Taylor & Francis
Taylor & Francis Group

Journal of Investigative Surgery

ISSN: 0894-1939 (Print) 1521-0553 (Online) Journal homepage: http://www.tandfonline.com/loi/iivs20

Apigenin Attenuates Inflammation in Experimentally Induced Acute Pancreatitis-Associated Lung Injury

Neofitos Basios, Pavlos Lampropoulos, Apostolos Papalois, Maria Lambropoulou, Michael K Pitiakoudis, Athanasia Kotini, Constantinos Simopoulos & Alexandra K Tsaroucha

To cite this article: Neofitos Basios, Pavlos Lampropoulos, Apostolos Papalois, Maria Lambropoulou, Michael K Pitiakoudis, Athanasia Kotini, Constantinos Simopoulos & Alexandra K Tsaroucha (2015): Apigenin Attenuates Inflammation in Experimentally Induced Acute Pancreatitis-Associated Lung Injury, Journal of Investigative Surgery, DOI: 10.3109/08941939.2015.1088603

To link to this article: http://dx.doi.org/10.3109/08941939.2015.1088603

Published online: 02 Dec 2015.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=iivs20

Download by: [Tufts University] **Date:** 04 December 2015, At: 17:29

Journal of Investigative Surgery, Early Online, 1–7, 2015
Copyright © 2015 Taylor & Francis Group, LLC
ISSN: 0894-1939 print / 1521-0553 online
DOI: 10.3109/08941939.2015.1088603

Taylor & Francis
Taylor & Francis Group

ARTICLE

Apigenin Attenuates Inflammation in Experimentally Induced Acute Pancreatitis-Associated Lung Injury

Neofitos Basios,[1] Pavlos Lampropoulos,[2] Apostolos Papalois,[3] Maria Lambropoulou,[4] Michael K Pitiakoudis,[1] Athanasia Kotini,[5] Constantinos Simopoulos,[1] Alexandra K Tsaroucha[1]

[1] Demokritos University of Thrace, Second Department of Surgery, University Hospital of Alexandroupolis, Alexandroupolis, Greece [2] Henry Dunant Hospital Center, Athens, Greece [3] Experimental – Research Center, ELPEN Pharmaceuticals, Pikermi, Greece [4] Demokritos University of Thrace, Department of Histology, University Hospital of Alexandroupolis, Alexandroupolis, Greece [5] Demokritos University of Thrace, Laboratory of Medical Physics, University Hospital of Alexandroupolis, Alexandroupolis, Greece

ABSTRACT

Background: Acute pancreatitis is associated with acute lung injury. The aim of the present study is to evaluate alterations of lungs in an experimental model of acute pancreatitis (AP) following both bilio-pancreatic duct obstruction close to the duodenum. Acute pancreatitis is a common disease with significant mortality. This situation makes the need of finding protective factors for the lung parenchyma, imperative. In the present study there is an effort to clarify the role of apigenin, a substance which is well known for its antioxidant and anti-inflammatory effects, on lung injury, following acute pancreatitis in rats. *Materials and methods:* In the present study, 126 male Wistar-type rats 3–4 months old and 220–350 g weight were used. At time 0 we randomly assigned the following groups: Group Sham: Rats were subjected to virtual surgery. Group Control: Rats were subjected to surgery for induction of acute pancreatitis. Group Apigenin: Rats were subjected to surgery for induction of acute pancreatitis and enteral feeding with apigenin. Immunochemistry for TNF-α and IL-6 as well as MPO activity were measured at predetermined time intervals 6, 12, 24, 48, and 72 h, in order to evaluate architectural disturbances of the lung tissue. *Results:* From the pathological reports we realized that comparing the control group with the apigenin group, there is an improvement of lung tissue damage following apigenin administration, with statistical significance. Apigenin reduces most histopathological alterations of the pulmonary tissue, reduces MPO and TNF-α activity at 48 hours and, furthermore, reduces IL-6 activity at 72 hours post-administration. *Conclusions:* Oral Apigenin administration in rats, following experimental induced acute pancreatitis, seems to be protective on the lung tissue. Apigenin administration to humans could potentially ameliorate acute lung injuries. However, special caution is required for humans' use, as more detailed studies are needed.

