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THE EFFECT OF MICROTUBULE STABILIZER ON RAT CAERULEIN-INDUCED PANCREATITIS*

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INDEXING WORDS

exocrine pancreas; microtubules; enzyme secretion

SYNOPSIS

Inhibition of pancreatic digestive enzyme secretion in the acinar cell is a significant phenomenon which can trigger acute pancreatitis. It has been shown that microtubules are responsible for the intracellular vesicular transport. The effect of taxol, a microtubule stabilizer, was examined in a model of acute edematous pancreatitis induced in rats by supramaximal stimulation with cholecystokinin analogue, caerulein. Prophylactic administration of taxol ameliorated inhibition of pancreatic digestive enzyme secretion, increased level of serum amylase, pancreatic edema, and histological alterations in this model. Immunofluorescence studies revealed that taxol stabilized the arrangement of microtubules by promoting tubulin polymerization. On the other hand, microtubule disorganization was found in rats without prophylactic taxol treatment. In caerulein-treated rats, there is microtubule disorganization

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**The abbreviations used are : CCK, cholecystokinin; DMSO, dimethylsulfoxide; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

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which causes interference with intracellular vesicular transport leading to inhibition of pancreatic digestive enzyme secretion - a forerunner of acute pancreatitis. Taxol was found to prevent these events.

INTRODUCTION

It was suggested that inhibition of pancreatic digestive enzyme secretion in the acinar cell is a significant phenomenon associated with acute pancreatitis and this has been the current subject of intense consideration.[20] This secretory blockade was observed in various experimental models of acute pancreatitis[12] and in most patients of clinical acute pancreatitis.[11] For example, in a model of acute edematous pancreatitis induced in rats by supramaximal stimulation with CCK** analogue, caerulein,[9,20] flow of pancreatic juice was markedly reduced,[1] secretion of pancreatic digestive enzyme was blocked,[9,13,14] and large vacuoles were observed in the cytoplasm of acinar cells.[1,9,22] These large vacuoles contain both digestive and lysosomal enzymes.[15,22] The intra-acinar cell activation of digestive enzymes resulting from their colocalization with lysosomal enzymes may lead to pancreatic autodigestion. But the mechanism of inhibition of digestive enzyme secretion is not known, and it is obscure whether the inhibition of digestive enzyme secretion is a cause or an effect of caerulein-induced pancreatitis.

Recent experiments have shown that microtubules are involved in the intracellular vesicular transport.[6] Secretory vesicles leave the Golgi region carrying newly synthesized proteins to the cell surface along microtubule tracks. Vinblastine and colchicine have been shown to inhibit the amylase release from *in vitro* mouse pancreas by their microtubule-disrupting action.[23] It is conceivable that inhibition of the digestive enzyme secretion in acute pancreatitis occurs due to disorder of intracellular vesicular transport, and that microtubules may be involved in this action.

Taxol, a plant product isolated from *Taxus brevifolia* has been shown to have antitumor and antimitotic activities.[21] Unlike other antimicrotubule agents that induce microtubule

disassembly such as colchicine and the vinca alkaloids, taxol promotes the polymerization of tubulin and stabilizes microtubules *in vitro*[8,18] and in cultured cells.[2,19]

In this study, the effect of the microtubule stabilizer, taxol in a model of rat caerulein-induced pancreatitis was investigated, and the role of microtubules in the process of the onset of caerulein-induced acute pancreatitis was discussed.

MATERIALS AND METHODS

Materials

Male Wistar rats (200-250 g wt) were employed. Caerulein was obtained from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Taxol was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Maryland, USA). Intramedic polyethylene tubing (PE-10) for pancreatic duct drainage was purchased from Clay Adams, Division of Becton Dickinson and Company (New Jersey, USA). Anti-β-tubulin monoclonal antibody was from Amersham Corp. Anti-trypsin polyclonal antibody was from Cambridge Medical Technology Corp. Other chemicals were obtained from commercial sources.

