

# Concentration of tissue angiotensin II increases with severity of experimental pancreatitis

著者	古河 浩之
著者別表示	Furukawa Hiroyuki
journal or publication title	博士論文本文Full
学位授与番号	13301甲第3977号
学位名	博士(医学)
学位授与年月日	2013-12-31
URL	<a href="http://hdl.handle.net/2297/41607">http://hdl.handle.net/2297/41607</a>

doi: 10.3892/mmr.2013.1509



## Concentration of tissue angiotensin II increases with severity of experimental pancreatitis

HIROYUKI FURUKAWA<sup>1</sup>, ATSUSHI SHINMURA<sup>1</sup>, HIDEHIRO TAJIMA<sup>1</sup>, TOMOYA TSUKADA<sup>1</sup>, SHIN-ICHI NAKANUMA<sup>1</sup>, KOICHI OKAMOTO<sup>1</sup>, SEISHO SAKAI<sup>1</sup>, ISAMU MAKINO<sup>1</sup>, KEISHI NAKAMURA<sup>1</sup>, HIRONORI HAYASHI<sup>1</sup>, KATSUNOBU OYAMA<sup>1</sup>, MASAFUMI INOKUCHI<sup>1</sup>, HISATOSHI NAKAGAWARA<sup>1</sup>, TOMOHARU MIYASHITA<sup>1</sup>, HIDETO FUJITA<sup>1</sup>, HIROYUKI TAKAMURA<sup>1</sup>, ITASU NINOMIYA<sup>1</sup>; HIROHISA KITAGAWA<sup>1</sup>, SACHIO FUSHIDA<sup>1</sup>, TAKASHI FUJIMURA<sup>1</sup>, TETSUO OHTA<sup>1</sup>, TOMOHIKO WAKAYAMA<sup>2</sup> and SHOICHI ISEKI<sup>2</sup>

<sup>1</sup>Department of Gastroenterological Surgery, Division of Cancer Medicine; <sup>2</sup>Department of Histology and Embryology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Ishikawa 920-8641, Japan

Received January 23, 2013; Accepted May 30, 2013

DOI: 10.3892/mmr.2013.1509

**Abstract.** Necrotizing pancreatitis is a serious condition that is associated with high morbidity and mortality. Although vasospasm is reportedly involved in necrotizing pancreatitis, the underlying mechanism is not completely clear. In addition, the local renin-angiotensin system has been hypothesized to be involved in the progression of pancreatitis and trypsin has been shown to generate angiotensin II under weakly acidic conditions. However, to the best of our knowledge, no studies have reported elevated angiotensin II levels in tissue with pancreatitis. In the present study, the concentration of pancreatic angiotensin II in rats with experimentally induced acute pancreatitis was measured. Acute pancreatitis was induced by retrograde injection of 6% sodium taurocholate into the biliopancreatic duct. Control rats were sacrificed without injection into the biliopancreatic duct. The concentration of tissue angiotensin II was measured using the florisol method. Angiotensin II concentration in tissues with acute pancreatitis measured at 3, 6, 12 and 24 h following taurocholate injection were significantly higher than that of normal pancreatic tissue. In addition, the concentration of angiotensin II increased in a time-dependent manner. The results demonstrated that the angiotensin II generating system is involved in the transition from edematous to necrotizing pancreatitis in experimental animals. We hypothesize that locally formed angiotensin II affects the microenvironment in pancreatitis.

### Introduction

Acute pancreatitis, particularly the necrotizing type, is associated with high morbidity and mortality. Necrotizing pancreatitis is characterized by parenchymal non-enhancement on contrast-enhanced computed tomography images (1). Although necrosis is irreversible, certain patients exhibiting this pancreatic parenchymal non-enhancement recover with normal pancreatic conditions (2). Vasospasm has been implicated in the development of pancreatic ischemia and necrosis (3); however, the precise underlying mechanism is unclear.

