate PPARα both in an *in vitro* cell-based transactivation assay and *in vivo* using both wild-type and PPARα-deficient mice (data not shown).[32,33] Thus, if the effect of P-407 is assumed to be similar to tetrahydrolipstatin with regard to HDL hydrolysis (i.e. no HDL hydrolysis, due to inactivation of EL), then PPARα would not be activated *in vivo*. Without hydrolysed HDL to stimulate PPARα activation *in vivo*, which in turn represses the expression of VCAM-1, then VCAM-1 expression should be upregulated (as well as the concentration of the soluble, shedded form of VCAM-1 in the plasma, i.e. sVCAM-1) and, therefore, leukocyte adhesion should be enhanced (Figure 1). With continued leukocyte adhesion, atheroma formation would be expected to eventually occur. In summary, in the P-407-induced mouse model of dyslipidaemia and atherosclerosis, EL activity is inhibited *in vitro* (present investigation) and *in vivo*,[31] both human and mouse PPARα (as well as PPARγ) are neither activated nor inhibited *in vitro* and *in vivo*,[31–33] plasma sVCAM-1 (as well as plasma sICAM-1 and sE-selectin) concentrations significantly increase to a maximum in mice 48 h after a single dose of P-407 (Figure 5),[34] and, if treated for 12–16 weeks with P-407, C57BL/6 mice of either sex develop aortic atherosclerotic lesions in the same size and numbers as those observed in classic diet-induced mouse models of atherogenesis.[19,22,23]

Another concept discussed by Ahmed *et al*.[10] was that they had previously reported that LPL acts on triglyceride-rich lipoproteins, such as VLDL, to also potently activate PPARα. Since we have previously shown that P-407 inhibits LPL,[26] then LPL would not be able to hydrolyse VLDL (of which there is an abundance in the P-407-induced mouse model of dyslipidaemia and atherosclerosis) and, therefore, could not

potently activate PPARα and subsequently repress VCAM-1 expression. This should presumably lead to increased VCAM-1 expression, as seen by an increase in the plasma concentration of sVCAM-1 (Figure 5),[34] and, subsequently, lead to eventual atherosclerotic lesion formation in P-407-treated wild-type mice. These findings (inhibition of LPL,[26] no activation of PPARα[32] and increased expression of VCAM-1, as reflected by an increase in plasma sVCAM-1[34]) and aortic atherosclerotic lesion formation (after 12–16 weeks)[18,22,23] are all observed in mice treated with P-407.

Conclusions

It has been shown that EL is inactivated *in vitro* in the present investigation. According to one hypothesis advanced by Ahmed *et al*.,[10] the inhibition in activity of both EL and LPL by P-407 does not result in the hydrolysis of HDL and VLDL, respectively, and, thus, PPARα is not potently activated by the dual hydrolytic action of EL and LPL. This, in turn, does not result in the inhibition of VCAM-1 expression and may potentially initiate the atherogenic cascade, wherein leukocytes adhere to the vascular endothelial cells. With continued leukocyte adherence, the formation of atherosclerotic lesions is accelerated. All of these physiological- and biochemical-based processes (inactivation of EL both *in vitro* and *in vivo*, HL and LPL by P-407, reduced plasma levels of HDL cholesterol, no activation of either human or mouse PPARα (as well as PPARγ) *in vitro* or *in vivo*, increased plasma concentrations of sVCAM-1 (as well as sICAM-1 and sE-selectin), and aortic atherosclerotic lesion formation after 12–16 weeks) are observed in the P-407-induced mouse model of dyslipidaemia and atherosclerosis. Future work will investigate whether leukocyte adherence is greater when P-407 is used to inhibit the capacity of EL to hydrolyse HDL, which in turn cannot stimulate PPARα and repress leukocyte adhesion.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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