

## ORIGINAL ARTICLE

# Octreotide negates the benefit of galantide when used in the treatment of caerulein-induced acute pancreatitis in mice

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## Abstract

**Background:** We have previously shown that galantide, a non-specific galanin receptor antagonist, ameliorates acute pancreatitis (AP) induced in mice. Octreotide, a somatostatin analogue, has been used in the treatment of AP with inconsistent outcomes. This study set out to compare the efficacy of a combined treatment of galantide and octreotide with the efficacy of each agent individually in experimental AP.

**Methods:** Acute pancreatitis was induced in mice with 7-hourly caerulein injections. Galantide and/or octreotide were co-administered with each caerulein injection commencing with the first injection. Control animals received galantide, octreotide or saline alone. Pancreata were harvested for histological examination and estimation of myeloperoxidase (MPO) activity. Plasma amylase and lipase activities were measured.

**Results:** Galantide significantly reduced AP-induced hyperenzymaemia by 39–45%. Octreotide alone, or in combination with galantide, did not significantly alter AP-induced hyperenzymaemia. Plasma enzyme activity in the control groups was comparable with pre-treatment activity. Galantide and octreotide administered individually reduced MPO activity by 79% and 50%, respectively; however their combination was without effect. Galantide, octreotide and their combination significantly reduced the percentage of abnormal acinar cells by 28–45%.

**Conclusions:** Treatment with galantide alone ameliorated most of the indices of AP studied, whereas treatment with octreotide reduced pancreatic MPO activity and acinar cell damage. Combining the two peptides appears to negate their individual benefits, which suggests an interaction in their mechanism of action.

## Keywords

peptide, somatostatin, inflammation

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## Introduction

Acute pancreatitis (AP) is a disease with high morbidity and mortality and a complex pathophysiology.<sup>1</sup> Despite extensive investigation, to date there exists no specific medical treatment for AP.<sup>2</sup> Given the numerous factors involved in the pathogenesis of AP, it appears that successful treatment of this disease may depend upon combining more than one therapeutic agent and on each agent targeting apparently different steps in AP. In this study, we investigated two possible therapeutic agents.

We have previously demonstrated that galanin is involved in the pathogenesis of caerulein-induced AP in mice and the Australian possum.<sup>3–7</sup> Mice carrying a loss-of-function mutation in the galanin gene (galanin knockout, Gal KO) have less severe caerulein-induced AP.<sup>5</sup> Galantide, a non-specific galanin receptor antagonist, is a peptide consisting of fragments of galanin and substance P (galanin-[1-12]-Pro-substance P-[5-11] amide).<sup>8</sup> We and others have previously shown that galantide is able to antagonize the effects of galanin on pancreatic function<sup>6,8</sup> and to ameliorate caerulein-induced AP in mice and the Australian possum.<sup>3–7</sup>

Octreotide, a somatostatin analogue, has been used clinically as a treatment for AP.<sup>9</sup> However, its beneficial results in AP have been inconsistent: some studies have shown an improvement in all parameters studied (plasma enzyme reduction, inflammatory markers, histological benefit),<sup>9–12</sup> whereas others suggest no benefit at all.<sup>13–15</sup> These inconsistencies have been ascribed to the application of different animal models of AP, different doses of octreotide and different treatment protocols.

Peptide therapeutics is a growing field<sup>16</sup> and the application of peptides in the treatment of AP has been very limited. Peptides offer several advantages as therapeutic agents, particularly the potential for minimal central side-effects.<sup>16</sup> Galantide is one of several galanin receptor antagonists that are chimeric peptides.<sup>8</sup> Similarly, octreotide is an octapeptide that mimics somatostatin pharmacologically.

In this study, we tested the hypothesis that a somatostatin analogue in combination with galantide, delivered at the induction of AP, would be a more effective treatment for AP than either agent alone. We aimed to investigate the effects of octreotide alone and in combination with galantide on the biochemical (plasma amylase and lipase and pancreatic myeloperoxidase [MPO]) and histological indices of AP induced by caerulein hyperstimulation in mice.

## Materials and methods

All experiments were approved by the Animal Welfare Committee of Flinders University. For these studies we used the caerulein mouse model originally described by Niederau *et al.*<sup>17</sup> Male Swiss mice aged 6–8 weeks and weighing 25–30 g were used. The mice were maintained in a facility with a controlled temperature (21–24 °C) and lighting (12 : 12 h light : dark cycle). Standard laboratory chow and drinking water were provided *ad libitum*. A minimum period of 2 days was allowed for animals to acclimatize before any experimental manipulations were undertaken. Mice were fasted for 12 h before the induction of AP and had free access to water.

