Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/

Evidence of use: A selective COX-2 inhibitor in the treatment of experimental chronic pancreatitis induced by Dibutyltin dichloride

Sergij Sukhodolya[1], Tetyana Falalyeyeva[2*], Olena Kuryk[3], Victoriya Petrushenko[1], Tetyana Beregova[2]

[1] National Pirogov Memorial Medical University, Vinnitsa, Ukraine
[2] Taras Shevchenko National University of Kyiv, Volodymyrska, 64/13, Kyiv, 01601, Ukraine
[3] State Scientific Institution "Scientific and Practical Center Preventive and Clinical Medicine The State Administration"

ARTICLE INFO
Received 23 January 2015
Accepted 24 March 2015

Keywords:
chronic pancreatitis,
selective COX-2 inhibitor,
Dibutyltin dichloride.

ABSTRACT

The search for new prophylactic and therapeutic drugs for the treatment of chronic pancreatitis (CP) is an urgent problem in current pancreatology. A promising direction in CP therapy may be the use of nonsteroidal anti-inflammatory drugs. Hence, the aim of the present study was to investigate the selective inhibition properties of COX-2 rofecoxib on the development of pancreas fibrosis in rats with experimental chronic pancreatitis induced by Dibutyltin dichloride (DBTC). The 60 male albino Wistar rats of our study were placed into three groups of 20 animals in each: I – the intact control; II – that which received an intraperitoneal injection (i.p.) of DBTC (6 mg/g); III – those which, 28 days after administration of DBTC (6 mg/g, i.p.), received a two-week course of treatment of rofecoxib (5 mg/kg, i.p). One day after rofecoxib treatment was completed, analysis was undertaken regarding the level of amylase, as well as the pancreatic amylase and lipase in the blood serum and the prostaglandin E2 in the pancreatic tissue. In addition, the morphological condition of the pancreas was ascertained. The obtained data suggest that administration of Rofecoxib reduces the development of fibrosis and improves the morpho-functional state of the pancreas in rats with chronic pancreatitis induced by DBTC. Thus, treatment with a selective COX-2 inhibitor could be a possible strategy for improving the clinical outcome of patients with CP.

INTRODUCTION

Chronic pancreatitis (CP) is characterized by progressive fibrosis, pain and loss of exocrine and endocrine functions. Hence, quality of life is impaired and life expectancy is reduced [1]. Two main clinical manifestations of CP are pancreatic insufficiency and (chronic) abdominal pain. Pancreatic insufficiency is marked by exocrine dysfunction (resulting in impaired food digestion and absorption), and by endocrine dysfunction (resulting in diabetes mellitus). Pain, either in the form of recurrent attacks of pancreatitis (representing paralysis of the apical exocytosis in acinar cells) or in the form of constant and disabling pain, is usually the main symptom [2]. While, morbidity and mortality are secondary to chronic pain and complications (e.g., diabetes, pancreatic cancer), the mortality ratio is higher than that of the general population. At the 10th year after the onset of the disease, survival is estimated to be at 69-80% [1]. The average age at diagnosis is 35 to 55 years.

Management begins with lifestyle modifications (e.g., cessation of alcohol and tobacco use) and dietary changes, followed by analgesics and pancreatic enzyme supplementation. However, therapeutic endoscopy or surgery is often necessary. Yet, before proceeding with endoscopic or surgical interventions, physicians and patients should weigh the risks and benefits of each procedure. Therapeutic endoscopy is indicated for symptomatic or complicated pseudocyst, biliary obstruction, and decompression of pancreatic duct [3]. Surgical procedures include decompression for large duct disease (pancreatic duct dilatation of 7 mm or more) and resection for small duct disease. Lateral pancreaticojejunostomy is the most commonly performed surgery for patients with large duct disease [6], while,

* **Corresponding author**
e-mail: tfalalyeyeva@mail.ru

DOI: 10.1515/cipms-2015-0059
© 2015 Medical University of Lublin. All rights reserved

pancreatoduodenectomy is indicated for the treatment of chronic pancreatitis with pancreatic head enlargement [5]. Of note, patients with CP are at increased risk of pancreatic neoplasm; sometimes surveillance is advocated, but formal guidelines and evidence of clinical benefit are lacking [8].

