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Serum concentrations of substance P in cholestasis

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ABSTRACT

Substance P (SP) is an excitatory neuropeptide that acts via the neurokinin-1 receptor (NK-1) in the nervous system. Pruritus, a complication of cholestasis, is a nociceptive stimulus; thus, we hypothesized that cholestasis would be associated with increased neurotransmission via SP as evidenced, in part, by increased serum concentrations of this neuropeptide. Accordingly, the aim of this study was to determine the serum concentration of SP in patients with pruritus secondary to cholestasis and in the serum of rats with cholestasis secondary to bile duct resection (BDR). The mean serum SP concentration of patients with chronic liver disease (CLD) and pruritus was 9.09 pg/mL SD ± 6.5, significantly higher than 0.74 pg/mL SD ± 0.77, the mean serum concentration of SP from patients with CLD without pruritus (p = 0.0001), and from that of the control group, which was 0.65 pg/mL SD ± 0.37 (p = 0.0001). The mean serum SP concentration from six rats with cholestasis secondary to BDR six and fourteen days after surgery was 57.9 pg/mL, SD ± 17.3, and 56.3 pg/mL, SD ± 21.4, respectively, as compared to the concentration from the sham resected control group, which was 3.5 pg/mL SD ± 0.59 (p = 0.002) at six days post surgery. In conclusion, in cholestasis, there is increased availability of SP. These data provide a rationale for the study of SP release and metabolism in cholestasis, and in the mediation of the pruritus.

Key words. Cholestasis. Pruritus. Neurotransmission. Bile duct resection.

INTRODUCTION

Pruritus is a complication of cholestasis.[1] There is evidence to suggest that the pruritus of cholestasis is mediated, at least in part, by increased opioidergic neurotransmission; a central mechanism is proposed.[2] The efficacy of opiate antagonists in the treatment of this type of pruritus supports the idea that this symptom is opioid receptor mediated.[3]

Pruritus is a sensation that has been defined as the second order of nociception, the first one being pain.[4] In this context, substance P is an excitatory neurotransmitter synthesized by primary afferent nociceptors and released into the spinal cord after noxious stimuli.[5] Substance P participates in the central sensitization associated with hyperalgesia and it acts through the receptor NK-1, which is expressed in various tissues including neural.[5] It was reported that the pharmacological increase in opioidergic tone in association with sustained administration of opiates activates mechanisms that promote pain, instead of analgesia, partly through the NK-1 receptor;[6] thus, as cholestasis is associated with increased opioidergic tone,[2] increased neurotransmission via substance P may participate in the mediation of pruritus, a nociceptive stimulus. One possible way by which substance P may mediate this type of pruritus is by its increased availability at its receptor; accordingly, we measured the serum concentrations of substance P in patients with chronic liver disease and pruritus from cholestasis. In addition, we measured substance P concentrations in the serum of rats with cholestasis secondary to bile duct resection to determine whether this model would be suitable to study this neuropeptide in human cholestasis.

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EXPERIMENTAL PROCEDURES

The study was approved by the Institutional Review Board. All subjects signed informed consent prior to recruitment into the study. Samples were

obtained from patients who were being followed in the clinic by the investigators. The study groups were comprised of patients with liver disease and pruritus and with liver disease without pruritus, as a disease control group. Chronic liver disease was defined as at least six months of abnormal serum liver profile. The etiology of the liver disease had been determined by complete work up prior to the recruitment to the study. The non disease control group consisted of subjects who had been referred to the endoscopy service for a screening colonoscopy, and who did not have documented history or evidence of liver disease. Blood was immediately placed on ice, centrifuged and the serum stored at minus 80 °C until used.

Serum substance P concentrations were also measured in stored sera from a preparation of bile duct and sham resected rats obtained from Zivic Miller six and fourteen days after the procedure. The bile duct resection model is an established model of cholestasis.[7] The samples had been collected as described above.

Serum substance P concentration was measured at Cayman Chemical Company (Ann Arbor, Michigan) by a commercially available competitive enzyme immunoassay manufactured by that company. The intraassay and interassay coefficient of variation is less than 20%. The measurement of substance P was supported by a non-restricted donation to the institution for research in the laboratory of the principal investigator by Nisshin Kyorin Pharmaceutical Company, LTD, Japan.

