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Dear Dr. Nemeth:

Your manuscript (12-107-R) has been accepted in Clinical Hemorheology and Microcirculation.

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Dear Dr. Nemeth,

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Effects of various drugs (flunixin, pentoxifylline, enoxaparin) modulating micro-rheological changes in cerulein-induced acute pancreatitis in the rat

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Running title: Flunixin, pentoxifylline and enoxaparin in experimental acute pancreatitis

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Abstract: Previously we have investigated the cerulein-induced acute pancreatitis and provided data on its micro-rheological impact in the rat. We hypothesized that non-steroid anti-inflammatory agent flunixin, the xanthine-derivate pentoxifylline and the low molecular weight heparin enoxaparin may have various beneficial effects improving microcirculatory and rheological parameters. In female rats, under general anesthesia, 10 μg/kg cerulein s.c. was administered and 2 hours afterwards microcirculation was tested by laser Doppler flowmetry on the tongue and after performing laparotomy on the small intestine, liver and pancreas prior to terminal blood sampling. From blood samples hematological parameters, blood pH, lactate concentration, erythrocyte deformability, osmoscan parameters and erythrocyte aggregation were tested. Compared to normal control in acute pancreatitis group we found severe deterioration in tissue microcirculation together with impaired erythrocyte deformability and enhanced aggregation, accompanied by acidic pH and increasing lactate concentration. Improvement was found when using flunixin (s.c.), pentoxifylline (i.p.) or enoxaparin (s.c.). These drugs could partly improve the blood flux on the surface of the investigated organs, and the flunixin had the most expressed improving effects on micro-rheological parameters. Surprisingly, the improving effect of pentoxifylline on micro-rheological parameters was not obvious (red blood cell deformability did not improved better than in the other treated groups), however, microcirculatory parameters improved.

Keywords: red blood cell deformability, red blood cell aggregation, osmoscan, microcirculation, cerulein-induced acute pancreatitis, rat model, various drug therapy

1. Introduction

Although the role of microcirculation disturbances in the pathogenesis of acute pancreatitis is well known [6, 14, 17, 19, 25, 34, 44, 47], there are only limited number of studies dealing with general hemorheological or micro-rheological changes [e.g., 5, 7, 8, 22]. In the development of microcirculatory disturbance in severe acute pancreatitis numbers of cytokines, inflammatory mediators and reactive oxygen species play role [5, 9, 13, 15, 23, 25, 28, 34, 36, 38, 39, 45, 46]. Microcirculatory disturbances of the pancreas and other organs may alter, or in turn, can be caused by impaired hemorheological parameters [2, 5, 7, 22, 31, 32, 36, 42, 45]. However, it is also obvious that influencing the pancreatic microcirculation can be an effective option in the treatment of acute pancreatitis [12-14, 26, 27, 41].

In our previous study, we have investigated the cerulein-induced acute pancreatitis and provided data on its micro-rheological impact in the rat [22]. It was demonstrated that single dose of 5 or 10 μg/kg cerulein-induced acute pancreatitis in rats. The presence of acute pancreatitis was confirmed by the rise in amylase enzyme activity (1 and 2 hours after cerulein administration), as well as by histomorphological evidences. The largest magnitude of changes was observed in red blood cell deformability and red blood cell aggregation in the group with 10 μg/kg cerulein, mostly in female animals [22].

Therefore, as continuation, in this study we used the cerulein in 10 μg/kg in female animals, to compare the effects of various drugs potentially affecting tissue microcirculation and micro-rheological parameters. We applied tissue microcirculatory measurements and more complete hemorheological examination using two types of aggregometry, ektacytometry as well as osmotic gradient ektacytometry [10, 18, 21, 30].

We hypothesized that the non-steroid anti-inflammatory agent flunixin, the xanthine-derivate pentoxifylline and the low molecular weight heparin enoxaparin may have various beneficial effects on improving microcirculatory and rheological parameters in this experimental cerulein-induced acute pancreatitis model.

2. Materials and methods

2.1. Experimental animals and groups

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (Nr.: 16/2008), in accordance with the relevant Hungarian Animal Protection Act (Law XVIII/1998).

Fifty female Sprague-Dawley rats (bodyweight: 292.1 ± 13.8 g) (Janvier Co., France) were randomly involved into the following experimental groups:

I. Control group (C, n=10): tissue microcirculation measurements, blood and tissue samplings.
II. Acute pancreatitis group (AP, n=10): administration of 10 μg/kg cerulein s.c., 2 hours later tissue microcirculation measurements, blood and tissue samplings.
III. Acute pancreatitis + Flunixin group (AP+Fl, n=10): AP group's procedure and administration of 2.5 mg/kg flunixin (Flunixin®) s.c.
IV. Acute pancreatitis + Pentoxifylline group (AP+Pe, n=10): AP group's procedure and administration of 50 mg/kg pentoxifylline (Pentoxifyllin®) i.p.
V. Acute pancreatitis + Clexane group (AP+Cle, n=10): AP group's procedure and administration of 2 mg/kg enoxaparin (Clexane®) s.c.