Therefore, the search for new prophylactic and therapeutic drugs is an urgent problem in current pancreatology. One of the promising directions in today's approach to CP correction may be the use of nonsteroidal anti-inflammatory drugs. Indeed, positive indications for their use are increasing day by day. Nonsteroidal anti-inflammatory drugs (NSAIDs) are abundant medications, with tens million annual prescription cases and billion dollars of profit for drug-makers. Both the therapeutic and sides effects of NSAIDs are generated by inhibiting the ability of cyclooxygenase (COX) to produce prostaglandins from arachidonic acid. This leads to suppression of inflammation as a desired property [14]. Chronic inflammatory diseases have been successfully treated by specifically targeting COX-2, and elevated COX-2 levels have been identified in pancreatic tissue from patients with CP. What is more, it is known that a selective inhibition of the cyclooxygenase-2 (COX-2) inhibitor suppresses chronic pancreatitis in an animal model (WBN/Kob rats). Therein, significant reduction of macrophage infiltration and fibrosis [12] came about. Furthermore, recently, a rat model of fibrosis has been reported that requires injection of dibutyltin dichloride (DBTC), resulting in an initial phase of inflammation with neutrophils and macrophages, followed by fibrotic changes [9]. This raises the possibilities of undertaking more detailed research. Hence, the aim of the present study is to investigate the influence of selective inhibition of COX-2 rofecoxib on the development of the pancreas fibrosis in rats with experimental chronic pancreatitis induced by DBTC.

MATERIAL AND METHODS

The study was carried out on 60 male albino Wistar rats, weighing 200-250 g, in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, of the National Institutes of Health, with the approval from the Animal Ethical Research Committee of Taras Shevchenko National University of Kyiv. The rats were kept in collective cages at controlled conditions of temperature (22±3°C), light (12 h light/dark cycle) and relative humidity (60±5%). The animals were fed with laboratory chow and tap water ad libitum.

Firstly, CP in rats was induced by injection of DBTC [11]. The rats were then placed into three groups of 20 animals in each of them: I – the intact control; II – that which received an intraperitoneal injection (i.p.) of DBTC (6 mg/g); III – that which were administered DBTC (6 mg/g, i.p.) + rofecoxib (5 mg / kg, i.p) (see experiment design, Table 1).

Rofecoxib was administrated twenty-eight days after injection of DBTC, to the rats of the main group in the subsequent 14 days. One day after rofecoxib treatment completion (the 43rd day of the experiment), the state of the rats' pancreas was analyzed, regarding the level of amylase, pancreatic amylase and lipase in the blood serum, while the morphological condition of the pancreas was investigated.

Table 1. Experiment design and experimental groups

Animal groups		
Control group (n=20)	Dibutyltin dichloride (DBTC) (n=20)	DBTC + rofecoxib (n=20)
i.p. 0.4 ml /200 g of water	i.p. 0.4 ml/200 g of DBTC (6 mg/g)	i.p. 0.4 ml /200 g of DBTC (6 mg/g)
Water in a volume of 0.4 ml /200 g (two-week course).	28 days after administration of DBTC, these rats received by i.p., injections of water in a volume of 0.4 ml/200 g (two-week course).	28 days after administration of DBTC rats were administered by i.p., injections of rofecoxib (5 mg/kg, i.p) (two-week course).

We used a lethal dose of urethane (3 g/kg, intraperitoneally) for the rats sacrifice. Their pancreas tissue was extracted and fixed in 10% formalin, embedded in paraffin, then sectioned and stained with hematoxylin and eosin. What is more, serum amylase and lipase levels were determined by biochemical analysis. Moreover, the concentration of prostaglandin E2 in the pancreatic tissue of all groups was assessed by way of ELISA analysis (Assay Pro, USA).

The histological preparations were analyzed at magnification X100 and h400. Furthermore, color micrographs were obtained using a digital camera Olympus C-5050 Zoom and microscope Olympus BX-41 (Olympus Europe GmbH, Japan).

Statistical analysis. Statistical analysis of all data was performed by the "Statistica 8.0" software package, and Shapiro-Wilk W criterion was used for the analysis of the data distribution type. The Student test was used for the comparison of independent samples if the results were normally distributed. In addition, Mean of value (M) and Standard Error of the Mean (SEM) were calculated. Significant differences were considered at $p \leq 0.05$.