The statistical significance of the difference in serum substance P concentrations among the study groups was sought by the unpaired T-test.

RESULTS AND DISCUSSION

Forty-three subjects were included. Fourteen patients had chronic liver disease and pruritus, and seventeen patients had chronic liver disease without pruritus; the number of patients, their diagnoses, and the mean activity of alkaline phosphatase, one of the liver derived enzymes that tends to be increased in cholestasis, are included in tables 1 and 2. Twelve subjects were included in the control group. The mean serum substance P concentration was 9.09 pg/mL SD ± 6.5 in the group of patients with chronic liver disease and pruritus, significantly higher than 0.74 pg/mL SD ± 0.77, the mean serum substance P concentration from the group of patients with chronic liver disease without pruritus ($p = 0.0001$), and from that of the control group, which was 0.65 pg/mL SD ± 0.37 ($p = 0.0001$) (Figure 1).

Table 1. Type of liver disease and mean alkaline phosphatase activity per diagnosis group in patients with pruritus.

Diagnosis	Median Alkaline Phosphatase (IU/L) [Range]
PBC (n = 8)	516 [381-710]
PSC (n = 2)	489 [349-629]
PSC recurrent post liver transplant	369
ETOH induced Cirrhosis (n = 1)	192
Secondary Sclerosing Cholangitis (n = 1)	735

Normal serum activity of alkaline phosphatase < 116 IU/L.

Table 2. Type of liver disease and mean alkaline phosphatase activity per diagnosis group in patients without pruritus.

Diagnosis	Median Alkaline Phosphatase (IU/L) [Range, normal 34-104 U/L]
Hepatitis B (n = 2)	108 [97-119]
Hepatitis C (n = 11)	90 [65-129]
Cryptogenic Cirrhosis (n = 1)	74
Autoimmune Hepatitis (n = 1)	242
PBC (n = 1)	857
Cholestasis of unknown etiology (n = 1)	383

Normal serum activity of alkaline phosphatase < 116 IU/L.

The mean serum concentration in the serum from six rats with cholestasis secondary to bile duct resection six days after surgery was 57.9 pg/mL, SD ± 17.3, and 56.3 pg/mL, SD ± 21.4, fourteen days after surgery, as compared to the concentration from the sham resected control group, which was 3.5 pg/mL SD ± 0.59 ($p = 0.002$) at day six post surgery, and below the assay detection threshold in the sham resected group, fourteen days post surgery (Figure 2).

In this study, the mean serum concentration of substance P was significantly higher in the group of patients with cholestasis and pruritus than that of the group of patients with cholestasis without pruritus, and that of the control group. The mean serum concentration of substance P in patients with cholestasis without pruritus was similar to that of the control group. These results suggest that in patients with cholestasis and pruritus there is increased availability of this nociceptive peptide, which may be associated with increased neurotransmission mediated by substance P; thus, substance P may contribute to the pathophysiology of cholestasis, in-

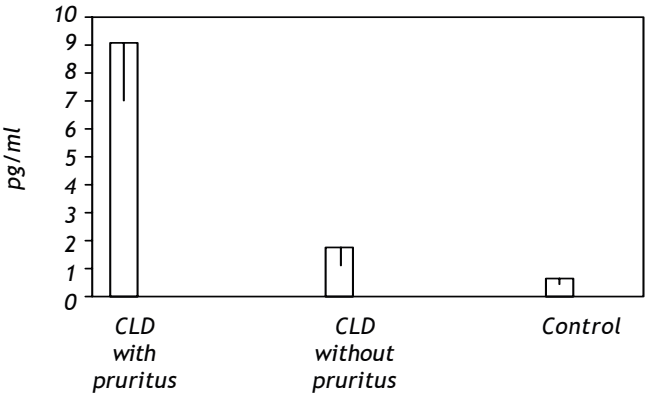

Figure 1. *Mean ± SE serum concentrations of substance P in the study groups. The mean serum concentration of substance P in the group of patients with chronic liver disease (CLD) and pruritus (n = 13) was significantly higher than that of patients with chronic liver disease without pruritus (n = 17), (p < 0.0001), and that of the control group of subjects (n = 12) (p < 0.0001). The mean serum concentration of substance P from the group of patients with cholestasis without pruritus was not significantly different from that of the control group of subjects.*