The mice were allocated to seven groups (Table 1). On the day prior to AP induction, a blood sample was collected by retro-orbital sinus bleeding under anaesthesia and used to measure the basal plasma enzyme activity.<sup>3,5,18</sup>

**Table 1** Experimental groups: protocols and animal numbers

Group	Protocol	Mice per group
1	Control, no AP (saline)	4
2	Control, AP	9
3	GT alone	6
4	OT alone	4
5	AP + GT	10
6	AP + OT	9
7	AP + OT + GT	12

AP, acute pancreatitis; GT, galantide (dose = 66 µg/kg/h); OT, octreotide (dose = 250 µg/kg/h)

Peptide stock solutions were prepared in 0.01% bovine serum albumin (BSA; Sigma-Aldrich Corp., St Louis, MO, USA) in saline. On the day of the experiment, AP was induced by seven intraperitoneal (i.p.) injections of caerulein (50 µg/kg) (American Peptide Co., Sunnyvale, CA, USA) administered in 150 µl of 0.9% NaCl at hourly intervals over 0–6 h. Each mouse was given a single subcutaneous injection of 100 µg/kg of buprenorphine-HCl (Temgesic Inject; Reckitt Benckiser Health Care UK, Hull, UK) at the induction of AP. This dose provided analgesia for the duration of the experimental period.<sup>18</sup>

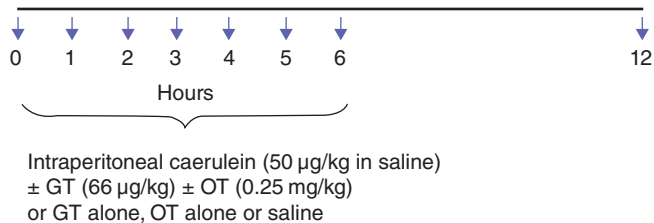
## Treatment protocols

Galantide (Bachem AG, Bubendorf, Switzerland) at a dose of 66 µg/kg was co-administered with each injection of caerulein commencing with the first caerulein injection (Fig. 1). Octreotide (American Peptide Co.) at a dose of 250 µg/kg was co-administered with caerulein at hourly intervals over 6 h (for a total of seven injections). The i.p. injection dose of octreotide (250 µg/kg) was previously used by Yuan *et al.* in a caerulein model of AP in mice.<sup>19</sup> In the experiments in which the two peptides were combined, each peptide, including caerulein, was administered separately.

Mice were killed 6 h after the final caerulein injection under anaesthesia as previously described.<sup>3,5,18</sup> Each pancreas was then harvested and divided into two pieces; one piece was placed in 10% buffered formalin solution for subsequent histological examination and the other piece was quickly blotted on gauze to remove excess blood and stored at –70 °C for subsequent MPO extraction and assay.<sup>18,20,21</sup> Myeloperoxidase activity was expressed as IU per mg of protein. The plasma samples were assayed for amylase and lipase activity using an enzymatic colorimetric assay (Roche/Hitachi, Roche Diagnostics GmbH, Mannheim, Germany) using a Roche/Hitachi Modular Analyser (Hitachi High-Technologies Corp., Tokyo, Japan).

## Histological analysis of pancreatic damage

Fixed pancreatic tissue was processed for standard haematoxylin and eosin histology. The histological analysis was performed using a point-counting morphometry method as previously



**Figure 1** Diagram illustrating the treatment protocol for administration of antagonists. The timeline shows doses of caerulein, galantide (GT) and octreotide (OT). GT and OT were administered at hourly intervals for 6 h starting from the first injection of caerulein. Mice were killed at 12 h

described.<sup>3,18,22</sup> The number of abnormal acinar cells was expressed as the percentage of total acinar cells counted. Interstitial space was expressed as a proportion of the number of points counted. Group data were expressed as mean  $\pm$  standard error (SE) ( $n = 4-12$  mice).

### Statistical analysis

Statistical analysis was performed using spss Version 11.5 (SPSS, Inc., Chicago, IL, USA). The Mann–Whitney  $U$ -test was used and a  $P$ -value of  $<0.05$  was regarded as significant.