All animals were anesthetized using 60 mg/kg, Thiopenthal® intraperitoneally. For inducing acute pancreatitis, cerulein (Sigma-Aldrich Co., Budapest) dissolved in sterile physiological saline solution was used subcutaneously (right abdominal region) in 10 μg/kg dose. In Control group the same-volume sterile physiological saline solution was given subcutaneously.

2.2. Microcirculatory measurements and sampling protocol

Two hours after administration of cerulein in Group II-V., and at the same time in Control Group a laser Doppler tissue flowmetry (LD-01 laser Doppler flowmeter, Experimetria Ltd., Hungary) with standard pencil probe was used to measure blood flux units (BFU) on the inferior surface of the tongue, and after completing a median laparotomy, on the front surface of the liver's middle lobe, on the anti-mesenterial surface of a jejunum loop and on the middle region (body) of the pancreas. At all measurement points after stabilization of the signal, BFU values were recorded for 20 sec, and the average values of these periods were analyzed off-line. In parallel rectal temperature was measured.

Following microcirculatory measurements, blood sampling of 3-5 ml blood was completed by cardiac puncture (24 G needle; anticoagulant: K_3-EDTA, 1.5 mg/ml).

2.3. Laboratory investigations

A Sysmex F-800 semi-automated microcell counter (TOA Medical Electronics Co., Japan) was used to determine *hematological parameters*, from which white blood cell count (WBC [$x10^3/\mu l$]), red blood cell count (RBC [$x10^6/\mu l$]), hematocrit (Hct [%]), mean corpuscular volume (MCV [fl]) and platelet count (Plt [$x10^3/\mu l$]) were analyzed in this study.

An ABL555 blood gas analyzer automate (Radiometer Copenhagen, Denmark) was used to measure *blood pH and lactate concentration* [mmol/l].

A LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands) was used to measure red blood cell deformability. For *regular red blood cell deformability tests* 5 µl blood sample was gently mixed in 1 ml of isotonic polyvinyl-pyrrolidone solution (360 kDa PVP in normal PBS; viscosity = 27 mPa.s, osmolarity = 290-300 mOsm/kg ; pH ~7.3). During the measurements, the laser diffraction pattern was analyzed while the device generated shear stress (SS) range from 0.3 to 30 Pa. The elongation index (EI) is equal to (L-

W)/(L+W), where L is the length and W is the width of the diffractogram. EI increases with red blood cell deformability [3, 18]. The tests were carried out at constant temperature of 37 °C. For comparison of the results, Lineweaver-Burk analyses were also performed, calculating the maximal elongation index (EI_{max}) and the shear stress at half EI_{max} ($SS_{1/2}$ [Pa]) values, according to the following formula: $1/EI = SS_{1/2} / EI_{max} \times 1/SS + 1/EI_{max}$ [4].

For the *osmotic gradient ektacytometry (osmoscan) measurements* 250 µl blood was required. During the measurements a constant shear stress of 30 Pa was used for continuous measurement of EI values, while the osmotic environment is changed by the device, using various, controlled mixture of 0 and 500 mOsmol/kg PVP solutions. In this study we analyzed the minimal elongation index values measured at low osmolar environment (minimal EI), maximal elongation index values (maximal EI), half of the maximal elongation index values at high osmolar environment (EI_{hyper}) and the relevant osmolarity values [10, 18].

Red blood cell aggregation was tested based on light-transmittance method using a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) with determination of aggregation index values M (at shear rate of 0 s^{-1}) and M1 (at shear rate of 3 s^{-1}) 5 or 10 seconds after disaggregation. The indices (M 5 s, M1 5s, M 10 s, M1 10 s) increase with enhanced red blood cell aggregation [3, 18]. In parallel the LoRRca device was also used to measure red blood cell aggregation parameters by syllectometry: amplitude (Amp), aggregation index (AI) and the aggregation half-time ($t_{1/2}$ [s]) [18]. The aggregation measurement were carried out in native blood samples without hematocrit adjustment.

2.3. Statistical analyses

Data are presented as mean ± standard deviation (S.D.). Depending on the normality of data distribution, Student's t-test or Mann-Whitney RS test was used for inter-group comparison. A $p<0.05$ was considered as statistically significant.

3. Results

3.1. Microcirculatory and body temperature changes

The comparisons have two dimensions: control versus acute pancreatitis, and comparing acute pancreatitis groups without or with various treatments.

When measuring blood flux on tongue, liver, intestine and pancreas, we found that acute pancreatitis presented lower BFU values compared to healthy control animals (Figure 1). On the tongue all the groups with acute pancreatitis showed lower BFU values compared to Control ($p<0.001$), but did not express statistically significant differences (Figure 1A).

On the small bowel the same situation could be observed compared to Control ($p<0.001$) (Figure 1B). On the liver, compared to Control significant difference was found in AP group ($p<0.001$), in AP+Pe ($p=0.026$) and in AP+Cle group ($p<0.001$) (Figure 1C). These observation suggest that on remote organs the drugs used could not improve the blood flux values, expect for flunixin when measuring surface BFU values of the intestine and the liver.