The local pancreatic renin-angiotensin system (RAS) may be a significant etiological factor of pancreatitis (4-9). Angiotensin II receptors have been detected in the endothelium of blood vessels and the epithelium of the pancreatic ductal system (8). Previous studies have revealed that experimental pancreatitis upregulates the RAS components in the pancreas (10,11). However, elevated local angiotensin II levels in pancreatitis tissues have not been reported. Trypsin may directly induce the generation of the pressor angiotensin II from angiotensinogen at a weakly acidic pH, independent of the action of angiotensin-converting enzyme (12,13).

In the present study, pancreatic angiotensin II concentration was measured in experimental animals with pancreatitis to evaluate the involvement of the local angiotensin II generating system in acute pancreatitis.

### Materials and methods

*Animal and experimental acute pancreatitis model.* The present study was conducted in compliance with the Division for Animal Research Resources, Institute of Kanazawa University. The experiments and procedures were approved by the Animal Care and Use Committee of the Kanazawa University (Ishikawa, Japan).

Male Wistar rats (weight, 250-350 g) were maintained on a 12-h light/dark cycle at an ambient temperature of 23°C.

---

*Correspondence to:* Dr Hiroyuki Furukawa, Department of Gastroenterological Surgery, Division of Cancer Medicine, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8641, Japan  
E-mail: keijuhospital@hotmail.co.jp

*Key words:* pancreatitis, angiotensin II, trypsin, taurocholate

The rats were fasted with free access to water for 12 h prior to surgery. Rats were anesthetized with diethyl ether inhalation and laparotomy was performed using a midline abdominal incision. Acute pancreatitis was induced by retrograde injection of 6% sodium taurocholate (Wako Pure Chemical Industries, Osaka, Japan) into the biliopancreatic duct at a dose of 0.4 ml/kg body weight to induce acute pancreatitis. Time 0 indicates the time of injection of sodium taurocholate. The rats were divided into groups and sacrificed at 3, 6, 12, 24 or 36 h following the induction of acute pancreatitis. Control rats were sacrificed without injection into the biliopancreatic duct.

**Sample collection and preparation.** Blood samples were collected by cardiac puncture for the measurement of plasma amylase levels. Pancreatic tissue samples were fixed in 10% neutral-buffered formalin and embedded in paraffin for histological examination. Parallel samples of pancreatic tissue were prepared for the measurement of angiotensin II concentration by immediately freezing in liquid nitrogen, followed by storage at  $-80^{\circ}\text{C}$  until the time of assay.

**Measurements of angiotensin II in tissues.** Frozen tissue samples were homogenized at  $4^{\circ}\text{C}$  in saline containing 0.1% NHC1 and 5% urinastatin. The homogenate was centrifuged at  $10,000 \times g$  for 30 min at  $4^{\circ}\text{C}$  and the supernatant was collected for radioimmunoassay of angiotensin II using the florisil method, as described previously (14).

**Statistical analysis.** Data were analyzed using the Welch's t-test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Plasma amylase levels.** Plasma amylase levels were measured to confirm the successful induction of acute pancreatitis. Following this induction, plasma amylase levels were elevated (Fig. 1).

**Histological examination.** In the early stages of pancreatitis, histological examination indicated subcapsular and interlobular edema, slight inflammatory neutrophilic infiltration and a small number of intralobular necrotic lesions. The extent of inflammatory cell infiltration and areas of necrosis progressed with time. At 24 h following taurocholate injection, marked inflammatory and necrotic changes were noted (Fig. 2A-E). In addition, intravenous thromboses were observed, but there was no evidence of arterial thrombosis (Fig. 2F).

**Angiotensin II concentration in experimental pancreatitis tissue.** Angiotensin II concentration was measured in pancreatic tissue samples obtained at 3, 6, 12, 24 and 36 h following taurocholate injection. Up to 24 h, the angiotensin II concentration was significantly higher in rats with taurocholate-induced pancreatitis compared with controls and the enzyme levels were found to increase in a time-dependent manner. However, the angiotensin II concentration in pancreatitis tissue at 36 h following taurocholate injection was lower than that in pancreatitis tissue at 24 h following injection (Fig. 3).