Keywords: apigenin; experimentally induced acute pancreatitis; lung injury; TNF-α; IL-6; MPO

Journal of Investigative Surgery

INTRODUCTION

Acute pancreatitis (AP) is a common inflammatory disease with a quite variable clinical outcome. The incidence rate in various European countries has been reported to be 22–100 cases per 100 000 population with an increase rate of 3% per year, especially in young women [1–3].The overall mortality rate is 5%, however, in cases of severe acute pancreatitis, the rate dramatically increases up to 30% [4]. Respiratory distress syndrome, acute lung injury and multiple organ failure are the main causes of death in most cases of severe AP [5].

The precise mechanism by which severe pancreatitis causes acute lung injury remains to be clarified. Many studies have shown that acute pancreatitis leads to the release of various proinflammatory cytokines including TNF-α and interleukines, proteases and reactive oxygen species (ROS). These molecules may cause acute lung injury which is characterized by widespread lung inflammation and alveolar capillary destruction [6].

Received 27 May 2015; accepted 25 August 2015.

Address correspondence to Dr Pavlos Lampropoulos, Henry Dunant Hospital Center, 107 Mesogion Av, Athens, 11526 Greece. E-mail: pav.lampropoulos@gmail.com

It becomes apparent that it is crucial to find pharmaceutical agents that stop the progression of AP to acute lung injury, not only because it is a common disease but also due to its high mortality rates. Ideally, since inflammation is the key component of the disease, one might hypothesize that anti-inflammatory drugs could serve as potential drugs of treatment. Indeed, some anti inflammatory agents are promising in preventing post ERCP pancreatitis including rectally administered indomethacin [7]. Others have investigated interleukin-10, as a potent anti-inflammatory cytokine [8], while others have investigated the combined effects of hyperbaric oxygen (HBO) and N-acetylcysteine (NAC) on acute necrotizing pancreatitis in rats [9].

Recently, we have shown in an experimental model of AP, that apigenin might be of some benefit in ameliorating the severity of AP [10]. Apigenin (4,5,7trihydroxyflavone) is found in various plants, vegetables and fruits, and has established anti-inflammatory, antioxidant, anti-allergic, anti-osteoporotic, and anticancerous properties [11–14]. Thus, we next hypothesized that apigenin could ameliorate injuries to the lung in the process of AP, which is usually the first distant organ affected. Therefore, we continued our previous experiment of an established model of AP in rats, to study the expression of TNF-α, IL-6 (important proinflammatory cytokines) and myeloperoxidase MPO activity in the lung parenchyma. Hence, we test the hypothesis that apigenin not only protects the pancreatic parenchyma from the inflammatory cascade of reactions but additionally restores homeostasis in the lung as well.

Journal of Investigative Surgery

MATERIALS AND METHODS

Animals and Design of the Study

For the purpose of the experiment, one hundred and twenty six Wistar male rats, aged 3–4 months old, weighing 220–350 g were utilized. All rats were housed in controlled temperature (22–25°C), humidity (55%–58%), lighting (12 h light/12 h dark). The animals were left to be acclimatized for at least 72 hours and were provided with free access to water and rat chow diet, in our laboratory. The animals were purchased from Pasteur Institute, Athens, Greece. Experimental Research Center of ELPEN Pharmaceuticals, Athens, Greece, provided all the necessary equipment and facilities for the purpose of the experiment, while the pathological and biochemical investigations were accomplished at the Laboratories of the Medical School of Democritus University of Thrace. The experiments were conducted according to National Research Council Guide for the Care and Use of Laboratory Animals and Directive 86/609 of the European Union, protocol number (K/2284), as required. All necessary precautions were taken to avoid animal suffering.

Experimental Procedure

Experimental AP was induced as previously reported [10]. Briefly, the animals under general anesthesia were placed in supine position. The rats were anesthetized in a special glass box with the use of isoflurane, following a subcutaneous injection of 0.25 cc of butorphenol (Dolorex). Soon after endotracheal intubation, anesthesia was maintained with a mixture of 93% O_2, 5% CO_2, and 2% isoflurane. No antibiotics were used. A midline incision was performed under sterile conditions with betadine solution 10% and shaving of the abdomen right before the incision. After entering the abdominal cavity, we performed a bilio-pancreatic duct ligation close to the duodenum. For the Sham operated animals, we only maneuvered the abdominal contents with no further action. For the Control operated group, we performed the ligation of the duct. For the Apigenin operated group, we performed ligation of the duct and administered apigenin (4cc solution) orally soon after recovery. In order to maintain proper analgesia for the animals, (2cc/kg butorphenol, Dolorex) was given subcutaneously in predetermined time intervals, every 4 hours and subsequently according to the animal's need.