RESULTS

It was found that the body weight of the rats of all experimental groups, before the experiment, averaged 201±7.62 g, while the weight of the rats in the control group during the 43 days of the study increased by 38% ($p < 0.01$). In the control group of animals, we observed a 100% survival rate. Following DBTC administration, the body weight of rats decreased by 14% ($p < 0.01$), in comparison with the control group, probably because of pancreatic dysfunction. In this group, there was 50% mortality, which, we put forward, was the result of a severe form of CP. This opinion has been confirmed by other researchers [15].

However, the selective COX-2 inhibitor Rofecoxib prevented loss of body weight (Fig. 1) in that treated group. In addition, there was no difference in body weight at baseline between this group and the three other groups (Fig. 2). In the study group, we also recorded only one death case.

Regarding the analysis of the level of enzymes which characterize the state of the pancreas in all groups (amylase, pancreatic amylase, lipase), in the control group, the studied parameters in serum were within normal limits, while the concentration of serum enzymes in rats with CP was reduced. The difference in these figures, when compared with the control group, are: amylase – 77% ($p < 0.001$), pancreatic amylase – by 76% ($p < 0.001$) and lipase – by 86% ($p < 0.001$) (Table 2).

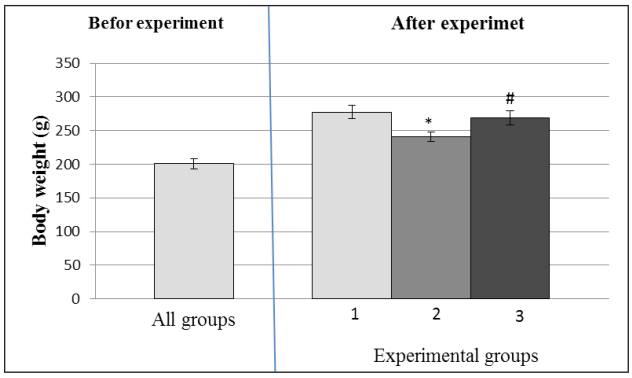


Figure 1. The body weight of all rats in all experimental groups: 1 – Control group; 2 – Group with Dibutyltin dichloride (DBTC); 3 – Group with DBTC + Rofecoxib. Values are mean (SEM). * – $p < 0.05$ comparison with control, # – $p < 0.05$ comparison with DBTC

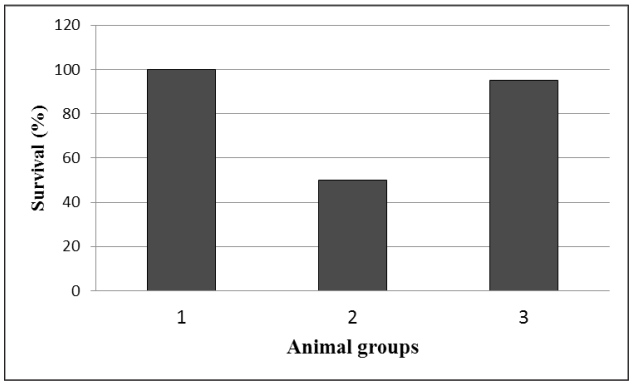


Figure 2. The survival of all rats of all experimental groups: 1 – Control group; 2 – Group with Dibutyltin dichloride (DBTC); 3 – Group with DBTC + Rofecoxib. Values are mean (SEM)