Methods

Taxol, dissolved in DMSO at a concentration of 10 mM and stored at -20°C, was injected subcutaneously to rats at a dosage of 5 mg/kg of body wt. An equal volume of DMSO was given to control rats. Rats were anesthetized with a subcutaneous injection of carbamic acid ethyl ester (urethan) at a dosage of 1.5 g/kg of body wt. A midline laparotomy was performed and a PE-10 cannula was introduced into the common biliopancreatic duct for a distance of 0.5 cm by extraduodenal incision of the duct. The jugular vein was exposed through a small cervical incision and a polyethylene catheter was placed in the vein. After 2 h of the administration of taxol, rats were infused with saline alone or with saline containing caerulein (0.2 μg/kg/h or 5 μg/kg/h) at a rate of 1.0 ml/h for 4 h. Pancreatic secretions were collected at 1-h intervals. After infusion with this agent for 4 h, blood was collected for

serum amylase determination and the pancreas was rapidly removed. Amylase activity was measured by the method of Irie *et al.*[4] Pancreatic edema was quantitated by measuring the weight of the pancreas immediately after harvesting (wet wt) and after desiccation for 48 h at 60°C (dry wt). Pancreatic water content was calculated as (wet wt-dry wt)/wet wt and was expressed in percent. Pancreatic tissue fragments were fixed with 10% formalin. After paraffin embedding and sectioning, tissues were stained with hematoxylin and eosin.

Immunofluorescence Studies

The rats were perfused transcardially with 2% paraformaldehyde in 50 mM phosphate buffer containing 8% sucrose. Pancreas was removed, cut into small blocks, and further immersed in the same fixative for 2 h at 4°C. The fixed tissues were cryoprotected through a range of increasing sucrose concentrations (10, 15, and 20%), mounted in OCT embedding medium, quick-frozen, and sectioned on a cryostat. The 5-μm thick frozen sections collected on poly L-lysine-coated slides were incubated with PBS containing 5% BSA for 1 h at room temperature and then overnight with either anti-β-tubulin monoclonal antibody or anti-trypsin polyclonal antibody at a final dilution of 1:500 in PBS containing 5% BSA. Analogous cryosections were treated with either mouse or rabbit non-immune immunoglobulin (10 μg/ml), and served as controls. After washing four times with PBS, the sections were incubated for 4 h at room temperature with fluorescein-labeled goat anti-mouse or anti-rabbit immunoglobulin at a final dilution of 1:30. After washing four times with PBS, the sections were mounted in PBS containing 20% glycerine under a coverglass and examined by a fluorescence microscope.

RESULTS

Effect of Taxol on Pancreatic Secretion

The effect of taxol on pancreatic secretion (flow of pancreatic juice and amylase secretion) is shown in Table 1. Infusion of caerulein at 0.2 μg/kg/h for 4 h caused a maximal stimulation of pancreatic secretion, while caerulein at 5 μg

/kg/h for 4 h inhibited pancreatic secretion. These observations are comparable to those described previously.[1,9,13,14,22] Taxol did not stimulate pancreatic secretion, but with supramaximal caerulein stimulation (5 μg/kg/h for 4 h), taxol released the inhibition of flow of pancreatic juice partially and that of amylase secretion nearly completely.

Effects of Taxol on Serum Amylase Level and Pancreatic Water Content

The effects of taxol on serum amylase level and pancreatic water content are shown in Table 2. Infusion of caerulein at 5 μg/kg/h for 4 h caused hyperamylasemia and pancreatic edema as reported previously.[9,20] However, when caerulein at 5 μg/kg/h was infused for 4 h with preceded injection of taxol, serum amylase level stayed almost normal and pancreatic water content was reduced almost to normal range. On macroscopic inspection, pancreatic edema was not observed.

Light Microscopic Appearance of Pancreas

Infusion of caerulein at 5 μg/kg/h for 4 h caused interstitial edema, acinar cell vacuolization, and inflammatory cell infiltration in the pancreas (Fig. 1A) as reported previously.[9,20] By contrast, when caerulein at 5 μg/kg/h was infused for 4 h with preceded injection of taxol, these histological alterations were not observed (Fig. 1B).