## Results

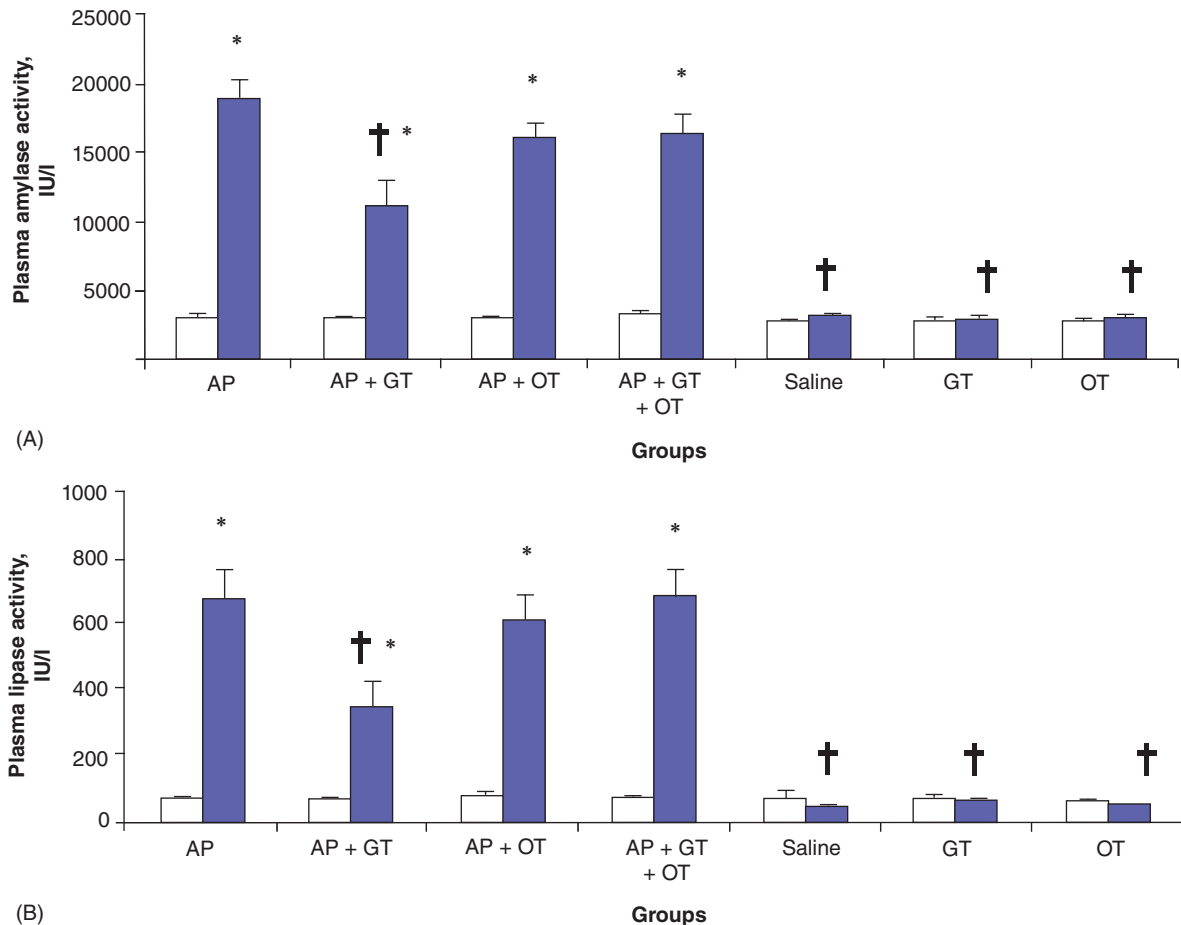
### Plasma enzymes

The induction of AP produced an approximately six-fold increase in plasma amylase activity and an approximately nine-fold increase in plasma lipase activity (Fig. 2A, B). Treatment with galantide

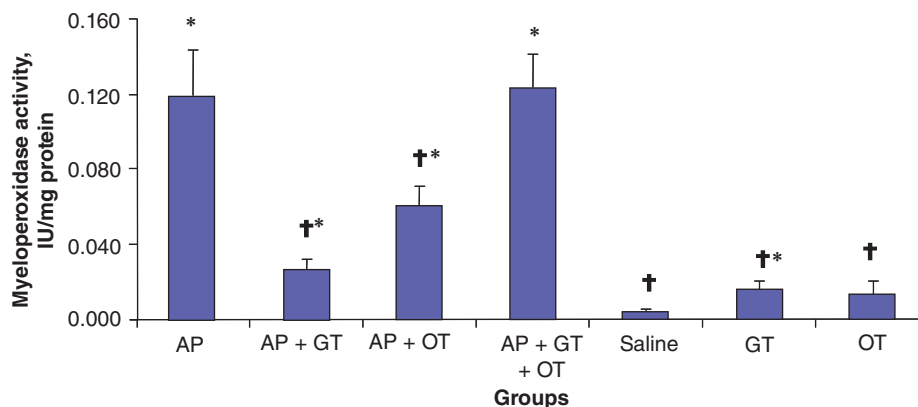
reduced the caerulein-induced elevation in plasma amylase and lipase activities by 39% and 45% ( $P < 0.007$  and  $P < 0.009$  compared with AP alone), respectively. Neither octreotide alone nor the combination of galantide and octreotide reduced the caerulein-induced hyperenzymaemia. Neither agent alone nor saline altered plasma enzyme activity compared with basal activity.