In case of the pancreas, significant difference could be seen only in the AP group versus Control ($p=0.039$), suggesting that here all of these drugs could moderately improve the BFU values (Figure 1D).

Since laser Doppler flowmetry results are affected by the temperature, we measured the body temperature: it was 35.29 ± 0.36 °C in Control group, 38.05 ± 0.66 °C in AP ($p<0.001$ vs. Control), 36.49 ± 1.34 °C in AP+Fl, 40.03 ± 0.78 °C in AP+Pe ($p<0.001$ vs. all groups), and 37.26 ± 0.94 °C in AP+Cle group ($p=0.002$ vs. Control). The high values in AP+Pe group was obvious even when touching the animals (room temperature was 25.95 °C).

3.2. Changes of hematological parameters

Table 1 summarizes the selected hematological parameters. Compared to Control, all acute pancreatitis groups showed lower white blood cell count (WBC [$x10^3/\mu l$]), except for

the group treated with enoxaparin (AP+Cle group), where WBC was elevated ($p<0.001$ vs. Control and vs. other groups).

Red blood cell count (RBC [$x10^6/\mu l$]) rose only in AP+Pe group ($p=0.008$ vs. Control) and AP+Cle group ($p<0.001$ vs. Control), which changes were accompanied by similar alterations in the hematocrit (Hct [%]). Hct values of AP+Pe and AP+Cle groups were significantly higher compared to Control ($p=0.001$ and $p<0.001$), AP ($p=0.007$ and $p=0.002$) as well as AP+Fl groups ($p=0.021$ and $p=0.027$), respectively. The mean corpuscular volume (MCV [fl]) did not show important differences.

Platelet counts (Plt [$x10^3/\mu l$]) were elevated in all acute pancreatitis groups, showing the highest values in AP+Cle group ($p<0.001$ vs. Control; $p<0.001$ vs. AP; $p=0.022$ vs. AP+Fl; $p=0.001$ vs. AP+Pe).

3.3. Changes of blood pH and lactate concentration

Blood pH significantly dropped in AP group ($p=0.022$ vs. Control), while the values of the treated groups were closer to the Control ones (Figure 2A).

Lactate concentration elevated slightly in AP and AP+Fl group without significant difference, while in AP+Pe group it significantly showed the highest values ($p<0.001$ vs. Control; $p=0.006$ vs. AP; $p=0.007$ vs. AP+Cle). The AP+Cle group did not differ from the Control (Figure 2B).

3.4. Changes of red blood cell deformability

Elongation index (EI) values decreased in groups with acute pancreatitis, except for the one treated with flunixin. Compared to the healthy Control group, the calculated EI_{max} values were significantly lower in AP ($p=0.037$), in AP+Pe ($p<0.001$) and in AP+Cle

(p=0.014) groups. Values of AP+Fl group were significantly higher versus AP (p<0.001), AP+Pe (p<0.001) and AP+Cle (p<0.001) groups (Figure 3A).

The calculated $SS_{1/2}$ values [Pa] showed similar relations: they were significantly lower versus Control in AP (p<0.001), AP+Pe (p<0.001) and AP+Cle (p<0.001) groups, and in AP+Fl group the values were significantly higher versus AP (p<0.001), AP+Pe (p<0.001) and AP+Cle (p<0.001) groups (Figure 3B).

As a sum, both EI_{max} and $SS_{1/2}$ decreased in acute pancreatitis being without or with treatment, except for the group with flunixin, where the values were closed to the Control.

When testing osmoscan parameters (Table 2), we found that minimal EI values were the highest in AP+Cle group (p<0.001 vs. Control; p=0.002 vs. AP; p<0.043 vs. AP+Fl and p<0.001 vs. AP+Pe), while the maximal EI values were the lowest in AP+Fl group (p=0.002 vs. Control; p=0.026 vs. AP and p=0.002 vs. AP+Pe). EI_{hyper} values were also the lowest in Ap+Fl group (p=0.008 vs. Control; p=0.031 vs. AP and p=0.002 vs. AP+Pe).

Osmolarity values at maximal EI ('optimal' osmolarity) did not show important differences. Interestingly, we found significant differences in osmolarity values of AP+Fl group at minimal EI (p=0.013 vs. Control; p=0.01 vs. AP; p=0.004 vs. AP+Pe; and p=0.006 vs. AP+Cle). Osmolarity values at EI_{hyper} were the highest in Ap+Pe group (p=0.011 vs. Control; p=0.011 vs. AP; p=0.001 vs. AP+Fl and p=0.002 vs. AP+Pe).

3.5. Changes of red blood cell aggregation

Table 3 summarizes the results from light-transmission aggregometry and syllectometrial measurements.