Effect of Taxol on the Distribution of β-tubulin

The frozen sections of rat pancreas were analyzed by indirect immunofluorescence with an anti-β-tubulin monoclonal antibody. When caerulein at 5 μg/kg/h was infused for 4 h (caerulein-induced pancreatitis), in contrast with the image of normal pancreas, the fluorescence of tubulin was weak and diffuse, and the microtubule network seemed to be disassembled and disrupted relatively (Fig. 2A). By contrast, when caerulein at 5 μg/kg/h was infused for 4 h with preceded injection of taxol, the microtubule network fluorescence became stronger from the supranuclear regions to the acinar lumen, and radial microtubules seemed to be stabilized by polymerizing tubulin (Fig. 2B). No significant staining was seen in the control sections.

Table 1. Effect of taxol on pancreatic secretion.

Agent	Flow of Pancreatic Juice	Amylase Secretion
	μl/4 h	*IU/4 h*
Saline	90 ± 15	798 ± 99
Taxol + Saline	81 ± 21	638 ± 152
Caerulein 0.2 μg/kg/h	359 ± 66	4321 ± 174
Caerulein 5 μg/kg/h	54 ± 11	504 ± 85
Taxol + Caerulein 5 μg/kg/h	216 ± 14 [a]	3876 ± 648 [a]

Results are the mean ± S.E. of at least three independent experiments. [a]Significantly different from the value of caerulein 5 μg/kg/h group by *t* test ($P<0.01$).

Table 2. Effects of taxol on serum amylase level and pancreatic water content.

Agent	Serum Amylase Level	Pancreatic Water Content
	IU/ml	*%*
Saline	7 ± 3	74 ± 1
Taxol + Saline	11 ± 2	73 ± 1
Caerulein 0.2 μg/kg/h	8 ± 3	75 ± 1
Caerulein 5 μg/kg/h	56 ± 12	85 ± 1
Taxol + Caerulein 5 μg/kg/h	13 ± 2 [a]	78 ± 1 [a]

Results are the mean ± S.E. of at least three independent experiments. [a]Significantly different from the value of caerulein 5 μg/kg/h group by *t* test ($P<0.05$).