### Pancreatic MPO

The induction of AP resulted in a 28-fold increase in pancreatic MPO activity compared with saline alone (Fig. 3). Treatment with galantide or octreotide significantly reduced pancreatic MPO activity compared with AP alone by 79% and 50% (both  $P < 0.002$ ), respectively. The reduction in MPO activity by galantide was significantly greater than that caused by octreotide ( $P < 0.011$ ). Surprisingly, the combination of the two agents did not affect MPO activity compared with AP alone. Pancreatic MPO



**Figure 2** Effects of administration of galantide (GT) and octreotide (OT) on acute pancreatitis (AP)-induced (A) hyperamylasaemia and (B) hyperlipasaemia. A significant increase was noted in plasma amylase activity following the induction of AP. Treatment of AP with GT alone reduced plasma amylase and lipase activity. Treatment of AP with OT alone and GT + OT had no effect on AP-induced hyperenzymaemia. Treatment with either GT or OT alone had no effect on plasma enzyme activity. □ represents basal enzyme activity for each group; ■ represents post-treatment enzyme activity. Data are expressed as IU/l and are presented as mean + standard error ( $n = 4-12$ ). \* $P < 0.05$  compared with the saline group; † $P < 0.05$  compared with the AP-alone group



**Figure 3** Effects of administration of galantide (GT) and octreotide (OT) on acute pancreatitis (AP)-induced pancreatic myeloperoxidase (MPO) activity. Treatment of AP with either GT or OT alone reduced MPO activity compared with the AP-alone group. Treatment of AP with GT + OT did not reduce MPO activity compared with the AP-alone group. Treatment with GT alone did alter pancreatic MPO activity compared with the saline group, but treatment with OT alone did not. MPO activity is expressed as IU/mg protein. Data are presented as mean + standard error ( $n = 4-12$ ). \* $P < 0.05$  compared with the saline group; † $P < 0.05$  compared with the AP-alone group

activity in the galantide-alone group was low but significantly greater ( $P < 0.011$ ) than that in the saline group, whereas MPO activity in the octreotide-alone group did not significantly differ from that in the saline group (Fig. 3).

### Pancreatic damage

As expected, histological assessment of pancreatic tissue revealed abnormal acinar cells and interstitial oedema in the mice with AP (Figs 4A, B and 5). In the AP-alone group, 63% of the acinar cells were abnormal (Figs 4A, 5). Treatment with galantide significantly reduced the percentage of abnormal acinar cells by 34% ( $P < 0.001$ ) compared with the AP-alone group. Similarly, octreotide treatment significantly reduced the percentage of abnormal acinar cells by 45% compared with the AP-alone group ( $P < 0.001$ ). Treatment with the combination galantide + octreotide also significantly reduced the percentage of abnormal acinar cells by 28% ( $P < 0.001$ ); this reduction was significantly less than that caused by AP treatment with octreotide alone ( $P < 0.023$ ), but did not differ from that produced with galantide alone ( $P < 0.323$ ). The galantide- or octreotide-alone control groups displayed a minor but statistically significant increase in the percentage of abnormal acinar cells compared with the saline group.

Interstitial oedema was increased about three-fold in the AP-alone group compared with the saline control group ( $P < 0.005$ ) (Figs 4B, 5). Treatment with octreotide alone significantly reduced the interstitial oedema compared with AP alone by 29% ( $P < 0.04$ ), whereas treatment with galantide alone or the combination of the two peptides failed to alter the oedema compared with AP alone.