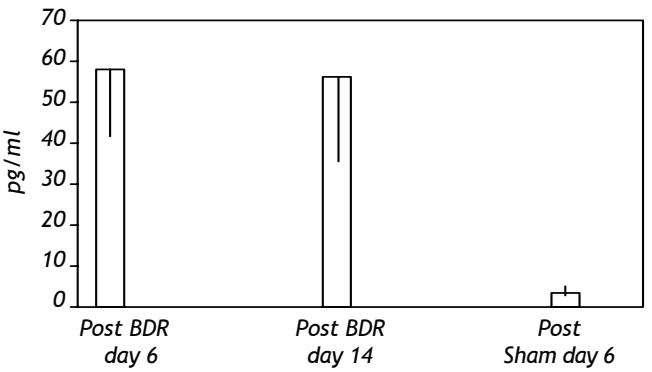

Figure 2. *Mean serum ± SE concentrations of substance P in rats with cholestasis secondary to bile duct resection (BDR) (n = 6) six and fourteen days after surgery, and from a sham resected control (SHAM) (n = 6), six days after the procedure. The mean serum concentration of substance P from the BDR group six and fourteen days after surgery, was significantly higher than that of the SHAM control group (p < 0.002). The mean serum concentration of substance P in the SHAM group (n = 6) fourteen days after surgery was below the assay detection threshold.*

cluding its associated pruritus. These data provide a rationale for the study of substance P release and metabolism in cholestasis. As substance P serum concentrations were significantly increased in rats with cholestasis secondary to bile duct resection, as compared to the control group, this animal model may be suitable to study substance P dynamics in human disease.

There isn't a model to study pruritus of cholestasis in human beings; however, behavioral studies in primates have shed light on the mechanism by which morphine, and other drugs that bind to the mu opioid receptor, mediate scratching behavior in this species.[8] These results have been interpreted as analogous to the pruritus and scratching associated with the administration of this type of drugs to human beings. In the context of substance P, however, this peptide was not reported to be associated with scratching in primates, in a recently published study.[8] Thus, it is not known at present whether these negative results also apply to human beings, or whether in human beings substance P can be pruritogenic.

The substance P antagonist aprepitant, has been approved for the treatment of nausea and vomiting secondary to chemotherapy .[9] The availability of this type of drug makes it feasible to test the hypothesis of substance P as a mediator of the pruritus of cholestasis in controlled studies of patients with liver disease.

The source of substance P in cholestasis is not known. There are published reports on the positive expression of substance P immunoreactivity in nerve fibers in human and rat livers[10,11] but, we could not find any publications on the expression of this type of immunoreactivity on hepatocytes or biliary epithelial cells.

The serum concentration of substance P reported in Egyptian children with chronic liver disease, as compared to controls, was one hundred fold higher than in the patients with cholestasis reported in this study (12); however, the proportional increase in the study reported here was much higher than in the study in children.[12] In our study, the serum substance P was thirteen fold higher in patients with cholestasis than in the normal and disease control groups, in contrast to the study in children, which was seven fold higher in the diseased group. Allowing for what may be technical differences, these two studies confirm that in liver disease, substance P concentration in serum is increased; accordingly, studies on the potential role of this peptide in liver disease merit consideration. In this context, the concentrations of substance P in sera from rats with cholestasis were much higher than that of patients in this study; this is likely secondary to the obstructive nature of the model. The fact that substance P concentrations are higher in rats with cholestasis than in the control group, however, suggests that the model may be useful to study the potential role of substance P in the pathophysiology of liver disease.

In summary, in this study, substance P concentrations were significantly higher in the serum from a group of patients with cholestasis and pruritus, and in the serum from a group of rats with cholestasis, than in their respective control groups. These results provide support for the study of the potential role of substance P in liver disease, including the mediation of the pruritus.

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