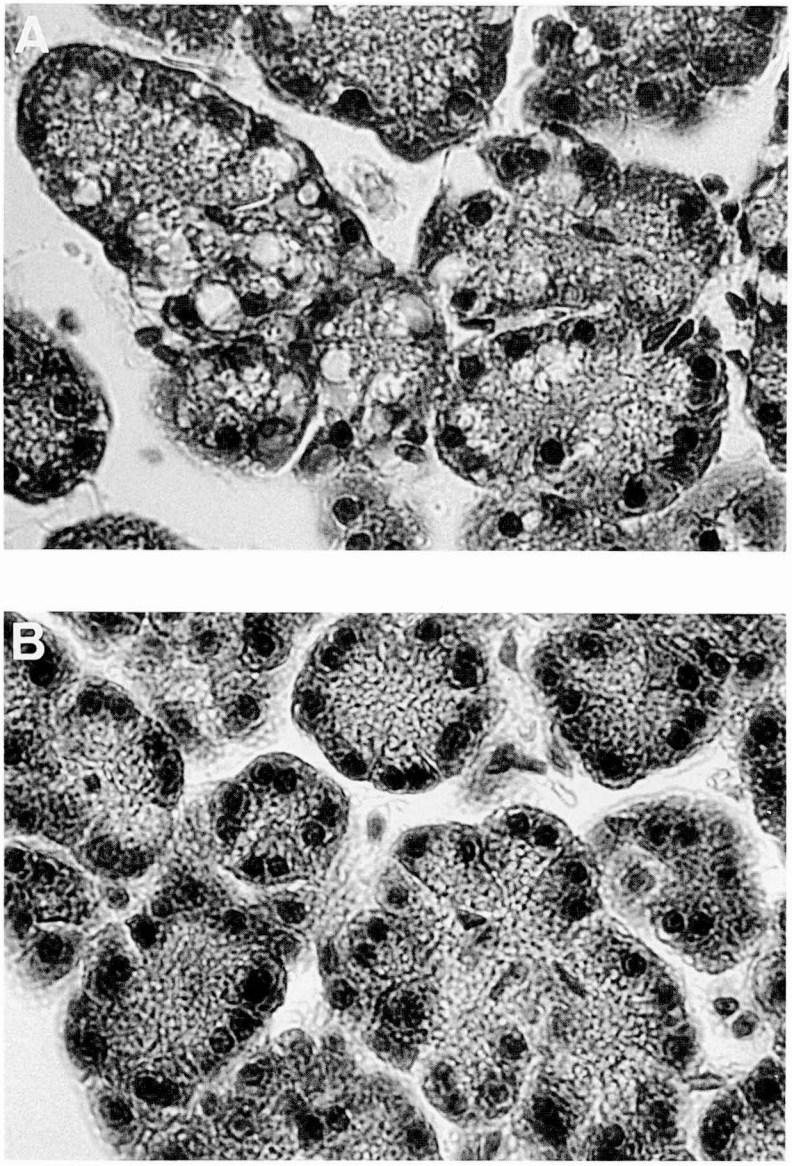

Fig. 1. Light microscopy of pancreatic acinar cells of caerulein-treated rats (5 µg/kg/h for 4 h) in the absence (A) and presence (B) of taxol. Magnification x 500.

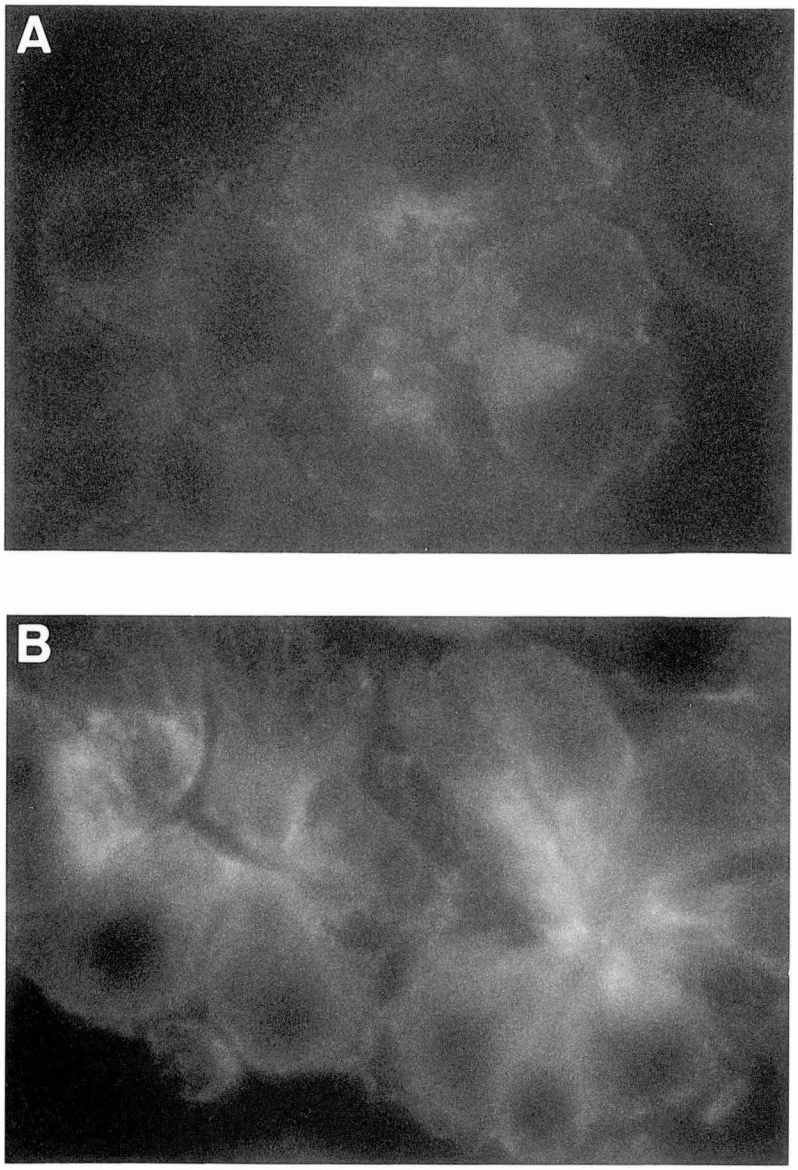

Fig. 2. Indirect immunofluorescence of exocrine pancreas showing the distribution of β-tubulin in caerulein-treated rats (5 μg/kg/h for 4 h) in the absence (A) and presence (B) of taxol. Magnification x 1250.