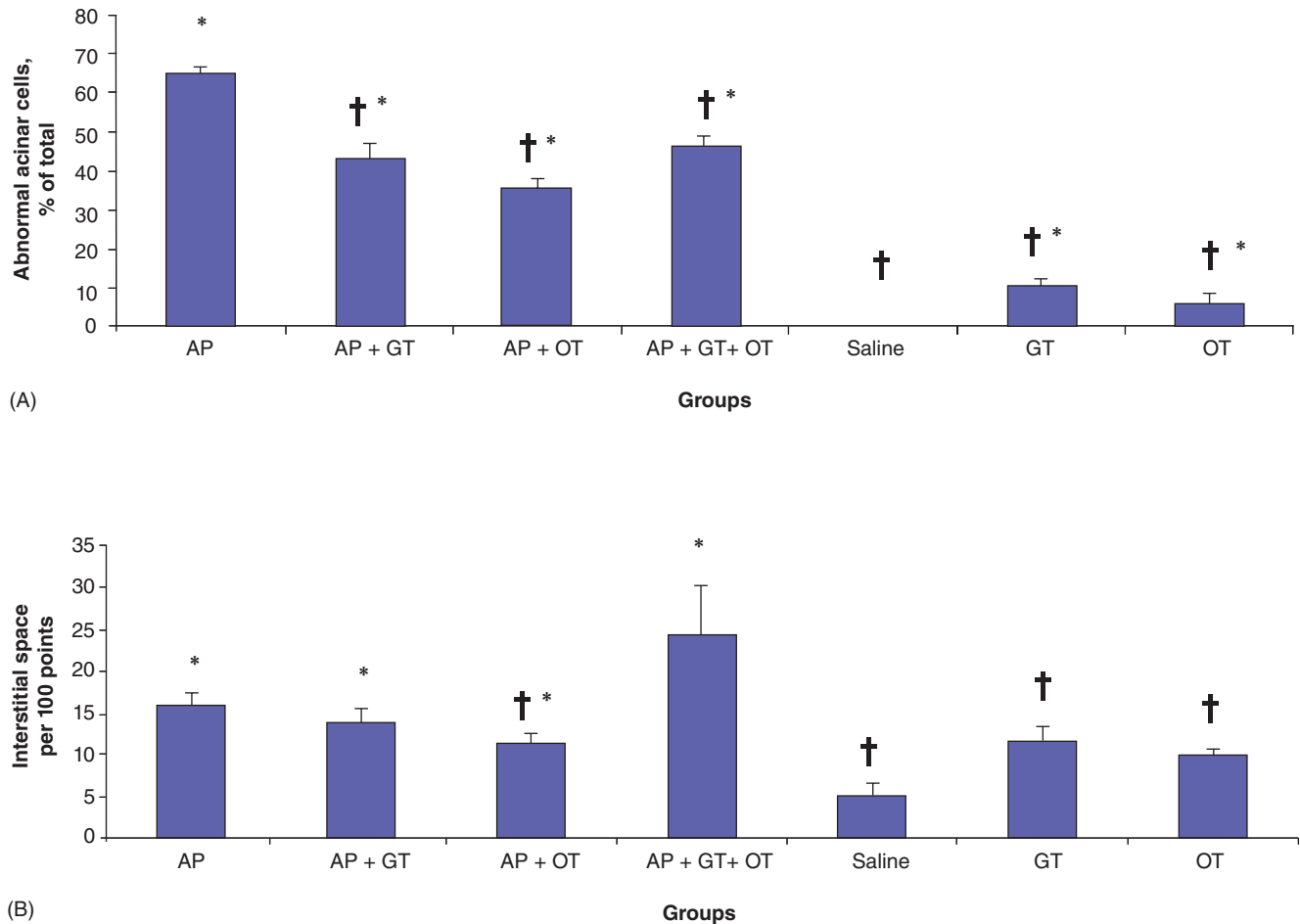
### Discussion

This study demonstrated that treatment of AP with either galantide or octreotide, administered at the induction of AP, reduced

most of the elevated markers of AP measured, including pancreatic damage. Interestingly, treatment with the combination of the two agents was much less effective.

To date there exists no specific treatment for AP. This may reflect the complexity of its pathophysiology, which involves multiple factors. Another important determinant of the course of the disease which may impact the success of any treatment is the time delay from the actual onset of the disease to the patient's presentation to the hospital. Although 50% of patients present in the first 12 h after the onset of disease, up to 30% of patients may present after 24 h of symptoms.<sup>23</sup> Hence any strategy targeting a single agent or pathway may be insufficient to control the progress of the disease. An alternative strategy is to target multiple steps in the pathogenesis of the disease by combining more than one therapeutic agent. In this study, we attempted to explore for the first time the benefit of administering the two peptides galantide and octreotide (previously shown individually to ameliorate the indices of AP) at the induction of AP. The impact of our findings is two-fold: firstly, this research may provide us with a potential novel therapy in the prevention of endoscopic retrograde cholangiopancreatography (ERCP)-induced AP, and, secondly, it provides the impetus for further experiments exploring the role of the combination in a delayed setting in AP (a situation that closely mimics the actual clinical scenario in AP arising from most other causes).