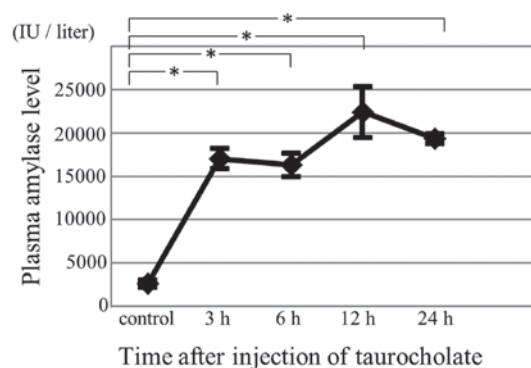


Figure 1. Plasma amylase levels in rats with taurocholate-induced pancreatitis were significantly higher than those of the control ( $P < 0.05$ ). Data are presented as the mean  $\pm$  SD.

## Discussion

Premature activation of trypsin within pancreatic acinar cells by various proteases, which in turn are activated by ectopic activation of trypsinogen, is hypothesized to represent the main etiological factor in pancreatitis. A number of studies in acute pancreatitis have examined the mechanisms by which edematous injury progresses to necrosis. In experimental models, pancreatic vascular perfusion failure has been implicated in the development of pancreatitis. Spormann *et al* (15) demonstrated that temporary ischemia significantly augmented the severity of pathology and Klar *et al* (16) revealed that vasoconstrictor administration has adverse effects in experimental acute pancreatitis. Intravascular thromboses have been indicated to represent a significant factor (17). Although intravenous thromboses were noted in the present study, arterial thromboses were not observed.

Deterioration of the balance between nitric oxide and endothelins has also been hypothesized to account for the development of necrotizing pancreatitis (18,19); however, the association between this abnormal balance and trypsin, the key molecule in the etiology of pancreatitis, is unclear.

It has been hypothesized that an intrinsic tissue RAS may be present in the pancreas (4). Chan *et al* (20) have demonstrated activation of the pancreatic RAS in a rat model of chronic hypoxia. Leung *et al* (10) revealed that experimental pancreatitis induced upregulation of several key RAS components in the pancreas. Several other studies have reported that RAS blockers are effective in the treatment of experimental pancreatitis (21-23).

Hypovascularity is a key characteristic of not only necrotizing pancreatitis but also pancreatic ductal cancer. The majority of pancreatic ductal cancers are hypovascular or avascular on angiography, but the angiography of surgically resected specimens cannot always accurately confirm poor circulation. Figarella *et al* (24) reported that cationic trypsinogen is spontaneously converted to trypsin under acidic conditions and Tajima *et al* (25) demonstrated that tumor-derived trypsin, activated by acidic conditions, was a key factor in tumor invasion. Ohta *et al* (26) found that angiotensin II acts on the pre-existing pancreatic arteries around the tumor leading to formation of hypovascular regions. Since the microenvironment around the tumor cells is more acidic than