Experimental Protocol

For the purpose of our experiment, the animals were randomly assigned into three groups: Sham group $n = 20$, Control group (induction of pancreatitis) $n = 56$, Apigenin group (induction of pancreatitis and apigenin administration) n=50. The statistical power of the experiment remained uncompromised, following randomization. Eight rats died before completion of the experiment and/or scheduled euthanasia, and were not included in the statistical analysis. The animals were sacrificed as described elsewhere [8], at predetermined intervals (6h, 12h, 24h, 48h, 72h) to collect lung tissues and other samples. Briefly, ketamine (Narcetan) 0.3–0.6 cc and xylazine (Rompun) 0.1–0.3 cc up to triple dosage, were used for euthanasia.

Immunohistochemistry Technique

Animals were sacrificed, exsanguinated and the lung tissues were harvested and sliced into small pieces. With the help of a microtome, we selected paraffin embedded 20 μm thick sections and left them in water bath at 65°C to avoid tissue folding. Soon

Table 1 Scoring of TNF-α immunochemistry, MPO activity, and IL-6 immunochemistry, in test subjects, in the three separate groups (Sham, Control, Apigenin)

	0 *n* (%)	1 *n* (%)	2 *n* (%)	3 *n* (%)
		TNF-α SCORE		
Sham	10 (50)	10 (50)	0 (0)	0 (0)
Control	9 (18.37)	12 (24.49)	21 (42.86)	7 (14.28)
Apigenin	11 (22.45)	21 (42.86)	16 (32.65)	1 (2.04)
Total	30 (25.42)	43 (36.44)	37 (31.35)	8 (6.78)
		MPO SCORE		
Sham	13 (65.00)	7 (35.00)	0 (0)	0 (0)
Control	14 (28.57)	20 (40.82)	11 (22.45)	4 (8.17)
Apigenin	19 (38.77)	19 (38.77)	10 (20.41)	1 (2.04)
Total	28 (23.73)	56 (47.46)	29 (24.58)	5 (4.23)
		IL-6 SCORE		
Sham	3 (15.00)	6 (30.00)	11 (55.00)	0 (0)
Control	4 (8.16)	12 (24.49)	18 (36.73)	15 (30.61)
Apigenin	11 (22.45)	19 (38.77)	15 (30.61)	4 (8.16)
Total	18 (15.25)	37 (31.35)	44 (37.29)	19 (16.10)

thereafter, we used the En Vision HRP mouse/rabbit detection system (K5007; DAKO, Carpinteria, CA) kit for the streptavidin biotin technique. Sections were left in the oven for 30 min at 80°C. Slides were then washed successively in xylene, a mixture of xylene and 96% ethanol, 96% ethanol, 100% ethanol, 96% ethanol and 100% ethanol baths for 5 minutes each. Rinsing in PBS buffer solution followed. The specimens were incubated in H_2O_2 solution (200 mL H_2O and 6 mL H_2O_2) for 15 min in a dark room to inhibit endogenous peroxidase activity, and rinsed once again in PBS buffer solution.

Determination of MPO Activity

MPO activity of the lung tissue was evaluated with immunohistochemisty, using MPO (rabbit polyclonal) antibody, DAKO (A 0398 DAKO, Carpinteria, CA) at 1:400 dilution, according to manufacturer's protocol. After incubation with primary anti-MPO antibody, two independent pathologists who were blinded to the study protocol, evaluated the tissue slides under light microscopy. Scoring was determined according to the proportion of the neutrophil cells with cytoplasmic staining in the entire section of the specimen. If no cells or less than 10% of cells were stained, the labeling index was negative and a score of zero was assigned. If ≥10% and <30% of cells were stained, the labeling index was weak and a score of one was assigned. If ≥30% and >70% of cells were stained, the labeling index was moderate and a score of two was assigned. If ≥70% of cells were stained, the labeling index was strong and a score of three was assigned (Table 1).

Determination of TNF-α

Immunohistochemical detection of TNF-α was performed with TNF-α (rabbit polyclonal), Acris (AP20373PU-N), dilution 1:200. Manufacturer's protocol was strictly followed on formalin-fixed paraffin-embedded sections. Staining for TNF-α was scored semi-quantitatively on a four scale system (0–3), as previously described [15]. A score 0 was assigned if no cells or <10% of cells were stained. A score one was assigned if ≥10% or <25% of cells were stained, and a score of two was assigned if ≥25% and <50% of cells were stained. Finally, a score of three was assigned if ≥50% of cells were stained. Differences of the blinded pathologists on scoring were resolved on mutual agreement **(**Table 1**)**.