Compared to the healthy Control, red blood cell aggregation index values M and M1 showed increase in all acute pancreatitis groups, with the largest magnitude in the untreated AP group. In case of M 5 s values the differences were significant in AP (p<0.001), in AP+Fl

($p<0.001$) and in AP+Cle ($p=0.002$) groups. M1 5s values showed definitive differences in AP ($p=0.001$) and in AP+Pe groups ($p<0.001$). Testing M 10 s values, the differences were similar, being significant versus Control in AP ($p<0.001$), in AP+Fl ($p=0.023$) and in AP+Cle ($p<0.001$) groups. Compared to Control, M1 10 s values were found to be significantly higher in AP ($p<0.001$), AP+Fl ($p=0.022$), in AP+Cle ($p=0.023$) and in AP+Pe groups ($p<0.001$). The latter values were much higher compared to the other acute pancreatitis groups, too ($p<0.001$ vs. all).

Using the LoRRca, we found that all group with acute pancreatitis expressed elevated AI values. Compared to the Control, the differences were significant in AP ($p=0.002$), in AP+Fl ($p=0.025$) and in AP+Pe ($p=0.014$) groups. AI values did not show significant differences, while $t_{1/2}$ values [s] expressed significant alteration only between AP+Pe and AP+Cle groups ($p=0.044$).

4. Discussion

The cerulein-induced acute pancreatitis model is widely used in rats, mice, Syrian hamsters and dogs [11, 16, 17, 36, 38, 39]. However, the ‘effective’ dosage of cerulein is controversial [5, 13, 22, 46]. Based on the results of our previous study [22] we used 10 µg/kg single-dose cerulein subcutaneously in rats, and had the samples 2 hours afterwards. By this protocol we could investigate the early phase of the acute pancreatitis [25, 34].

The most accepted theories in the pathomechanism of severe acute pancreatitis include pancreatic autodigestion, leukocyte activation induced inflammation, bacterial translocation and pancreatic microcirculatory disturbances [5, 13, 14, 24, 25, 28, 34, 35, 36, 38, 39]. It is known, that during the early phase of the acute pancreatitis there is vasoconstriction in the larger pancreatic vessels resulting in a reduced capillary flow with increased permeability, which is followed by capillary stasis, hemorrhage in the late phase [25, 34]. Inflammatory

cytokines and formation of microthrombi as well as the edematous changes also lead to the deteriorated microcirculation [1, 25, 37]. Zhou et al. noticed in cerulein-induced acute pancreatitis the decrease of blood flow, functional capillary density and intermittent irregular perfusion in the pancreas [47].

Our microcirculatory findings correlated well with the literature data, the blood flux in the pancreas decreased 2-hours after administration of the cerulein. In remote regions (tongue, liver, intestine) we could also observed decreased blood flux values, suggesting a generalized inflammatory response [1, 25, 37, 44]. Activation of inflammatory cells leads to deliberation of pro-inflammatory mediators (TNF-α, IL-1, IL-2, IL-6) as well as NO, reactive oxygen radicals and arachidonic acid metabolites, besides anti-inflammatory IL-10. These factors together with various chemochines, such as IL-8 and chemoattractant proteins, lead to a systemic inflammatory response [6, 17, 19, 25, 35, 44]. These generalized changes may support the observation of decreased blood flux values on the surface of the investigated remote organs, as well as the increased body temperature.

Concerning the blood rheological changes during acute pancreatitis, there are not so many articles in the available literature. In the early phase –together with microcirculatory changes– their investigation was an important aim of our study.

In the pathophysiology of the acute pancreatitis the cascade system starts by the expression of inflammatory and vasoactive mediators such as platelet activation factor, intracellular adhesion molecule-1, endothelin, tumor necrosis factor, nitric oxide, oxygen free radicals, thromboxanes, bradykinin, prostaglandins and others [15, 23, 26, 33-40]. Inflammatory processes, oxidative damage, changes in pH, lactate concentration, oxygenation, all may have impact on red blood cell micro-rheological properties [2, 5, 7, 29].

In our study we could see definitive decrease in blood pH of the acute pancreatitis group being without treatment. It is known that alteration is pH affect red blood cell

deformability and aggregation [2, 29]. During inflammatory processes the free radical reactions as well as local changes of fluid distribution and micro-environmental osmolarity may also alter the rheological parameters [2, 7, 9, 31]. And in turn, altered blood rheology has an impact on the microcirculation [32]. Thus, parallel investigation of micro-rheological and microcirculatory alterations may have informative values on better understanding the pathophysiological events and to compare therapeutic tools.

We found that red blood cell deformability decreased (note that both EI_{max} and $SS_{1/2}$ decreases, because of the shape of the EI-SS curve) and erythrocyte aggregation enhanced in the samples from acute pancreatitis group. We did not make hematocrit adjustment for the aggregation measurements. The average hematocrit varied between 39 and 43 °%, and the available blood samples volume did not allow to perform e.g. 40% red blood cell plasma suspensions for all the aggregation measurements (note that just the LoRRca needs approximately 1 ml of sample for the aggregation test).