Galantide has been shown to ameliorate caerulein-induced severe AP in mice and in the Australian possum.<sup>3,5-7</sup> Our studies to date indicate that galanin acts at several steps in the induction and progression of AP. The present study confirms our previous reports of the beneficial effects of galantide treatment in a mild model of AP. In the present study, galantide administered at AP induction ameliorated most of the indices of AP, except oedema. The effect of galantide on plasma amylase activity is consistent with an inhibition of the co-stimulatory/potentiating effect of galanin on caerulein-stimulated amylase secretion.<sup>24</sup>

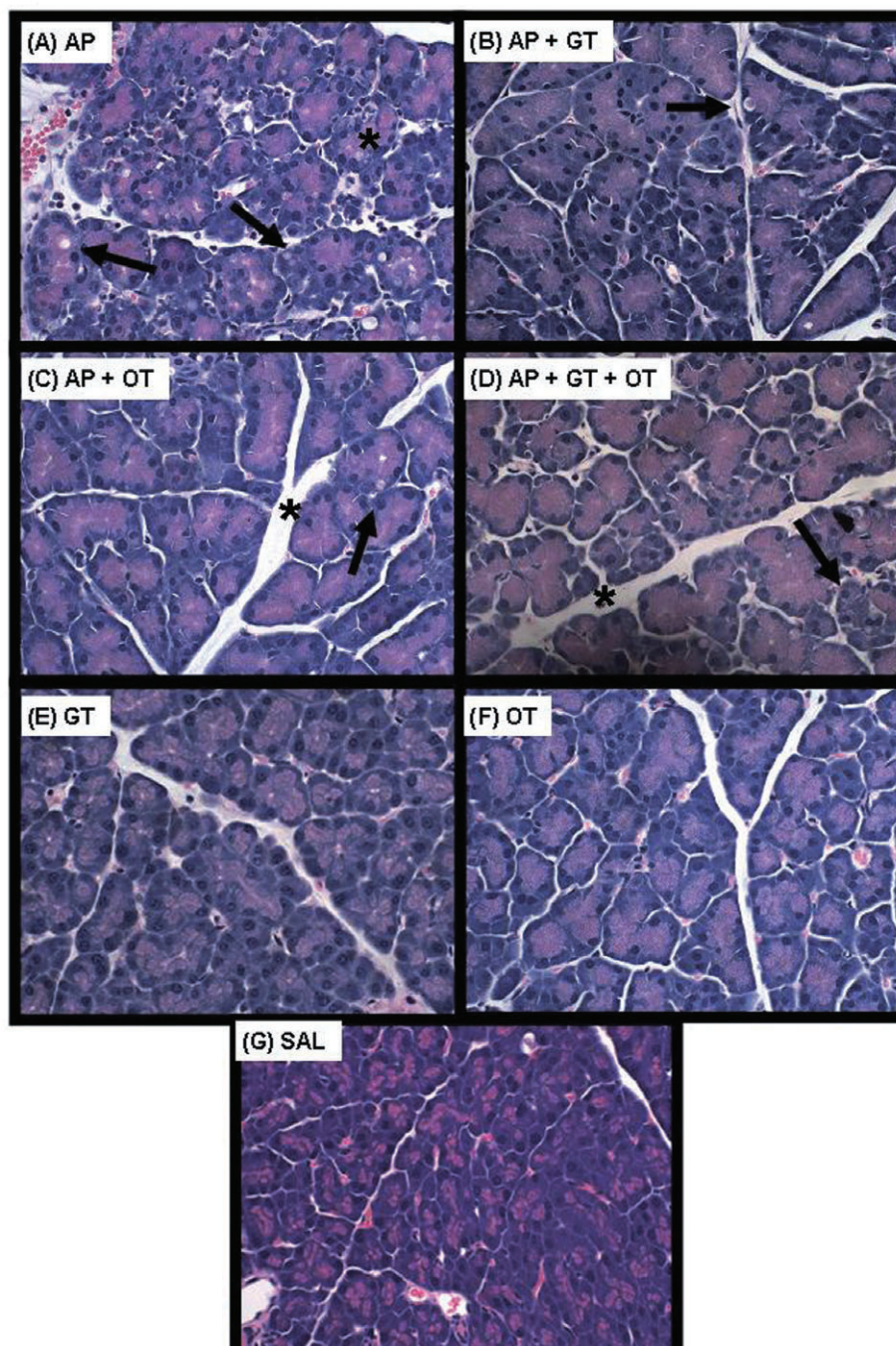


**Figure 4** Acute pancreatitis (AP)-induced pancreatic damage showing percentages of (A) abnormal pancreatic acinar cells and (B) pancreatic interstitial oedema in different experimental groups. Induction of AP resulted in a significant increase in the percentage of abnormal acinar cells. Treatment with galantide (GT) or octreotide (OT), individually and in combination, reduced the percentage of abnormal acinar cells compared with that in the AP-alone group. The OT- or GT-alone control groups showed a small but statistically significant increase in the percentage of abnormal pancreatic acinar cells compared with the saline group. The saline group displayed no abnormal pancreatic acinar cells. Only the treatment with OT significantly reduced pancreatic interstitial oedema compared with AP alone. Oedema in the OT- or GT-alone control groups did not significantly differ from that in the saline group. Data are presented as mean + standard error ( $n = 4-12$ ). \* $P < 0.05$  compared with the saline group; † $P < 0.05$  compared with the AP-alone group

Ischaemia involving the pancreatic microvasculature is a key trigger in the progression of pancreatic damage after the onset of AP.<sup>25</sup> Galanin has been shown to induce pancreatic microvascular vasoconstriction<sup>6</sup> and galantide has been shown to be vasodilatory, which may prevent the development of pancreatic necrosis. Moreover, galanin is known to inhibit the secretion of insulin.<sup>8</sup> Therefore, it is also possible that galantide, by antagonizing galanin's inhibition of insulin release, may increase local concentrations of insulin. Insulin is important for the regeneration of acinar cells following AP<sup>26</sup> and potentiates the expression of heat shock proteins including hsp 70.<sup>27,28</sup> Insulin is also known to induce actin-cytoskeletal reorganization in order to mediate a wide variety of biological actions, including cell growth, migration and metabolism.<sup>29</sup>