Determination of IL-6

Immunochemical detection of IL6 was accomplished with the use of a IL-6(rabbit polyclonal) antibody ABCAM (ab6672), dilution 1:500. Once again formalin-fixed paraffin-embedded sections of lung tissue were used according to the manufacturer's protocol. A score was assigned according to the percentage of cells stained by two blinded pathologists **(**Table 1**)**.

Statistical Analysis

Statistical analysis was performed using Minitab version 17 for Windows statistical package. We had initially included 126 rats into our experiment. Eight rats died prior to termination of the experiment, thus 118 rats were included in our analysis. Our results lacked normality in distribution and had ordinal nature, thus we used non-parametric methods of statistical analysis: Fisher's exact test, Kruskal–Wallis test, and finally, Mann–Whitney test for independent sample results. The Kruskal–Wallis test was used in all group' comparisons and the Mann–Whitney test was used to compare groups in pairs. Results were also expressed as mean ± SD. Values of $p < 0.05$ was considered statistically significant.

RESULTS

Histopathological Changes of the Lungs in Our Experimental Model of AP

Histopathological evaluation of the pulmonary parenchyma showed no or mild abnormalities in the sham operated group, while in the control and apigenin groups we observed significant pulmonary architectural disturbance. Early manifestations of the architectural disturbance included congestion,

© 2015 Taylor & Francis Group, LLC

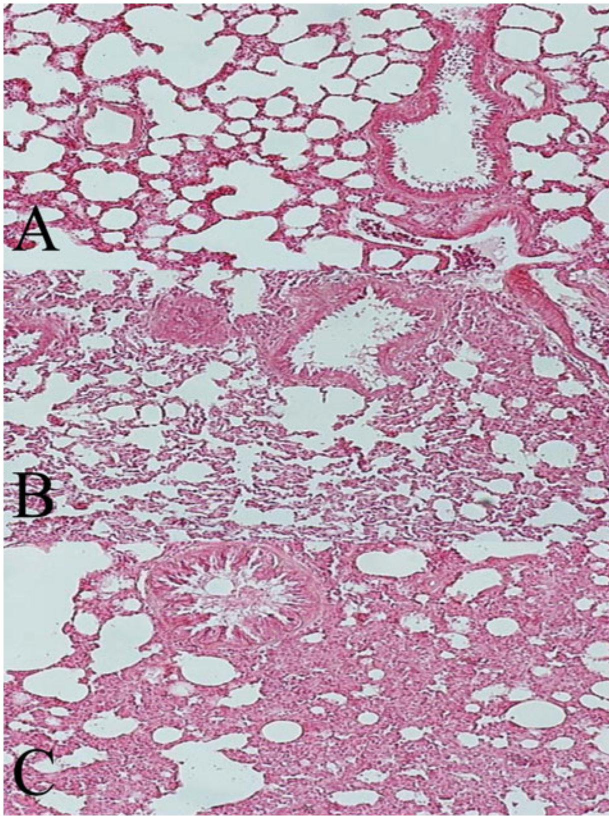

Figure 1. Representative results of H&E staining in rat pulmonary tissue: Sham group, ×100 at 72 hours (a), Apigenin group ×100 at 48 hours (b), and Control group ×100 at 48 hours (c).

pulmonary edema and intraalveolar hemorrhage. Late manifestations included interstitial edema and inflammatory cells infiltration. Furthermore, we noticed that these changes are milder in the apigenin group compared with the control group **(**Figure 1**)**.

MPO Activity is Reduced at 48 Hours Following Apigenin Administration in Rats with Experimental AP

The MPO activity remained constantly low in the sham operated group. In the control group, the MPO activity increased throughout all time intervals reaching maximal activity at 72 hours. On the contrary, in the apigenin group, the MPO activity reached maximal activity at 24 hours and thereafter started a significant decline. Statistical analysis revealed that at all time intervals there is significant difference of MPO activity between the control and apigenin groups ($p = 0.047$), between the control and sham group ($p < 0.001$), and between apigenin and sham group ($p < 0.001$). In addition, at 48 hours and thereafter, there is strong reduction of MPO activity in the apigenin group compared with the control group ($p = 0.026$ at 48 hours and $p = 0.015$ at 72 hours) **(**Figures 2, 3, 4**)**.