Further aim was to investigate whether various drugs may have improving effect on microcirculatory and micro-rheological changes induced by acute pancreatitis.

There are many possible ways (e.g., heparin, low molecular weight heparin [LMWH], pentoxifylline, allopurinol, baicalin, tirilazad mesylate, bovine hemoglobin, mesenteric lymph duct ligation, etc.) to affect the development of microcirculatory disturbances in experimental acute pancreatitis [5, 8, 13, 14, 16, 20, 33, 38, 39].

In our study, we found that the non-steroid anti-inflammatory agent flunixin, which is widely used in the veterinary practice, had beneficial impact to reduce the micro-rheological impairment, supposedly by reducing the general effects of the inflammatory processes [1, 25, 34, 37]. It is known that free radical reactions originated from the inflammatory events may have impair red blood cell micro-rheological properties [2, 7, 9]. Interestingly, both the red

blood cell deformability and aggregation parameters could be improved in the group treated with flunixin.

There are numerous clinical and experimental studies on the effects of pentoxifylline on acute pancreatitis [15, 16, 26, 27]. In experimental studies pentoxifylline –during the decrease of the secretion of inflammatory mediators– reduces systemic and local inflammatory responses and mortality [e.g., 15, 16]. Pentoxifylline as a phosphodiesterase inhibitor regulates the intra-pancreatic protein serine/threonine phosphatase activity and inflammatory mediators that play an important role in the pathomechanism of acute pancreatitis, too [15, 16, 26, 43]. Another well-known and important effect this drug is the improvement of red blood cell deformability, increase in filterability [e.g., 42]. However, in this current study we did not found direct evidence for the improving effect on red blood cell deformability in the present acute pancreatitis rat model. As an additional surprise, the body temperature was the highest in the AP+Pe group, probably due to the moderate microcirculatory and micro-rheological improvement but together with the existing inflammatory processes.

A positive effect of heparin was observed on the microcirculation of the pancreas in these studies [13, 14, 33]. In clinical studies, LMWH decreased the severity and the rate of encephalopathy as well as the mortality in severe acute pancreatitis [23, 24]. The possible effect of LMWH may be the downregulation of endothelin-1, TNF-α and IL-6 that reduced the formation of microthrombi in the pancreas and other organs [24, 33].

5. Conclusion

Investigation of microcirculation of local and remote tissues together with the analysis of hemorheological parameters and certain metabolic factors could be performed in this cerulein-induced acute pancreatitis model. Concluding the result: 2 hours after administration

of the cerulein (10 μg/kg, s.c.), we found severe deterioration in tissue microcirculation (tongue, liver, small intestine and pancreas), together with impaired red blood cell deformability and enhanced red blood cell aggregation, accompanied by acidic pH level and increasing lactate concentration compared to normal control. Improvement could be seen when using flunixin, pentoxifylline or enoxaparin, however, not in the same manner. These drugs could partly improve the blood flux on the surface of the investigated organs, however, the non-steroid anti-inflammatory drug flunixin had the most expressed improving effects on micro-rheological parameters (both on red blood cell deformability and aggregation). Surprisingly, the improving effect of pentoxifylline on micro-rheological parameters was not obvious (red blood cell deformability did not improved better than in the other treated groups), however, microcirculatory parameters improved.

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The authors comply with the Ethical Guidelines for Publication in *Clinical Hemorheology and Microcirculation* as published on the IOS Press website and in Volume 44, 2010, pp. 1-2 of this journal.

There is no conflict of interest.

7. References

[1] N. A. Al-Banna, D. Pavlovic, M. Gründling, J. Zhou, M. Kelly, S. Whynot, O. Hung, B. Johnston, T.B. Issekutz, H. Kern, V. Cerny and Ch. Lehmann, Impact of antibiotics on the microcirculation in local and systemic inflammation, *Clin. Hemorheol. Microcirc.* **53** (2013), 155-169.

[2] O.K. Baskurt, Mechanisms of blood rheology alterations, in: *Handbook of Hemorheology and Hemodynamics,* O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds., IOS Press, Amsterdam, The Netherlands, 2007, pp. 170-190.

[3] O.K. Baskurt, M. Boynard, G.C. Cokelet, P. Connes, B.M. Cooke, S. Forconi, M.R. Hardeman, F. Jung, F. Liao, H.J. Meiselman, G. Nash, N. Nemeth, B. Neu, B. Sandhagen, S. Shin, G. Thurston and J.L. Wautier; International Expert Panel for Standardization of Hemorheological Methods, New guidelines for hemorheological laboratory techniques, *Clin. Hemorheol. Microcirc.* **42** (2009), 75-97.

[4] O.K. Baskurt, M.R. Hardeman, M. Uyuklu, P. Ulker, M. Cengiz, N. Nemeth, S. Shin, T. Alexy and H.J. Meiselman, Parameterization of red blood cell elongation index - shear stress curves obtained by ektacytometry, *Scan. J. Clin. Lab. Inv.* **69** (2009), 777-788.