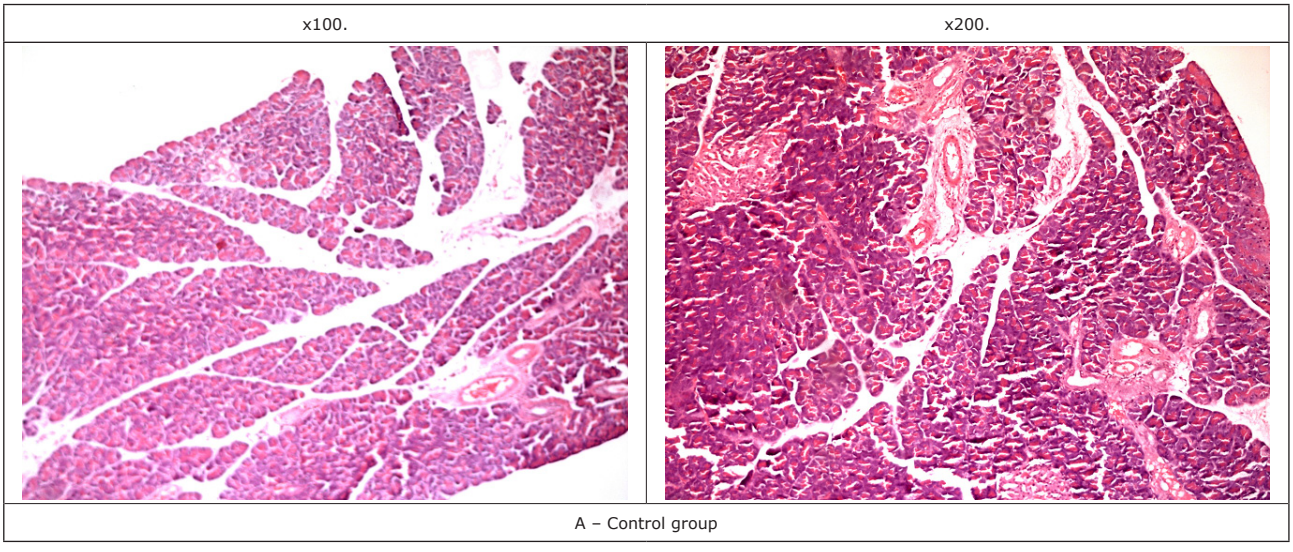
Table 2. The level of enzymes in serum (U/L) in the rats of the different experimental groups.

Enzymes / Groups	Amylase	Pancreatic Amylase	Lipase
Control group	61.5±4.8	22.2±1.5	17.5±1.5
Group with Dibutyltin dichloride (DBTC)	14.4±3.2*	5.3±1.7*	2.3±1.5*
Group with DBTC + Rofecoxib	36.8±5.9*/#	15.2±2.3*/#	11.4±1.9*/###

* – $p < 0.05$ comparison with control,
# – $p < 0.05$,
– $p < 0.001$ comparison with DBTC

The reduced enzyme activity in the rats of Group II confirms the development of pancreas exocrine insufficiency. The level of enzymes in rats with CP after the treatment with rofecoxib, however, significantly increased. Compared with the untreated rats, amylase increased by 155% ($p < 0.05$), pancreatic amylase – +186% ($p < 0.05$) and lipase – +395% ($p < 0.05$). These results suggest a partial restoration of exocrine pancreatic function in this group. Regarding the state of the pancreas, the pancreatic tissue in rats of I group have normal structure (Fig. 3, A), while a pancreatic fibrosis with loss of acinar cells was observed 28 days after a single administration of DBTC in rats. The pancreas tissue of the rats which received DBTC also showed changes within the pancreatic ducts in the form of dilatation of their lumen, as well as the flattening and focal atrophy of the ductal epithelium, with focal proliferation of epithelial cells. Moreover, administration of DBTC resulted in the development of fibrosis in the pancreas tissue (Fig. 3, B). Fibrosis of moderate degree was also seen around the ducts. In addition, fibrous tissue was loose, with blood vessels. Furthermore, there was a marked polymorphonuclear cell infiltration in the perivascular and periductal areas. The changes were accompanied by a mild inflammatory infiltrate composed of lymphocytes, plasma cells and scattered neutophils. Foci of degenerative changes and atrophy of acinar cells were also observed. What is more, areas of fibrosis were seen throughout the parenchyma of the exocrine pancreas. However, 14 days of treatment with Rofecoxib reduced the development of fibrosis and improved the morpho-functional state of the pancreas in rats with chronic pancreatitis induced by DBTC. In the pancreatic tissue of rats from this study group, we saw fixed moderate perivascular fibrosis and edema. We had found, as well, a significant reduction in the severity of inflammation (Fig. 3, C).

The content of COXs is showed indirectly by assessing prostaglandin (PG) E_2, the active secretory product of the enzymatic cascade initiated by COXs. In our work, the concentration of PGE_2 was increased by 200% ($p < 0.001$) in the pancreas of rats with experimental induced CP brought about by DBTC. However, a significant reduction of PGE_2 levels was evident in animals treated with rofecoxib (Fig. 4). Hence, systemic inhibition of COX-2 lowers PGE_2 secretion in the pancreas.