IL-6 Activity is Reduced at 72 Hours Following Apigenin Administration in Rats with Experimental AP

IL-6 immunochemistry in the sham operated group showed a slight disturbance in the pulmonary architecture of rats that reached maximal value at 24 hours and plateau throughout the end of our experiment. In the control group, we noticed a constant increase of the IL-6 immunostaining at all time intervals. In the apigenin group, IL-6 immunoreactivity increased up to 48 hours and started to decrease up to 72 hours. Statistical analysis showed that at all time intervals there is statistical difference in the IL-6 activity between the control and the apigenin groups ($p = 0.0345$). At specific time intervals, however, there is a strong reduction of immunostaining in the apigenin group compared to the control group ($p = 0.019$ at 72 hours) **(**Figures 2, 3, 4**)**.

TNF-a Activity is Reduced at 48 Hours Following Apigenin Administration in Rats with Experimental AP

Measurements of TNF-a in the sham operated group showed no specific differences at all time intervals of our experiment. In the control group, we observed a constant increase of the immunostaining that reached maximal values at 72 hours. In the apigenin group, we noticed a dramatic drop of the TNF-a immunostaining after 48 hours of our experiment. Statistical analysis showed no significant changes at all times intervals between the control and the apigenin groups ($p = 0.231$). At specific time intervals, however, there is a significant reduction of immunostaining in the apigenin group compared to the control group at 48 and 72 hours ($p = 0.003$ at 48 hours and $p < 0.001$ at 72 hours) **(**Figures 2, 3, 4**)**.

DISCUSSION

Apigenin is chemically known as 4′, 5, 7,-trihydroxy flavone, with a molecular formula C15H10O5and a molecular weight of 270.24 [16]. It belongs in the family of flavonoids and more specifically in the subclass of flavones. Apigenin and luteolin are major representatives of the flavones subclass and are mostly found in chamomile, celery, parsley, thyme, onions, red pepper, lettuce, berries, etc [17]. Apigenin has been reported to have anti-mutagenic, anti-cancerous, anti-inflammatory, anti-proliferative, anti-oxidative and anti-progression properties, while at the same time there are no substantial evidence to indicate adverse reactions following in vivo consumption [17,18].

In our experiment, we tested the hypothesis that apigenin may ameliorate pulmonary damages in an

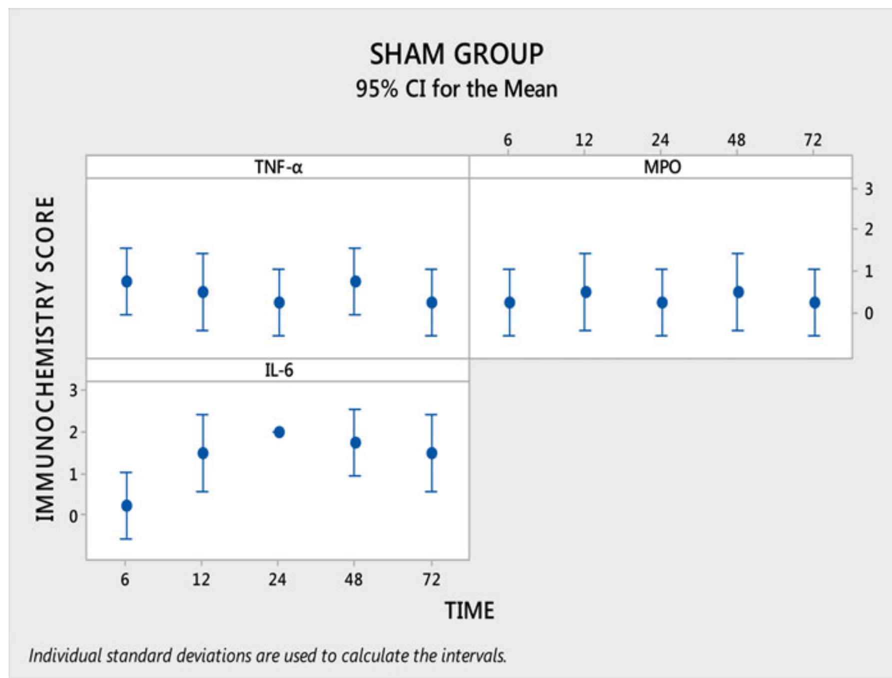

Figure 2. Immunohistochemical manifestations of experimentally induced acute pancreatitis on the lung parenchyma in the Sham group. The time course over 72 hours is shown for (a) TNF-α, (b) MPO, (c) IL-6. Statistical analysis revealed that TNF-α immunochemistry and MPO activity showed no specific variations at all time intervals. IL-6 immunochemistry in the sham operated group showed a slight disturbance in the pulmonary architecture of rats, that reached maximal value at 24 hours and plateau throughout the end of our experiment.