A recent study by Schmidhuber *et al.*<sup>30</sup> reported that adult mice carrying a loss-of-function mutation in the galanin gene (Gal KO) demonstrated an absence of the normal neurogenic inflammatory response upon treatment of the skin with either noxious heat or the vanilloid receptor 1 agonist capsaicin. These and other findings reported by the same authors suggest a role for galanin in neurogenic inflammation in murine skin.<sup>30</sup> Neurogenic inflammation is a contributor to AP.<sup>31</sup> Galantide may ameliorate AP by attenuating neurogenic inflammation by virtue of its antagonism of galanin's effects in the pancreas.<sup>3</sup>

Octreotide, a long-acting somatostatin analogue, has been extensively studied in relation to AP. The rationale behind the use of octreotide as a treatment for AP is based on several observations, including its inhibitory effects on pancreatic exocrine secre-



**Figure 5** Representative haematoxylin and eosin stained photomicrographs of pancreatic sections for the various groups. (A) The induction of acute pancreatitis (AP) resulted in abnormal acinar cells (arrow) and interstitial oedema (\*). Co-administration of (B) galantide (GT) (66  $\mu\text{g}/\text{kg}$ ), (C) octreotide (OT) (250  $\mu\text{g}/\text{kg}$ ) or (D) the combination of the two peptides with caerulein (AP + GT + OT) resulted in a significant reduction in abnormal acinar cells. Normal acinar cell architecture was seen following administration of (E) GT alone (66  $\mu\text{g}/\text{kg}$ ), (F) OT alone (250  $\mu\text{g}/\text{kg}$ ) and (G) saline (SAL). Original magnification  $\times 40$

tion<sup>32</sup> and its ability to reduce the degree of leukocyte infiltration and focal areas of pancreatic tissue necrosis in experimentally induced AP.<sup>12</sup> Its ability to increase the tone of the sphincter of Oddi has been used in the prevention and treatment of post-

ERCP pancreatitis, in which it is believed to reduce the reflux of duodenal contents into the pancreatic duct.<sup>33</sup>