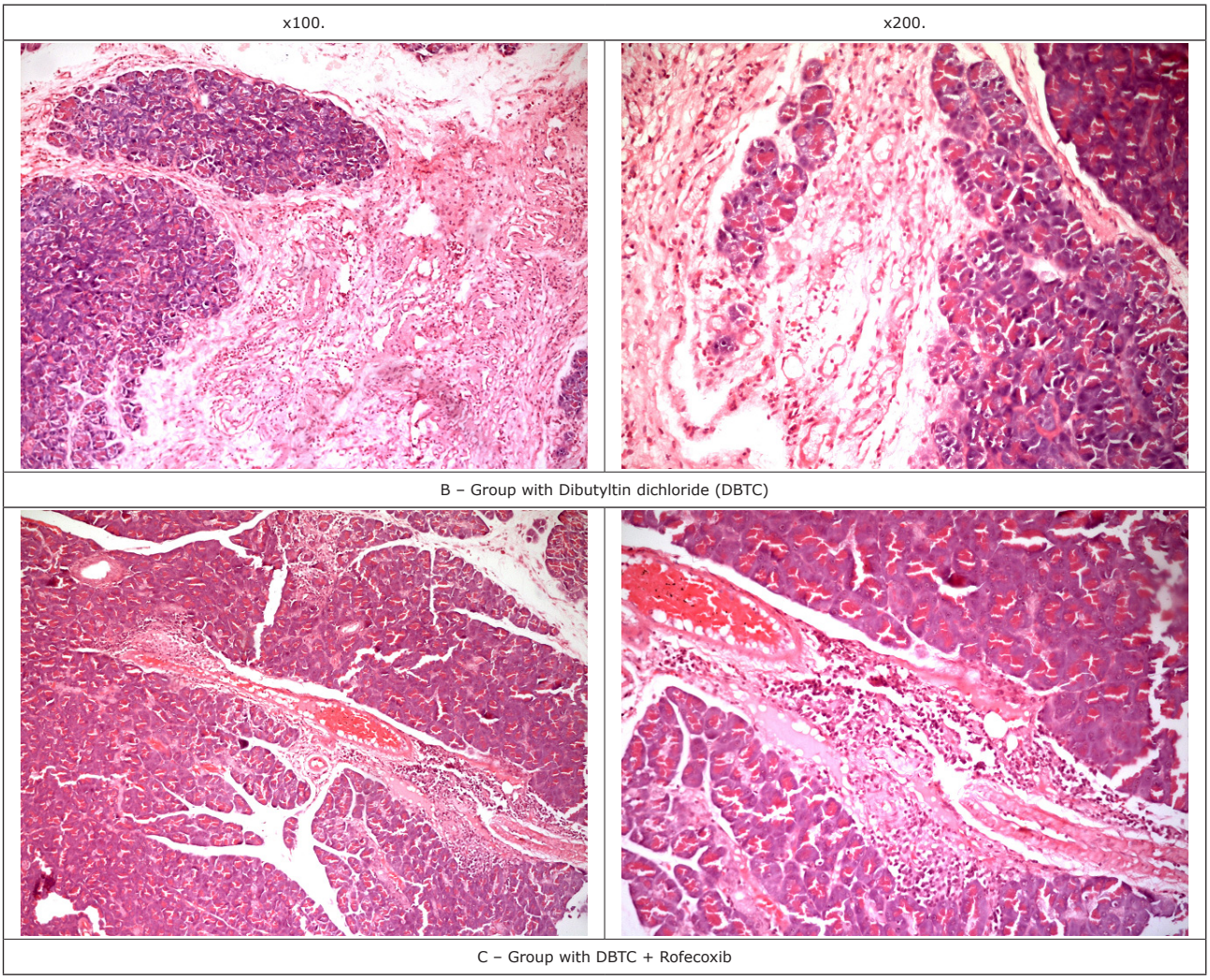


Figure 3. Light microscopic micrographs of the pancreas tissue of studied rats stained with hematoxylin and eosin