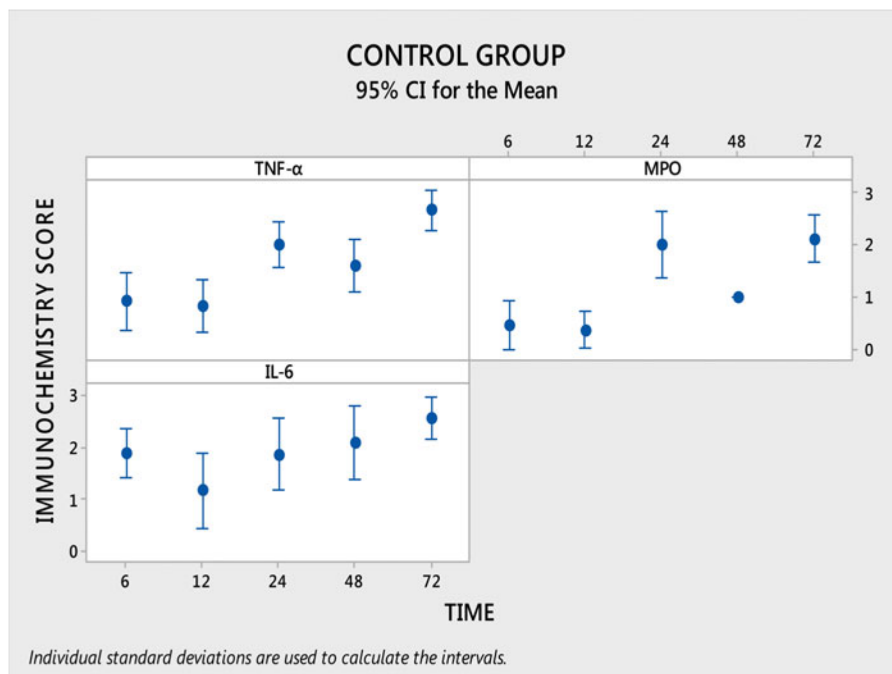

Figure 3. Immunohistochemical manifestations of experimentally induced acute pancreatitis on the lung parenchyma in the Control group. The time course over 72 hours is shown for (a) TNF-α, (b) MPO, (c) IL-6.Statistical analysis revealed that TNF-α immunochemistry showed a constant increase of the immunostaining that reached maximal values at 72 hours. The MPO activity and IL-6 immunochemistry showed an increased throughout all time intervals reaching maximal activity at 72 hours.

© 2015 Taylor & Francis Group, LLC

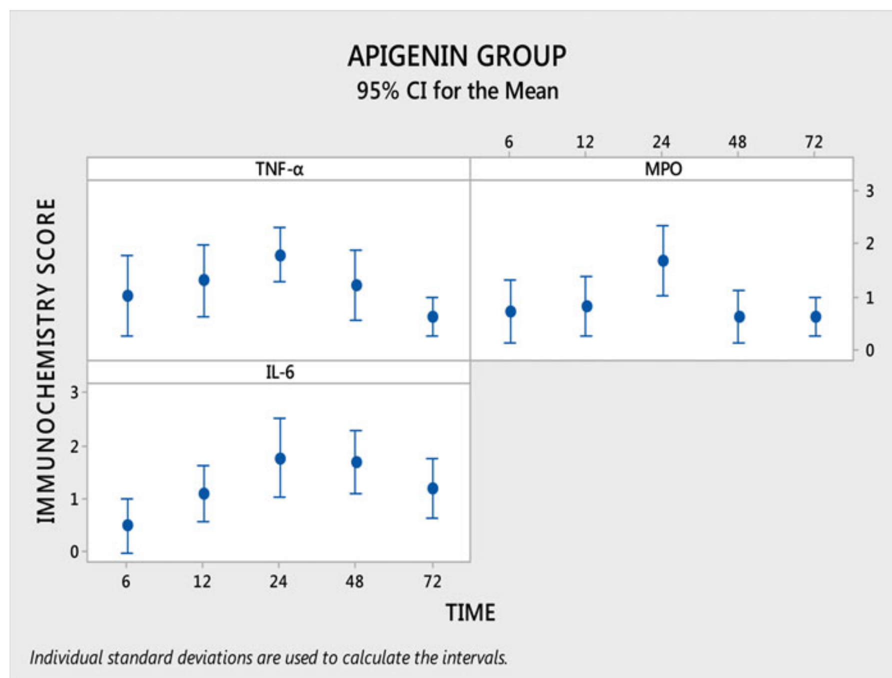

Figure 4. Immunohistochemical manifestations of experimentally induced acute pancreatitis on the lung parenchyma in the Apigenin group. The time course over 72 hours is shown for (a) TNF-α, (b) MPO, (c) IL-6. In the Apigenin group, we noticed a dramatic drop of the TNF-a immunostaining after 48 hours of our experiment. The MPO activity reached maximal activity at 24 hours and thereafter started a significant decline. IL-6 immunoreactivity increased up to 48 hours and started to decrease up to 72 hours.