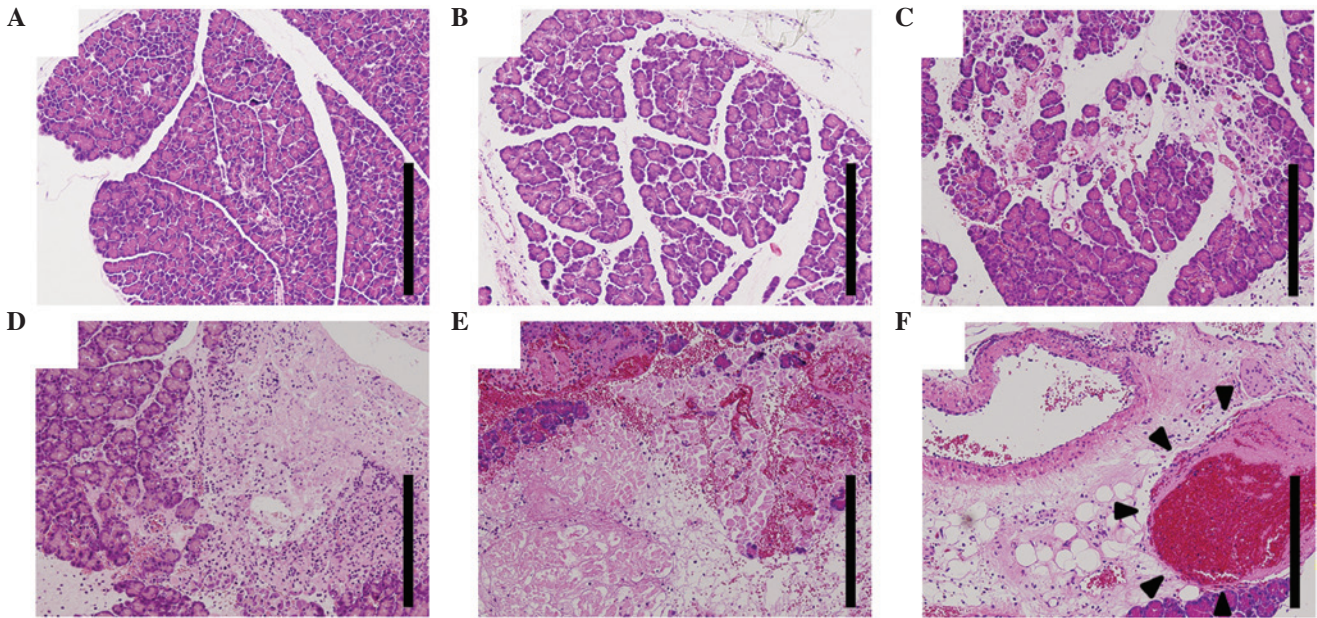


Figure 2. Histological examination of rat pancreatic tissue in taurocholate-induced pancreatitis. (A) Control, (B) 3, (C) 6, (D) 12 and (E) 24 h following taurocholate injection (H&E stain; magnification, x100; bar length, 250  $\mu$ m). Histopathological sections reveal (A) subcapsular and interlobular edema, slight inflammatory cell infiltration and (B) a small number of intralobular necrotic lesions; (C and D) inflammatory cell infiltration and areas of necrosis progressed in a time-dependent manner and (E) a large amount of necrosis and hemorrhage was observed. (F) Intravenous thromboses were observed (arrowheads), but arterial thrombosis was not noted. H&E, hematoxylin and eosin.