[5] T.L. Berezina, S.B. Zaets, D.J. Mole, Z. Spolarics, E.A. Deitch and G.W. Machiedo, Mesenteric duct ligation decreases red blood cell alterations caused by acute pancreatitis, *Am. J. Surg.* **190** (2005), 800-804.

[6] M. Bhatia, F.L. Wong, Y. Cao, H.Y. Lau, J. Huang, P. Puneet and L. Chevali, Pathophysiology of acute pancreatitis, *Pancreatology* **5** (2005), 132-144.

[7] B. Chmiel, R. Grabowska-Bochenek, D. Piskorska, A. Skorupa, L. Cierpka and S. Kusmierski, Red blood cells deformability and oxidative stress in acute pancreatitis, *Clin. Hemorheol. Microcirc.* **27** (2002), 155-162.

[8] B. Chmiel, J. Pajak, R. Grabowska-Bochenek and L. Cierpka, Decreased red blood cells deformability in acute pancreatitis in the rat. Effect of tirilazad mesylate, *Folia Med. Cracov.* **44** (2003), 109-115.

[9] M. Chvanov, O.H. Petersen and A. Tepikin, Free radicals and the pancreatic acinar cells: role in physiology and pathology, *Phil. Trans. R. Soc. B.* **360** (2005), 2273-2284.

[10] M.R. Clark, N. Mohandas and S.B. Shohet, Osmotic gradient ektacytometry: comprehensive characterization of red cell volume and surface maintenance, *Blood* **61** (1983), 899-910.

[11] A.P. Clemons, D.M. Holstein, A. Galli and C. Saunders, Cerulein-induced acute pancreatitis in the rat is significantly ameliorated by treatment with MEK1/2 inhibitors U0126 and PD98059, *Pancreas* **25** (2002), 251-259.

[12] L.I. Cosen-Binker and H.Y. Gaisano, Recent insights into the cellular mechanisms of acute pancreatitis, *Can. J. Gastroenterol.* **21** (2007), 19-24.

[13] P. Ceranowicz, A. Dembinski, Z. Warzecha, M. Dembinski, J. Cieszkowski, K. Rembisz, S.J. Konturek, B. Kusnierz-Cabala, R. Tomaszewska and Pawlik WW, Protective and therapeutic effect of heparin in acute pancreatitis, *J. Physiol. Pharmacol*. **59** (Suppl. 4) (2008), 103-125.

[14] M. Dobosz, L. Mionskowska, S. Hac, S. Dobrowolski, D. Dymecki and Z. Wajda, Heparin improves organ microcirculatory disturbances in caerulein-induced acute pancreatitis in rats, *World J. Gastroenterol.* **10** (2004), 2553-2556.

[15] J. Escobar, J. Pereda, A. Arduini, J. Sandoval, M.L. Moreno, S. Perez, L. Sabater, L. Aparisi, N. Cassinello, J. Hidalgo, L.A. Joosten, M. Vento, G. Lopez-Rodas and J. Sastre, Oxidative and nitrosative stress in acute pancreatitis. Modulation by pentoxifylline and oxypurinol, *Biochem. Pharmacol.* **83** (2012), 122-130.

[16] L. Gomez-Cambronero, B. Camps, J.G. de La Asuncion, M. Cerda, A. Pellin, F.V. Pallardo, J. Calvete, J.H. Sweiry, G.E. Mann, J. Vina and J. Sastre, Pentoxifylline ameliorates cerulein-induced pancreatitis in rats: role of glutathione and nitric oxide, *J. Pharmacol. Exp. Ther.* **293** (2000), 670-676.

[17] J. Granger and D. Remick, Acute pancreatitis: models, markers, and mediators, *Shock* **24** Suppl 1 (2005), 45-51.

[18] M.R. Hardeman, P.T. Goedhart and S. Shin, Methods in hemorheology, in: *Handbook of Hemorheology and Hemodynamics,* O.K. Baskurt, M.R. Hardeman, M.W. Rampling and H.J. Meiselman, eds., IOS Press, Amsterdam, The Netherlands, 2007, pp. 242-266.

[19] S.J. Harper and S. Cheslyn-Curtis, Acute pancreatitis, *Ann. Clin. Biochem.* **48** (2011), 23-37.

[20] A.T. Isik, M.R. Mas, L. Yamanel, S. Aydin, B. Comert, C. Akay, G. Erdem and N. Mas, The role of allopurinol in experimental acute necrotizing pancreatitis, *Indian J. Med. Res.* **124** (2006), 709-714.

[21] Z. Klarik, F. Kiss, I. Miko and N. Nemeth, Aorto-porto-caval micro-rheological differences of red blood cells in laboratory rats: further deformability and ektacytometrial osmoscan data, *Clin. Hemorheol. Microcirc.* 2012 March 7. Epub ahead of print. doi: 10.3233/CH-2012-1539

[22] R. Kotan, N. Nemeth, F. Kiss, J. Posan, K. Miszti-Blasius, L. Toth, I. Furka, I. Miko, P. Sapy and Zs. Szentkereszty, Micro-rheological changes during experimental acute pancreatitis in the rat, *Clin. Hemorheol. Microcirc.* **51** (2012), 255-264.