experimental model of acute pancreatitis. Our hypothesis was based on previous observations that apigenin has strong anti-inflammatory properties. Other investigators have shown that apigenin is a strong inhibitor of transcriptional activation of both cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages [19]. In addition, flavonoids strongly inhibit IL-4 expression by basophiles with an IC(50) value of 2–5 microM. This inhibition is mediated by inhibiting the activation of nuclear factors of activated T cells and AP-1[20]. Furthermore, flavones have been shown to block the TNF-α-induced activation of CAM expression through transcriptional activities. At a nontoxic dose of ≥ 10 μmol/L, the expression of VCAM-1, ICAM-1, and E-selectin proteins is blocked by a direct modulation of gene transcription [21]. Potential therapeutic properties of flavonoids were demonstrated on skin pathological conditions associated with inflammation, by inhibition of IFN gamma-induced ICAM-1 expression in a reconstructed human skin [22]. Recently, treatment with flavonoids also showed to attenuate neutrophils' infiltration, production of the pro-inflammatory cytokine TNF-α, and generation of the lipid peroxidation product MDA in an experimental model of acute colitis [23].

Results from our present experiment are in absolute agreement since we have shown that oral administration of apigenin alleviates the inflammatory complications on pulmonary parenchyma, resulting from acute pancreatitis. We have shown that apigenin reduces most histopathological alterations of the pulmonary tissue, reduces MPO and TNF-α activity at 48 hours and, furthermore, reduces IL-6 activity at 72 hours post-administration. Hence, we noticed that the beneficial effect of apigenin is time-dependent. This observation is in agreement with our previous experiment that the beneficial effect of apigenin on acute pancreatitis is also time dependent.

The main question that needs to be addressed is whether apigenin has a direct protective effect on lung parenchyma or if lung protection results from apigenin's proven beneficial effect on acute pancreatitis. Acute lung injury is caused by released pro-inflammatory cytokines and various cytokines from the injured pancreatic tissue. Since apigenin has a time dependent anti-inflammatory effect on pancreas, it becomes clear that apigenin has a protective effect on lung parenchyma indirectly. At the same time we have observed that apigenin reduces MPO activity, TNF-α and Il-6 immunoreactivity in the lung tissue. From our previous experiment, we noticed that apigenin exerts its effects on pancreas after 48 hours. In the present experiment apigenin shows milder pulmonary changes at 48 hours onwards. Hence, there is not enough time to see less lung injury due to milder pancreatitis from apigenin administration. Later on, in the course of the inflammatory cascade however, apigenin may

impose its antiinflammatory actions indirectly on lung tissue, since less provocative molecules are released from the pancreas. Therefore, we concluded that apigenin protects from acute pancreatitis associated lung injury both directly and indirectly. Results from our experiments showed that the protective effect of apigenin is biphasic. Directly, apigenin significantly inhibits the LPS-enhanced inflammatory activity in the lungs, and exhibits anti-inflammatory effect through the MAPK and NF-κB (IκB) pathways [24]. Indirectly, apigenin's lung protection is the result from lower level of cytokines and interleukines released from pancreas.

In conclusion, we proved that apigenin, an abundant natural derivative, has important beneficial effects in inflammation on the pancreatic and pulmonary tissues. However, we have a long way to go, in order to address issues about proper dosages, ways of administration, oral bioavailability and most importantly human trials. However, taking into account that apigenin has no major adverse effects, we are hopeful that scientists soon will start investigate these parameters.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

ACKNOWLEDGMENTS

This study was funded by Scholarship—Grant by the Experimental Research Center ELPEN Pharmaceuticals (E.R.C.E). Also we thank Mrs E. Karampela, Mrs M. Karamperi, and Mrs K. Tsarea for their technical support during the experiments.

REFERENCES

[1] Roberts SE, Williams JG, Meddings D, et al. Incidence and case fatality for acute pancreatitis in England: geographical variation, social deprivation, alcohol consumption and aetiology—a record linkage study. *Aliment Pharmacol Ther.* 2008;28:931–41.