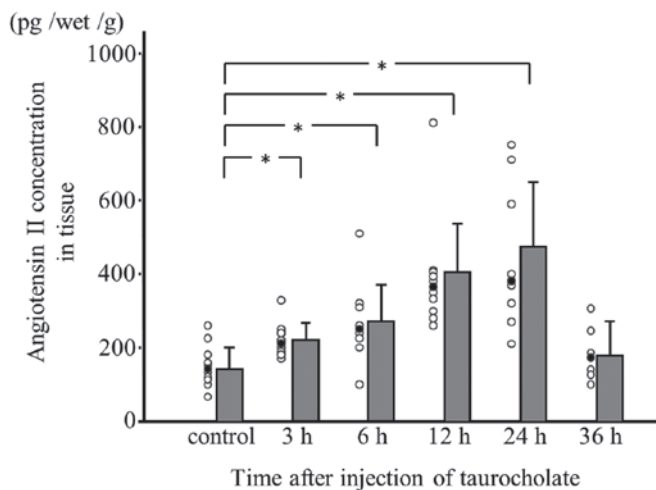


Figure 3. Pancreatic angiotensin II concentrations in rats with taurocholate-induced pancreatitis. Angiotensin II concentration was 151.2 ± 58.5 pg/g wet tissue in control pancreatic tissue and 225.5 ± 43.9, 270 ± 118.6, 414.5 ± 156.9 and 464.4 ± 192.9 pg/g wet tissue in pancreatic tissue at 3, 6, 12 and 24 h following injection of taurocholate, respectively. Tissue angiotensin II concentrations in pancreatitis at 3, 6, 12 and 24 h following injection were significantly higher than that in control pancreatic tissue ( $P < 0.05$ ). The concentration of angiotensin II in pancreatitis tissue at 36 h following injection was 182.4 ± 72.1 pg/g, lower than that at 24 h following injection. Each point represents the value and each bar represents the mean ± SD.

that of the majority of normal tissues, this phenomenon may be associated with tumor-derived trypsin, directly generating angiotensin II from angiotensinogen (27,28).

It was reported that the pH of the inflammatory exudate decreases with time (29-31) due to local acid production by glycolytic infiltrating neutrophils and low oxygen tension in inflammatory lesions. In the early phase of pancreatitis devel-

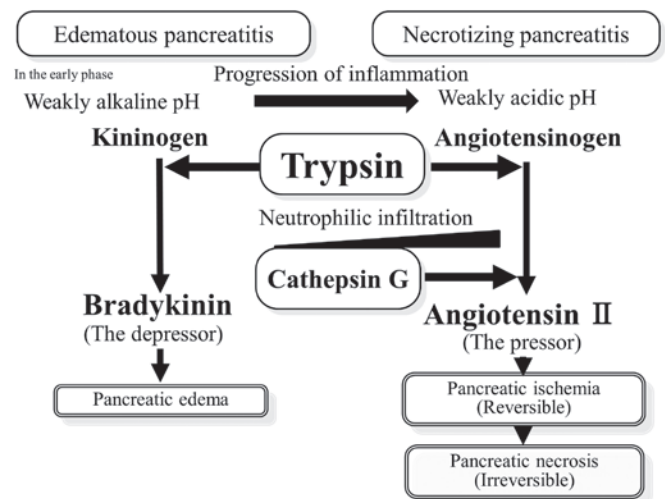


Figure 4. Proposed mechanism of the trypsin-related and cathepsin G-related angiotensin II generating system, and evolution of edematous pancreatitis into necrotizing pancreatitis. In the early phases of pancreatitis, the depressor bradykinin, is produced from kininogen by trypsin at a weakly alkaline pH. Thereafter, pancreatic hyperemia and edema develop (edematous pancreatitis). Inflammation progresses with time, as the pH decreases. Pressor angiotensin II is produced from angiotensinogen at a weakly acidic pH. Similarly, the inflammation progresses with time, as neutrophils infiltrate. Angiotensin II is produced by cathepsin G released by infiltrating neutrophils and pancreatic ischemia and necrosis develop (necrotizing pancreatitis).

opment, the depressor, bradykinin, was produced at a weakly alkaline pH. Therefore, pancreatic hyperemia and edema were observed. With time, the inflammation progressed, pH decreased and angiotensin II was produced under weakly acidic conditions by trypsin. In addition, pancreatic ischemia and necrosis were observed (Fig. 4). In our model of experimental pancreatitis, angiotensin II concentration decreased



at 36 h, as compared with that at 24 h. We hypothesize that pancreatic tissue destruction may be responsible for the degradation of angiotensin II.

Cathepsin G, one of the serine proteinases expressed in the azurophilic granules of neutrophils, has the potential to induce angiotensin II production (32). In the early phases of acute pancreatitis, the presence of subcapsular and intralobular edema, slight neutrophil infiltration and a small number of intralobular necrotic lesions were noted. The extent of neutrophil infiltration and areas of necrosis progressed over time. Cathepsin G released by infiltrating neutrophils may be involved in angiotensin II generation (33). As the inflammation progressed with time, angiotensin II was produced by cathepsin G released by infiltrating neutrophils (Fig. 4). A number of studies have reported that the neutrophil elastase inhibitor prevents neutrophil infiltration into the extravascular tissue (34,35). Although there are no cathepsin G inhibitors available for clinical use today, the neutrophil elastase inhibitor may suppress the angiotensin II generated by the cathepsin G released by infiltrating neutrophils.