The effects of octreotide on pancreatic exocrine secretion have been studied in animals<sup>9-15</sup> as well as humans<sup>34,35</sup> in terms of its

potential as a therapeutic agent for AP. The proposed mechanisms by which octreotide was shown to ameliorate AP in previous studies included the inhibition of basal and the stimulation of exocrine pancreatic secretion,<sup>36</sup> by which it 'puts the pancreas to rest', reduces the degree of leukocyte infiltration and limits focal areas of pancreatic tissue necrosis in experimentally induced AP,<sup>12</sup> the reduction of lipid peroxidation,<sup>37</sup> and the production of histological changes consistent with organization and healing in established cases of AP.<sup>9</sup> However, reports on the action of somatostatin and octreotide on pancreatic exocrine secretion are inconsistent and reflect the use of different species and concentrations and the complexity of the system under study.<sup>9,12,38,39</sup>

Our current findings of a lack of effect of octreotide on plasma enzyme levels in a caerulein model of AP are comparable with those of previous studies using a similar model.<sup>38,39</sup> However, the mechanisms responsible are unclear. One possible mechanism could be that offered by Jenkins *et al.*<sup>40</sup> These authors showed that following the initial inhibition of pancreatic enzyme and overall juice secretion by octreotide, continued administration of the drug resulted in a steep increase ('escape phenomenon') in the enzyme level despite a continued inhibition of overall juice secretion within 4–5 h. This 'escape phenomenon' may reflect the desensitization of the somatostatin receptors involved in the regulation of pancreatic amylase secretion. Using a mouse pancreatic lobule preparation under experimental conditions equivalent to those used in the present study, we recently showed that galanin potentiated amylase secretion induced by supramaximal concentrations of caerulein and that this response was partly mediated by endogenous somatostatin, indicating an interaction between galanin and somatostatin.<sup>24</sup> Thus the caerulein-induced release of endogenous somatostatin may occupy the somatostatin receptors regulating acinar cell secretion, thereby preventing the binding of octreotide and consequently resulting in no change in AP-induced hyperenzymaemia with octreotide treatment. Another explanation may involve the inability of octreotide to overcome cholecystokinin (CCK)-stimulated amylase secretion, as reported by Garvin *et al.*<sup>41</sup>

In the present study we also noted an unexpected reduction in the efficacy of the agents when used in combination, suggesting interactions at one or more sites. As outlined above, interactions between galanin and somatostatin can occur in the presence of supramaximal concentrations of caerulein. Another explanation may relate to the reported potentiation of CCK-stimulated amylase secretion by somatostatin,<sup>42</sup> which could mask or negate the effect of galantide and result in no significant change in hyperenzymaemia. Further studies are required to understand this unexpected result.

The lack of effect of the combination of these peptides on pancreatic MPO activity suggests opposing mechanisms of action, a proposal that is difficult to reconcile. These actions may occur at the neutrophil recruitment level and/or at the cellular/intracellular level to modulate the neutrophil activation state, and hence MPO activity, of the infiltrating neutrophils. As described

above, Schmidhuber *et al.*,<sup>30</sup> found that galanin is involved in neurogenic inflammation caused by substance P. Galantide contains moieties of galanin and substance P<sup>8</sup> and could thus exert its effect of reducing MPO activity by a mechanism involving either galanin and/or substance P. Somatostatin has been shown to block substance P receptors<sup>43</sup> and consequently may possibly prevent galantide from binding to these receptors and exerting its beneficial effects (via substance P antagonism). This may be an example of common receptor activity of these two peptides. Another reason for the decreased reduction in MPO activity seen with octreotide as well as with the combination of the two peptides may involve the contribution to MPO activity of activated monocytes and macrophages<sup>44</sup> as octreotide has been shown to promote activation of the monocyte–macrophage system.<sup>45,46</sup> Octreotide has also been shown to inhibit neutrophil chemotaxis induced by substance P.<sup>43</sup>

Our finding that the combination of the two peptides administered at the induction of AP has no benefit does not support further testing of the combination in a delayed setting (i.e. after the induction of AP).

## Conclusions

This study is the first to explore the efficacy of a combination of two peptides, galantide and octreotide, in the treatment of mild caerulein-induced AP in mice. From this study we conclude that treatment with galantide alone ameliorates mild caerulein-induced AP, whereas treatment with octreotide alone reduces pancreatic MPO activity and acinar cell damage. The combination of the two peptides appears to negate the individual benefits of the two agents, which may indicate complex interaction(s) in the mechanism of action of the two peptides at several sites.

## Acknowledgement

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## Conflicts of interest

None declared.

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