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Lysophosphatidic acid and atotaxin in patients with cholestasis and pruritus: Fine biology, anticipated discernment

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Article commented

Kremer AE, Martens JJ, Kulik W, Rueff F, Kuiper EM, van Buuren HR, van Erpecum KJ, Kondrackiene J, Prieto J, Rust C, Geenes VL, Williamson C, Moolenaar WH, Beuers U, Oude Elferink RP. Lysophosphatidic acid is a potential mediator of cholestatic pruritus. *Gastroenterology* 2010; 139: 1008-18.

Original abstract

Background & aims. Pruritus is a common and disabling symptom in cholestatic disorders. However, its causes remain unknown. We hypothesized that potential pruritogens accumulate in the circulation of cholestatic patients and activate sensory neurons. **Methods.** Cytosolic free calcium ([Ca(2+)] (i)) was measured in neuronal cell lines by ratiometric fluorometry upon exposure to serum samples from pruritic patients with intrahepatic cholestasis of pregnancy (ICP), primary biliary cirrhosis (PBC), other cholestatic disorders, and pregnant, healthy, and nonpruritic disease controls. Putative [Ca(2+)] (i)-inducing factors in pruritic serum were explored by analytical techniques, including quantification by high-performance liquid chromatography/mass spectroscopy. In mice, scratch activity after intradermal pruritogen injection was quantified using a magnetic device. **Results.** Transient increases in neuronal [Ca(2+)] (i) induced by pruritic PBC and ICP sera were higher than corresponding controls. Lysophosphatidic acid (LPA) could be identified as a major [Ca(2+)] (i) agonist in pruritic sera, and LPA concentrations were increased in cholestatic patients with pruritus. LPA injected intradermally into mice induced scratch responses. Autotaxin, the serum enzyme converting lysophosphatidylcholine into LPA, was markedly increased in patients with ICP *vs.* pregnant controls ($P < 0.0001$) and cholestatic patients with *vs.* without pruritus ($P < 0.0001$). Autotaxin activity correlated with intensity of pruritus ($P < 0.0001$), which was not the case for serum bile salts, histamine, tryptase, substance P, or mu-opioids. In patients with PBC who underwent temporary nasobiliary drainage, both itch intensity and autotaxin activity markedly decreased during drainage and returned to preexistent levels after drain removal. **Conclusions.** We suggest that LPA and autotaxin play a critical role in cholestatic pruritus and may serve as potential targets for future therapeutic interventions.

Key words. Itch. Scratching behavior. Animal models. Cholestasis. Phospholipids.

Comment

Pruritus is a complication of cholestasis. It can be severe leading to sleep deprivation and to suicidal ideations. It is inferred that the pruritogen(s) that mediate this form of pruritus is made in the liver, excreted in bile, and that, as a result of cholestasis (i.e. impaired secretion of bile), it accumulates in plasma and other tissues, causing pruritus. In support of a liver origin of the pruritogen(s) is the decrease or resolution of pruritus in patients who progress to liver failure, in the course of chronic li-

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ver disease (e.g. primary biliary cirrhosis),[1] and the disappearance of pruritus after liver transplantation.[2] The idea that the pruritogen(s) is excreted in bile is supported by the resolution of pruritus after the relief of extrahepatic biliary obstruction.[3] Intractable pruritus is an indication for liver transplantation, even when synthetic function is preserved, because of the marked negative impact that this symptom has on the quality of life of patients. Thus, studies that shed light on the pathogenesis of this terrible symptom are extremely relevant. In this regard, in a study entitled *Lysophosphatidic acid is a potential mediator of cholestatic pruritus* by Kremer, *et al*., published in the September issue of the journal Gastroenterology, the authors report that lipophosphatidic acid (LPA) is a potential mediator of the pruritus of cholestasis.[4] The hypothesis on which the study was reported to be based was that potential pruritogens accumulate in the circulation of cholestatic patients and activate sensory neurons.[4]

Lysophosphatidic acid (LPA) is a bioactive phospholipid produced extracellularly by the action of autotaxin (ATX), a plasma enzyme with lysophospholipase activity, on lysophospholipids.[5] LPA functions through specific G protein coupled receptors.[5,6] LPA participates in several biological processes including cell proliferation, survival, migration, adhesion, and differentiation, and in pathophysiological processes including platelet aggregation, wound healing, vasopressor activity, and angiogenesis.[5,6] In animal studies, it has been reported that LPA induces nociception through substance P release from peripheral nerve endings,[7] and it has been implicated as a mediator in animal and *in vitro* neural models that explore signals associated with pain.[8-10]

Changes in cytosolic calcium $[Ca^{2+}]$ i is a measure of neural activation.[11] SH-SY5Y is a human neuroblastoma cell line.[12] Kremer, *et al*.,[4] investigated the effects of human serum on $[Ca^{2+}]$ i in suspensions of cultured SH-SY5Y. The authors report that the addition of serum from patients with cholestasis and pruritus to a SH-SY5Y suspension was associated with an increase in $[Ca^{2+}]$ i greater than that associated with the addition of serum from healthy controls, suggesting neuronal activation by the biological preparations. The authors report that the addition of serum from pregnant women with cholestasis and pruritus to the cellular suspension was associated with a greater change in the concentration of $[Ca^{2+}]$ i than that secondary to the addition of sera from pregnant women without that complication, and also, that the addition of serum of patients with primary biliary cirrhosis and pruritus to the cell suspension was associated with a greater change in the $[Ca^{2+}]$ i than that secondary to the addition of serum from patients with primary biliary cirrhosis without pruritus.[4] It is not reported whether the samples where pooled, and is so, how, or whether they were individually tested for their effect on $[Ca^{2+}]$ i.