In conclusion, the present study indicates that angiotensin II generation is instrumental in the transition from experimental edematous pancreatitis to necrotizing pancreatitis. We hypothesize that locally formed angiotensin II may act on the pancreatic arteries leading to regional hypovascularity. Therefore, inhibition of the angiotensin II generating system by administration of angiotensin receptor blockers, serine protease inhibitors or elastase inhibitors may be useful for preventing the evolution of edematous pancreatitis into necrotizing pancreatitis.

## References

- Bollen TL, van Santvoort HC, Besselink MG, *et al*: The Atlanta Classification of acute pancreatitis revisited. *Br J Surg* 95: 6-21, 2008.
- Traverso LW and Kozarek RA: Pancreatic necrosectomy: definitions and technique. *J Gastrointest Surg* 9: 436-439, 2005.
- Takeda K, Mikami Y, Fukuyama S, *et al*: Pancreatic ischemia associated with vasospasm in the early phase of human acute necrotizing pancreatitis. *Pancreas* 30: 40-49, 2005.
- Paul M, Poyan Mehr A and Kreutz R: Physiology of local renin-angiotensin systems. *Physiol Rev* 86: 747-803, 2006.
- Chappell MC, Millsted A, Diz DI, *et al*: Evidence for an intrinsic angiotensin system in the canine pancreas. *J Hypertens* 9: 751-759, 1991.
- Chappell MC, Jacobsen DW and Tallant EA: Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* 16: 741-747, 1995.
- Ghiani BU and Masini MA: Angiotensin II binding sites in the rat pancreas and their modulation after sodium. *Comp Biochem Physiol A Physiol* 111: 439-444, 1995.
- Leung PS, Chan WP, Wong TP, *et al*: Expression and localization of the renin-angiotensin system in the rat pancreas. *J Endocrinol* 160: 13-19, 1999.
- Tahmasebi M, Puddefoot JR, Inwang ER, *et al*: The tissue renin-angiotensin system in human pancreas. *J Endocrinol* 161: 317-322, 1999.
- Leung PS, Chan WP and Nobiling R: Regulated expression of pancreatic renin-angiotensin system in experimental pancreatitis. *Mol Cell Endocrinol* 166: 121-128, 2000.
- Ip SP, Kwan PC, Williams CH, *et al*: Changes of angiotensin-converting enzyme activity in the pancreas of chronic hypoxia and acute pancreatitis. *Int J Biochem Cell Biol* 35: 944-954, 2003.
- Arakawa K, Ikeda M, Fukuyama J, *et al*: A pressor formation by trypsin from renin-denatured human plasma protein. *J Clin Endocrinol Metab* 42: 599-602, 1976.
- Arakawa K and Maruta H: Ability of kallikrein to generate angiotensin II-like pressor substance and a proposed 'kinin-tensin enzyme system'. *Nature* 288: 705-706, 1980.
- Morimoto T, Aoyama M, Gotoh E, *et al*: A method of radioimmunoassay of plasma angiotensin II using florisil. *Nihon Naibunpi Gakkai Zasshi* 59: 15-29, 1983 (In Japanese).
- Spormann H, Sokolowski A and Letko G: Effect of temporary ischemia upon development and histological patterns of acute pancreatitis in the rat. *Pathol Res Pract* 184: 507-513, 1989.
- Klar E, Rattner DW, Compton C, *et al*: Adverse effect of therapeutic vasoconstrictors in experimental acute pancreatitis. *Ann Surg* 214: 168-174, 1991.
- Menger MD, Plusczyk T and Vollmar B: Microcirculatory derangements in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 8: 187-194, 2001.