[23] X.S. Lu, F. Qiu, Y.X. Li, J.Q. Li, Q.Q. Fan and R.G. Zhou, Effect of lower-molecular weight heparin in the prevention of pancreatic encephalopathy in the patient with severe acute pancreatitis, *Pancreas* **39** (2010), 516-519.

[24] X.S. Lu, F. Qiu, J.Q. Li, Q.Q. Fan, R.G. Zhou, Y.H. Ai, K.C. Zhang and Y.X. Li, Low molecular weight heparin in the treatment of severe acute pancreatitis: a multiple centre prospective clinical study, *Asian J. Surg.* **32** (2009), 89-94.

[25] C. Mansfield, Pathophysiology of acute pancreatitis: potential application from experimental models and human medicine to dogs, *J. Vet. Intern. Med.* **26** (2012), 875-887.

[26] J. Marton, G. Farkas, T. Takacs, Z. Nagy, Z. Szasz, J. Varga, K. Jarmay, A. Balogh and J. Lonovics, Beneficial effects of pentoxifylline treatment of experimental acute pancreatitis in rats, *Res. Exp. Med. (Berl)* **197** (1998), 293-299.

[27] A.S. Matheus, A.M. Coelho, S. Sampietre, J. Jukemura, R.A. Patzina, J.E. Cunha and M.C. Machado, Do the effects of pentoxifylline on the inflammatory process and pancreatic infection justify its use in acute pancreatitis? *Pancreatology* **9** (2009), 687-693.

[28] R.F. Meirelles Jr., R. Ceneviva, J.L.F. Caboclo and M.M. Eisenberg, Pancreatic capillary blood flow during caerulein-induced pancreatitis evaluated by laser-doppler flowmeter in rats, *Acta Cir. Bras.* **18** (Suppl. 5) (2003), 18-22.

[29] N. Nemeth, I. Miko, A. Furka, F. Kiss, I. Furka, A. Koller and M. Szilasi, Concerning the importance of changes in hemorheological parameters caused by acid-base and blood gas alterations in experimental surgical models, *Clin. Hemorheol. Microcirc.* **51** (2012), 43-50.

[30] N. Nemeth, F. Kiss, Z. Klarik and I. Miko, Comparative osmotic gradient ektacytometry data on inter-species differences of experimental animals, *Clin. Hemorheol. Microcirc.* 2012 Oct. 17. Epub ahead of print. doi: 10.3233/CH-2012-1620

[31] S. de Oliveira and C. Saldanha, An overview about erythrocyte membrane, *Clin. Hemorheol. Microcirc.* **44** (2010), 63-74.

[32] A.S. Popel and P.C. Johnson, Microcirculation and hemorheology, *Ann. Rev. Fluid. Mech.* **37** (2005), 43-69.

[33] F. Qiu F, X.S. Lu and Y.K. Huang, Effect of low molecular weight heparin on pancreatic micro-circulation in severe acute pancreatitis in a rodent model, *Chin. Med. J. (Engl)* **120** (2007), 2260-2263.

[34] M.K. Russell, Acute pancreatitis: a review of pathophysiology and nutrition management, *Nutr. Clin. Pract.* **19** (2004), 16-24.

[35] R.P. Sah and A. Saluja, Molecular mechanisms of pancreatic injury, *Curr. Opin. Gastroenterol.* **27** (2011), 444-451.

[36] J. Schmidt, D. Ebeling, E. Ryschich, J. Werner, M.M. Gebhard and Klar E, Pancreatic capillary blood flow in an improved model of necrotizing pancreatitis in the rat, *J. Surg. Res.* **106** (2002), 335-341.

[37] A.S. Silva-Herdade and C. Saldanha, Effects of acetylcholine on an animal mode of inflammation, *Clin. Hemorheol. Microcirc.* **53** (2013), 209-216.

[38] T. Strate, O. Mann, H. Kleinhans, S. Rusani, C. Schneider, E. Yekebas, M. Freitag, T. Standl, C. Bloechle and J.R. Izbicki, Microcirculatory function and tissue damage is improved after therapeutic injection of bovine hemoglobin in severe acute rodent pancreatitis, *Pancreas* **30** (2005), 254-259.

[39] T. Strate, O. Mann, H. Kleinhans, C. Schneider, W.T. Knoefel, E. Yekebas, T. Standl, C. Bloechle and J.R. Izbicki, Systemic intravenous infusion of bovine hemoglobin significantly reduces microcirculatory dysfunction in experimentally induced pancreatitis in the rat, *Ann. Surg.* **238** (2003), 765-771.

[40] K.H. Su, C. Cuthbertson and C. Christophi, Review of experimental animal models of acute pancreatitis, *HPB* **8** (2006), 264-286.