By filtration methods, and subsequently, by lipid extraction, the factor associated with changes in $[Ca^{2+}]$ i was reported to be lipophosphatidic acid (LPA). This interpretation was supported further by the ability of a specific LPA receptor blocker to reduce significantly the change (the term increase in used in the manuscript) in $[Ca^{2+}]$ i in SH-SY5Y, although not to abolish it. The concentration of several LPA species was reported to be significantly higher in the sera from patients with cholestasis of pregnancy, than in the sera from pregnant women matched for gestation term.[4]

The investigators report that activity of ATX, the enzyme that generates LPA.[5] was higher in the serum from women with cholestasis of pregnancy, than in the serum from pregnant women without intrahepatic cholestasis of pregnancy, and from non pregnant control women. It is also reported that ATX activity was significantly higher in the sera of male patients with cholestasis and pruritus, than in the sera of male patients with cholestasis without pruritus, although the number of samples in the latter group was only five.[4] It can be appreciated from the figures, that there is overlap in the serum activity of ATX among all the groups studied although, the activity of ATX in the patients with intrahepatic cholestasis of pregnancy and pruritus, is depicted as being higher than that measured in all other groups. The investigators report that ATX activity correlated significantly with the intensity of itch, as assessed by a visual analogue scale, in contrast to the lack of correlation with the serum concentration of tryptase, histamine, substance P, bile acids, and mu opioid activity. They also report that in patients who underwent nasobiliary drainage for the treatment of intractable pruritus, serum ATX activity decreased along with the pruritus score and increased when the pruritus score increased. It is also reported that ATX was not detected in bile.[4]

Kremer, *et al*., reported that the intradermal injections of LPA and not the administration of the carrier were reported to be associated with scratching behavior in mice, and state that this finding

suggests that ATX and LPA play a causative role in the itch during cholestasis.[4]

The authors conclude that LPA is a potential mediator of itch in cholestasis.[4] They also conclude that endogenous opioids, histamine, bile acids, substance P, and tryptase are not involved in the mediation of the pruritus of cholestasis because the serum concentration, or activity, in the case of opioids, did not correlate with the patients' pruritus scores obtained by the use of a visual analogue scale at the time the blood was drawn for testing.[4]

The identity of the pruritogen(s) that mediates the pruritus of cholestasis is not known. In an effort to identify the pruritogen(s), concentration of substances have been measured in patients of cholestasis; these measures have tended to coincide with the availability of the assays.[13] This approach to the pruritus of cholestasis has not delivered results that have changed the treatment of this symptom. The scientific approach to the pruritus of cholestasis, however, changed after the development of an important observation reported by Thornton and Losowsky in their seminal paper of 1988.[14] In that publication, Thornton and Losowsky reported the development of what they named an opiate withdrawal-like reaction in patients with cholestasis and pruritus, after the administration of oral nalmefene, an opiate antagonist.[14] The patients also experienced relief of their pruritus in association with the administration of nalmefene.[14]

The opiate withdrawal-like reaction that patients with cholestasis and pruritus can experience after the administration of opiate antagonists[14-16] suggests that increased opioidergic tone contributes to the pathophysiology of cholestasis. The relationship between increased opioidergic tone and pruritus and scratching is well documented.[17-20] Thus, the ameliorating effect of opiate antagonists on the pruritus in patients with cholestasis, which has been reproduced in studies conducted in different parts of the world,[14-16, 21-25] some of which included behavioral methodology,[15,16,21,22] supports the hypothesis that the endogenous opioid system mediates, at least in part, the pruritus of cholestasis.[26]

Kremer, *et al*., state that their results question a major causative role of opioids in the pathogenesis of pruritus in cholestasis apparently for two reasons:[4]

- There was no correlation between plasma opioid activity and the sensation of pruritus, as measured by the visual analogue scale; this statement is not substantiated by the reported results because the visual analogue scale is not a reliable method to assess pruritus, and
- Patients with intrahepatic cholestasis of pregnancy did not have increased plasma mu opioid activity, as measured in a study conducted in a rat model of cholestasis,[27] and that only a few patients with primary biliary cirrhosis and pruritus had increased plasma opioid activity.