- Liu XH, Kimura T, Ishikawa H, *et al*: Effect of endothelin-1 on the development of hemorrhagic pancreatitis in rats. *Scand J Gastroenterol* 30: 276-282, 1995.
- Foitzik T, Faulhaber J, Hotz HG, *et al*: Endothelin mediates local and systemic disease sequelae in severe experimental pancreatitis. *Pancreas* 22: 248-254, 2001.
- Chan WP, Fung ML, Nobiling R, *et al*: Activation of local renin-angiotensin system by chronic hypoxia in rat pancreas. *Mol Cell Endocrinol* 160: 107-114, 2000.
- Tsang SW, Cheng CH and Leung PS: The role of the pancreatic renin-angiotensin system in acinar digestive enzyme secretion and in acute pancreatitis. *Regul Pept* 119: 213-219, 2004.
- Chan YC and Leung PS: Angiotensin II type I receptor-dependent nuclear factor-kB activation-mediated proinflammatory actions in a rat model of obstructive acute pancreatitis. *J Pharmacol Exp Ther* 323: 10-18, 2007.
- Oruc N, Ozutemiz O, Nart D, *et al*: Inhibition of renin-angiotensin systems in experimental acute pancreatitis in rats: A new therapeutic target? *Exp Toxicol Pathol* 62: 353-360, 2010.
- Figarella C, Mischuk-Jamska B and Barrett AJ: Possible lysosomal activation of pancreatic zymogens. Activation of both human trypsinogens by cathepsin B and spontaneous acid. Activation of human trypsinogen I. *Biol Chem Hoppe Seyler* 369: 293-298, 1988.
- Tajima H, Ohta T, Elnemr A, *et al*: Enhanced incasiveness of pancreatic adenocarcinoma cells stably transfected with cationic trypsinogen cDNA. *Int J Cancer* 94: 699-704, 2001.
- Ohta T, Amaya K, Yi S, *et al*: Angiotensin converting enzyme-independent, local angiotensin II-generation in human pancreatic ductal cancer tissue. *Int J Oncol* 23: 593-598, 2003.
- Montcourrier P, Silver I, Farnoud R, *et al*: Breast cancer cells have a higher capacity to acidify extracellular milieu by a ductal mechanism. *Clin Exp Metastasis* 15: 382-392, 1997.
- Ohta T, Numata M, Yagishita H, *et al*: Expression of 16 kDa proteolipid of vacuolar-type H(+)-ATPase in human pancreatic cancer. *Br J Cancer* 73: 1511-1517, 1996.
- Donald WE and Walter HS: The pH of inflammatory exudates. *Proc Soc Exp Biol Med* 137: 1328-1332, 1971.
- Simmen HP, Battaglia H, Giovanoli P, *et al*: Analysis of pH, pO<sub>2</sub> and pCO<sub>2</sub> in drainage fluid allows for rapid detection of infectious complications during the follow-up period after abdominal surgery. *Infection* 22: 386-389, 1994.
- Ward TT and Steigbigel RT: Acidosis of synovial fluid correlates with synovial fluid leukocytosis. *Am J Med* 64: 933-936, 1978.
- Wintroub BU, Klickstein LB and Watt KW: A human neutrophil-dependent pathway for generation of angiotensin II. Purification of the product and identification as angiotensin II. *J Clin Invest* 68: 484-490, 1981.
- Rykl J, Thiemann J, Kuzawski S, *et al*: Renal cathepsin G and angiotensin II generation. *J Hypertens* 24: 1797-1807, 2006.
- Kawabata K, Hagio T, Matsumoto S, *et al*: Delayed neutrophil elastase inhibition prevents subsequent progression of acute lung injury induced by endotoxin inhalation in hamsters. *Am J Respir Crit Care Med* 161: 2013-2018, 2000.
- Young RE, Voisin MB, Wang S, *et al*: Role of neutrophil elastase in LTB<sub>4</sub>-induced neutrophil transmigration in vivo assessed with a specific inhibitor and neutrophil elastase deficient mice. *Br J Pharmacol* 151: 628-637, 2007.