Effect of Taxol on the Distribution of Trypsin

In the same samples, immunofluorescence studies with an anti-trypsin polyclonal antibody were performed. When caerulein at 5 μg/kg/h was infused for 4 h (caerulein-induced pancreatitis), spherical granular fluorescence was seen in the apical regions of acinar cells. This image indicates that inhibition of pancreatic digestive enzyme secretion occurs in caerulein-induced pancreatitis as so far described.[9,13,14] By contrast, when caerulein at 5 μg/kg/h was infused for 4 h with preceded injection of taxol, fine fluorescence was seen in the acinar lumen, indicating that inhibition of pancreatic digestive enzyme secretion was removed. No significant staining was seen in the control sections.

DISCUSSION

In this study, it has been first described that stabilizing microtubules removes the inhibition of pancreatic digestive enzyme secretion induced by supramaximal caerulein stimulation and prevents the development of caerulein-induced pancreatitis. These results suggest that microtubule disorganization is the initiating event in caerulein-induced pancreatitis and that inhibition of pancreatic digestive enzyme secretion by interfering with intracellular vesicular transport due to microtubule disorganization causes caerulein-induced pancreatitis.

Two possible mechanisms have been proposed concerning the role of the microtubules in exocytosis; first, microtubules serve as the tracks along which exocytotic vesicles are guided to specific cell surface. Secondly, microtubules are involved in the maintenance of the structural organization of the Golgi complex. The disruption of the microtubule network would lead to the disorganization of the Golgi complex and would consequently result in an inhibition of protein transport. In a model of caerulein-induced pancreatitis, transport from the endoplasmic reticulum to the Golgi cisternae is not altered, but the maturation of condensing vacuoles into zymogen granules is found to be impaired.[13,14,22] Therefore, it is conceivable that disorganization of microtubule tracks in this specific part

results in the inhibition of digestive enzyme secretion and colocalization of digestive and lysosomal enzymes, and causes caerulein-induced pancreatitis.

It has been shown that caerulein-induced pancreatitis is mediated by low-affinity CCK receptors which inhibit the digestive enzyme secretion,[17] but the intracellular signal transduction system functioning beneath low-affinity CCK receptor has not been understood at all. From the results in this study it is conceivable that tubulin, which is a component of microtubules, is a final targetting protein in the signal transduction system of the low affinity receptor.

In the results obtained, taxol removed the inhibition of amylase secretion nearly completely, but that of flow of pancreatic juice incompletely, and normalized serum amylase level nearly completely, but pancreatic water content incompletely. Recent investigation has demonstrated that CCK downregulates vasoactive intestinal peptide and secretin receptors in rat pancreatic acini.[5] Since it is generally believed that secretin acts to stimulate a flow of bicarbonate- and electrolyte-rich ductular secretion, probably this discrepancy occurs as a result of the downregulation of secretin receptors by caerulein and this part of secretion is considered to be related to endogenous secretin.

In another model of acute hemorrhagic pancreatitis induced by feeding young female mice a choline-deficient, ethionine-supplemented (CDE) diet,[10] pancreatic digestive enzyme secretion is blocked and colocalization of digestive enzyme zymogen with lysosomal hydrolases is observed as well as in caerulein-induced pancreatitis.[3,7] In this model, it has been shown that fusion of the zymogen granules with lysosomes (crinophagy) causes the formation of large vacuoles.[7] Moreover, a recent study has demonstrated that pancreatic duct obstruction also leads to this colocalization phenomenon.[16] In this model, which seems to be applicable to clinical gall stone pancreatitis, colocalization is found in the zymogen granule-sized organelles. Therefore, it is suggested that interference in the process of intracellular vesicular transport occurs in these experimental models. Clinical acute pancreatitis does not appear to be involved in excessive CCK stimulation. But the re-

sults of this study raised the possibility that microtubule disorganization mediated by some other signal transduction system may cause acute pancreatitis under the condition where excessive CCK stimulation is absent. However, the involvement of microtubule disorganization in other experimental models and clinical cases is still unknown. Hence, further investigations are necessary to clarify the role of microtubules in other models and clinical cases.

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