In this context, it is the behavioral manifestation of increased opioidergic tone, i.e. the opiate withdrawal-like reaction precipitated by opiate antagonists,[14-16] and the amelioration of scratching activity, the behavioral manifestation of pruritus, in association with the administration of opiate antagonists[15,16,21,22] what supports a role of the endogenous opioid system in the mediation of the pruritus of cholestasis, and not the correlation of serum levels of opioids or their activity with the perception of itch. Furthermore, a central mechanism in the mediation of the pruritus of cholestasis is hypothesized;[26] this hypothesis is viable in the absence of any opioid activity measures in the periphery. In regards to the central component of this type of pruritus, a brain scan study that applied SPECT and fMRI methodology in patients with pruritus of cholestasis during periods of itch and no itch has been published in abstract form.[28] Itch was reported not to be associated with sensory cortex activation; increasing itch severity was reported to correlate with activity in the prefrontal cortex, orbital frontal cortex, putamen, globus pallidus, insular cortex, and orbital anterior and posterior cingulated cortices. The pattern of activation suggested to the authors that the limbic system is the primary central nervous system pathway involved in the perception of itch, and stated that the findings supported a central origin for this type of pruritus or itch.[28] This interpretation supports the hypothesis of centrally mediated pruritus in cholestasis.[26]

The lack of edema and erythema that classically accompany histamine associated pruritus in the skin of patients with cholestasis suggests that histamine is not the mediator of the pruritus of cholestasis. The relief of pruritus reported in association with antihistamines by some patients is likely due to sedation.

Kremer, *et al*., report that the lack of correlation between the concentration of serum bile acids and the pruritus scores obtained with the visual analogue scale, does not support a role of these

substances in the mediation of this symptom.[4] A role of bile acids in the mediation of the pruritus of cholestasis has not been confirmed.[13] Beuers, *et al*., concluded that the transient relief of intractable pruritus in association with biliary drainage was independent from serum bile acids concentrations.[29] In this context, the relief of pruritus in association with nasobiliary drainage may be due to a decrease in the degree of cholestasis; the mechanism mediating the relief is not known. We must expect a large placebo effect as the mediator of the relief of pruritus in association with therapeutic interventions.[30] We must also leave room for the unexpected, and, in the study of Kremer, *et al*., the unexpected might have been the movement in serum ATX activity, a decrease and an increase in association with relief and recurrence of pruritus, respectively, in patients who had undergone nasobiliary drainage for the treatment of pruritus. This finding may be a reflection of enzyme activity in different degrees of cholestasis, but cannot be interpreted as being directly related to the pruritus that those patients experienced at the time. These results, however, provide a rationale to explore the behavior of LPA and ATX in cholestasis. The time over which the samples were stored is not documented in the report; this information may be relevant because the concentration of LPA and the activity of ATX may change in storage.

In contrast to the results reported by Kremer, et al, mean substance P concentration was significantly higher in the serum from a group of patients with cholestasis and pruritus, than that in the serum from a group of patients with chronic liver disease without pruritus, and of control subjects.[31] These findings supported the idea that antagonists of the NK1 receptor, to which substance P binds, such as aprepitant, may be useful in the treatment of patients with pruritus from cholestasis.[31] A study published on line reports that the administration of aprepitant was associated with a decreased in pruritus, assessed by the visual analogue scale, in a heterogeneous group of patients with chronic pruritus;[32] this study, however, requires confirmation in controlled clinical trials. The difference between the serum concentrations of substance P between the two studies may be explained[4,31] by the differences in the assays used to measure the peptide, and by the pattern of substance P secretion.

The identification of substances as potential mediators of the pruritus of cholestasis must be followed by behavioral studies that explore the connection between the identified substance(s) and the sensation of pruritus, and its behavioral manifestation, scratching.

Kremer, *et al.,* confirmed that the administration of LPA could be associated with scratching in laboratory animals.[4,33] LPA was associated with scratching behavior on C57BL/6J female mice.[4] It is not reported why the authors used this breed of mice. The relevance of this result to the scientific study of the pruritus of cholestasis is not apparent, as scratching behavior in association with the intradermal injection of substances, including LPA, is not a model of scratching in cholestasis.

The study of Kremer, *et al*., provides impetus to explore the biology of LPA and ATX in liver disease; however, the data reported do not give insight into the pathogenesis of the pruritus of cholestasis. The authors are commended for including patients with cholestasis of pregnancy in their study, as there is a need to understand the pathogenesis of this complication; the relevance of LPA and ATX in the pathophysiology of cholestasis of pregnancy merits exploration. Kremer, *et al*., state that ATX inhibitors and LPA receptor blockers are being developed for human use, and that these medications may represent a novel class of antipruritic drugs. Indeed, the use of antagonists is a strong methodology that clarifies the role of a receptor in a given function, as opiate antagonists have done in the pruritus of cholestasis.[14-16,21-25]

The scientific study of pruritus, including that associated with cholestasis, is a research priority as there is tremendous need to develop effective, and specific therapeutic interventions to treat patients. Evidence to support a role of LPA and ATX in the pathogenesis of the pruritus of cholestasis is awaited with much enthusiasm.

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