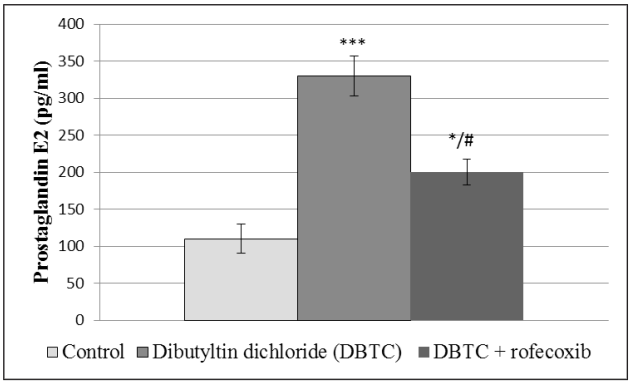


Figure 4. The concentration of prostaglandin E_2 in rat pancreas with experimental chronic pancreatitis induced by DBTC, with and without Rofecoxib treatment. Values are mean (SEM). * – $p < 0.05$, *** – $p < 0.001$ comparison with control, # – $p < 0.05$ comparison with DBTC

DISCUSSION

CP is recognized as a chronic inflammatory disease, and is characterised by progressive loss of parenchyma and subsequent loss of pancreatic exocrine and endocrine function. Morphological findings in CP, such as inflammatory infiltration of pancreatic tissue, fibrosis, atrophy of acinar cells, calcification, pancreatic duct strictures, etc., can be found at the very beginning of the disease. Moreover, the second stage of the disease is characterised by various degrees of exocrine dysfunction, and later on endocrine dysfunction [13].

For choice of an adequate model of chronic pancreatitis, we took into account the etiological factors of this disease. The etiology of CP is multifactorial. Many patients have this disease as a result of a complex mix of environmental (alcohol, cigarettes, and occupational chemicals) and genetic factors (mutation in a trypsin-controlling gene or the cystic fibrosis transmembrane conductance regulator); a few patients have hereditary or autoimmune disease [2]. Alcohol abuse, however, constitutes the major etiology. Yet, animal models based on the application of alcohol to induce chronic inflammation have not been very successful [11]. Genetic mouse models were not available for us. Furthermore, these have proven to be of only limited value [11]. Hence, the majority of experimental studies use Wistar Bonn/Kobori (WBN/Kob) rats which develop pancreatitis spontaneously. Of note, the WBN/Kob rat, exhibits spontaneous inflammation and fibrosis that can be used to investigate early pathophysiologic events [12]. More recently, a rat model of fibrosis has been reported that requires a single injection of DBTC, resulting in an initial phase of inflammation with

neutrophils and macrophages, followed by fibrotic changes [10]. In the present study, we used this model of CP.

Therapeutic strategies to treating CP are mostly symptomatic and very limited, yet, other chronic inflammatory diseases have been successfully treated by specifically targeting COX-2. Elevated COX-2 levels have been identified in pancreatic tissue from patients with CP [4]. Thus, we examined the effects of the selective COX-2 inhibitor Rofecoxib on the intensity of inflammatory reaction and fibrosis in the pancreas in DBTC induced CP. Our results suggest that Rofecoxib administered (as a two-week course) at the dose of 5 mg/kg, body wt, i.p. had good successful therapeutic effect. Rofecoxib reduced the development of fibrosis and improved the morpho-functional state of the pancreas in rats with CP caused by DBTC.

The secretory products of the COX system are prostaglandins (PG), primarily PGE2, acting in an autocrine or paracrine fashion. However, it is unclear whether PGE2 produced by pancreatic cells promotes inflammation. Furthermore, it is unclear whether the infiltrating inflammatory cell population of the pancreas (for example, neutrophils, lymphocytes, and macrophages) expresses COX-2. These inflammatory cells are attracted to the pancreas and promote the destruction of the parenchyma, and by their phagocytic activity remove dying cells and cell debris.

Yet, the presence of COXs was demonstrated indirectly by assessing PGE_2. In our work, the concentration of PGE_2 was increased in rats pancreas with experimental CP brought about by administration of DBTC. Moreover, we established that a significant reduction of PGE_2 levels comes about in animals treated with rofecoxib. Accordingly, systemic inhibition of COX-2 lowers PGE_2 secretion in the pancreas.