[2] Roberts SE, Akbari A, Thorne K, A.et al. The incidence of acute pancreatitis: impact of social deprivation, alcohol consumption, seasonal and demographic factors. *Aliment Pharmacol Ther.* 2013;38:539–548.

[3] Gluszek S, Koziel D. Prevalence and progression of acute pancreatitis in the swietokrzyskie voivodeship population. *Pol Przegl Chir*. 2012;84:618–25.

[4] Phillip V, Steiner JM, Algül H. Early phase of acute pancreatitis: assessment and management. *World J Gastrointest Pathophysiol.* 2014;5:158–68.

[5] Lundberg AH, Granger DN, Russell J, et al. Quantitative measurement of P- and E-selectin adhesion molecules in acute pancreatitis: correlation with distant organ injury. *Ann Surg.* 2000;231:213–22.

[6] Lin WC, Chen CW, Huang YW, et al. Kallistatin protects against sepsis-related acute lung injury via inhibiting inflammation and apoptosis. *Sci Rep.* 2015;5:12463.

[7] Wong LL, Tsai HH. Prevention of post-ERCP pancreatitis.*World J Gastrointest Pathophysiol.* 2014;5:1–10.

[8] Keceli M, Kucuk C, Sozuer E, et al. The effect of interleukin-10 on acute pancreatitis induced by cerulein in a rat experimental model.*J Invest Surg.* 2005;18:7–12.

[9] Onur E, Paksoy M, Baca B, et al. Hyperbaric oxygen and N-acetylcysteine treatment in L-arginine-induced acute pancreatitis in rats. *J Invest Surg.* 2012;25:20–8.

[10] Lampropoulos P, Lambropoulou M, Papalois A, et al. The role of apigenin in an experimental model of acute pancreatitis.*J Surg Res.* 2013;183:129–37.

[11] Di Carlo G, Mascolo N, Izzo AA, et al. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci* 1999;65:337–53.

[12] Kim HP, Son KH, Chang HW, et al. Anti-inflammatory plant flavonoids and cellular action mechanisms. *J Pharmacol Sci.* 2004;96:229–45.

[13] Garcıa-Lafuente A, Guillamon E, Villares A, et al. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm Res* 2009;58:537–52.

[14] Park JA, Ha SK, Kang TH, et al. Protective effect of apigenin on ovariectomy-induced bone loss in rats. *Life Sci* 2008;82:1217–23.

[15] Barrera P, Joosten LA, den Broeder AA, et al. Effects of treatment with a fully human anti-tumour necrosis factor alpha monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNF alpha in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2001;60:660–9.

[16] Shukla S, Gupta S. Apigenin: a promising molecule for cancer prevention. *Pharm Res*. 2010;27:962–78.

[17] Patel D, Shukla S, Gupta S. Apigenin and cancer chemoprevention: progress, potential and promise (review). *Int J Oncol.* 2007;30:233–45.

[18] Birt DF, Walker B, Tibbels MG, et al. Anti-mutagenesis and anti-promotion by apigenin, robinetin and indole-3-carbinol. *Carcinogenesis*. 1986;7:959–63.

[19] Liang YC, Huang YT, Tsai SH, et al. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis*. 1999;20:1945–52.

[20] Kawai M, Hirano T, Higa S, et al. Flavonoids and related compounds as anti-allergic substances. *Allergol Int.* 2007;56:113–23.

[21] Choi JS, Choi YJ, Park SH, et al. Flavones mitigate tumor necrosis factor-alpha-induced adhesion molecule upregulation in cultured human endothelial cells: role of nuclear factor-kappa B. *J Nutr.* 2004;134:1013–9.

[22] Bito T, Roy S, Sen CK, et al. Flavonoids differentially regulate IFN gamma-induced ICAM-1 expression in human keratinocytes: molecular mechanisms of action. *FEBS Lett.* 2002;520:145–52.

[23] Joo M, Kim HS, Kwon TH, et al. Anti-inflammatory effects of flavonoids on TNBS-induced colitis of rats. *Korean J Physiol Pharmacol.* 2015;19:43–50.

[24] Li KC, Ho YL, Hsieh WT, et al. Apigenin-7-glycoside prevents LPS-induced acute lung injury via downregulation of oxidative enzyme expression and protein activation through inhibition of MAPK phosphorylation. *Int J Mol Sci.* 2015;161:1736–54.

© 2015 Taylor & Francis Group, LLC