Reding T. *et al.* [12] have employed the WBN/Kob rat model to study the regulation and localization of secretory pancreatic stress proteins which are highly up-regulated during the inflammatory disease. They found that cyclooxygenase (COX-2) is highly increased in these animals, indicating that this disease might be a target for treatment with COX -2 inhibitors such as Rofecoxib. Furthermore, their work has shown that WBN/Kob rats treated with Rofecoxib exhibited a marked reduction and delay of histopathological parameters of acute and chronic pancreatic changes. In addition, cytokines and chemoattractants were strongly reduced. On a cellular level, they noted that the infiltration of macrophages was blocked. In addition, subsequent fibrosis was strongly reduced, suggesting that the inhibitor had an effect on inflammation and fibrosis. To test whether this inhibitor had a direct effect on macrophage migration, they used chemotaxis assays. In so-doing, they revealed that the directional response to a chemoattractant was disrupted in the presence of Rofecoxib.

In summary, in our work, administration of Rofecoxib prevented the progression of pancreatic fibrosis induced by DBTC in rats. The obtained results substantiate the possibility of nonsteroidal anti-inflammatory drugs employment in CP treatment. Thus, the use of a selective COX-2 inhibitor in such treatment could be a possible strategy for improving clinical outcome in patients with CP.

REFERENCES

1. Bang U.C. *et al.*: Mortality, cancer, and comorbidities associated with chronic pancreatitis: A Danish nationwide matched-cohort study. *Gastroenterol.*, 146, 989-994, 2014.
2. Braganza J.M. *et al.*: Chronic pancreatitis. *Lancet*, 377, 1184-1197, 2011.
3. Dumonceau J.M. *et al.*: Treatment for painful calcified chronic pancreatitis: extracorporeal shock wave lithotripsy versus endoscopic treatment: A randomised controlled trial. *Gut*, 56, 545-552, 2007.
4. Hashimoto T. *et al.*: Apoptosis of acinar cells is involved in chronic pancreatitis in WBN/Kob rats: Role of glucocorticoids. *Pancreas*, 21, 296-304, 2000.
5. Jimenez R.E. *et al.*: Outcome of pancreaticoduodenectomy with pylorus preservation or with antrectomy in the treatment of chronic pancreatitis. *Ann Surg.*, 231, 293-300, 2000.
6. Kalady M.F. *et al.*: Immediate and long-term outcomes after lateral pancreaticojejunostomy for chronic pancreatitis. *Am Surg.*, 67, 478-483, 2001.
7. Kono H. *et al.*: Development of an animal model of chronic alcohol-induced pancreatitis in the rat. *Am J Physiol Gastrointest Liver Physiol.*, 280, 1178-1186, 2001.
8. Malka D. *et al.*: Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut*, 51, 849-852, 2002.
9. Mydro A. *et al.*: RAS inhibitors decrease apoptosis of acinar cells and increase elimination of pancreatic stellate cells after in the course of experimental chronic pancreatitis induced by Dibutyltin Dichloride. *J of Physiol. and Pharmacol., 59, 2, 239-249, 2008.*
10. Nair R.J., Lawler L, Miller M.R.: Chronic pancreatitis. *Am Fam Physician.*, 1, 76, 11, 1679-1688, 2007.
11. Otsuki M., Yamamoto M., Yamaguchi T.: Animal Models of Chronic Pancreatitis Gastroenterol. *Research and Practice*, 8 pages, 2010.
12. Reding T. *et al.*: A Selective COX-2 inhibitor suppresses chronic pancreatitis in an animal model (WBN/Kob rats): Significant reduction of macrophage infiltration and fibrosis. *Gut*, 55, 8, 1165-1173, 2006.
13. Strate T. *et al.*: Pathogenesis and the natural course of chronic pancreatitis. *Eur J Gastroenterol Hepatol*, 14, 929-934, 2002.
14. Wyatt J.E., Pettit W.L., Harirforoosh S.: Pharmacogenetics of nonsteroidal anti-inflammatory drugs. *Pharmacogenomics J.*, 12, 6, 462-467, 2012.
15. Wang Luo-Wei *et al.*: Sonic hedgehog expression in a rat model of chronic pancreatitis. *World J Gastroenterol.*, 28, 20, 16, 4712-4717, 2014.