[41] Z. Szentkereszty, R. Kotan, J. Posan, P. Arkossy and P. Sapy, Therapeutic tactics in the treatment of acute necrotizing pancreatitis, *Hepatogastroenterol.* **55** (2008), 1172-1174.

[42] O. Uskudar, A. Erdem, H. Demiroglu and N. Dikmenoglu, Decreased erythrocyte deformability in Behçet's disease, *Clin. Hemorheol. Microcirc.* **33** (2005), 89-94.

[43] M.M. Yada-Langui, E.A. Anjos-Valotta, P. Sannomiya, M. Rocha e Silva and R. Coimbra, Resuscitation affects microcirculatory polymorphonuclear leukocyte behavior after hemorrhagic shock: role of hypertonic saline and pentoxifylline, *Exp. Biol. Med. (Maywood)* **229** (2004), 684-693.

[44] X.P. Zhang, Z.J. Li and J. Zhang, Inflammatory mediators and microcirculatory disturbance in acute pancreatitis, *Hepatobil. Pancreat Dis. Int.* **8** (2009), 351-357.

[45] X. Zhang, H. Tian, C. Wu, Q. Ye, X. Jiang, L. Chen, Y. Cai, R. Xu and W. Yuan, Effect of baicalin on inflammatory mediator levels and microcirculation disturbance in rats with severe acute pancreatitis, *Pancreas* **38** (2009), 732-738.

[46] W. Zhou, B.A. Levine and M.S Olson, Platelet-activating factor: a mediator of pancreatic inflammation during cerulein hyperstimulation, *Am. J. Pathol.* **142** (1993), 1504-1512.

[47] Z.G. Zhou, Y.D. Chen, W. Sun and Z. Chen, Pancreatic microcirculatory impairment in experimental acute pancreatitis in rats, *World J. Gastroenterol*. **8** (2002), 933-936.

8. Tables

Table 1. Changes of selected hematological parameters

Variable	Control	AP	AP+Fl	AP+Pe	AP+Cle
WBC [x10^3/μl]	3.74 ± 1.28	3.22 ± 1.18	3.4 ± 1.05	2.93 ± 0.69	5.15 ± 1.6 *#
RBC [x10^6/μl]	6.85 ± 0.51	6.99 ± 0.41	6.91 ± 0.81	7.34 ± 0.58 *	7.47 ± 0.47 *
Hct [%]	39.03 ± 2.29	39.9 ± 1.98	39.14 ± 4.54	42.23 ± 3.49 *#	42.29 ± 2.5 *#
MCV [fl]	57 ± 1.78	57.08 ± 1.79	56.66 ± 1.34	57.47 ± 1.25	56.62 ± 1.77
Plt [x10^3/μl]	775.6 ±170.4	831.3 ± 87.4	866.1 ± 132.9*	835.9 ± 116.2	954 ± 96.1 *#

means ± S.D.; * p<0.05 vs. Control, # vs. other groups

Table 2. Parameters of osmotic gradient ektacytometry measurements at shear stress of 30 Pa.

Variable	Control	AP	AP+Fl	AP+Pe	AP+Cle
Minimal EI	0.103 ± 0.008	0.104 ± 0.008	0.107 ± 0.011	0.103 ± 0.008	0.116 ± 0.005*#
Maximal EI	0.514 ± 0.008	0.511 ± 0.005	0.497 ± 0.014*	0.516 ± 0.006	0.508 ± 0.007
EI_{hyper}	0.257 ± 0.004	0.256 ± 0.003	0.248 ± 0.007*	0.258 ± 0.003	0.254 ± 0.004
Osmolarity at min. EI [mOsm/kg]	162.3 ± 5.5	164.4 ± 6.5	136.5 ± 33.6*#	165.1 ± 4.8	162.1 ± 3.9
Osmolarity at max. EI [mOsm/kg]	312.5 ± 10.3	319.4 ± 14.9	313.6 ± 13.9	313.8 ± 9.1	318 ± 13.3
Osmolarity at EI_{hyper} [mOsm/kg]	412.9 ± 6.9	412.7 ± 6.8	408.3 ± 8.6	420.9 ± 6.1*#	411.8 ± 4.9

means ± S.D.; * p<0.05 vs. Control, # vs. other groups

Table 3. Red blood cell aggregation parameters measured by LoRRca (Amp, AI, $t_{1/2}$) or Myrenne MA-1 aggregometer (M and M1 index values at 5 and 10 sec)

Variable	Control	AP	AP+Fl	AP+Pe	AP+Cle
Amplitude [AU]	6.59 ± 1.81	9.76 ± 2.05 *	9.16 ± 2.78 *	8.8 ± 1.78 *	8.4 ± 2.19
AI [%]	70.77 ± 11.9	69.37 ± 5.29	71.39 ± 9.22	73.38 ± 4.97	76.62 ± 6.07
$t_{1/2}$ [sec]	0.66 ± 0.62	0.94 ± 0.47	1.04 ± 0.75	0.85 ± 0.41	0.52 ± 0.25
M 5 sec [AU]	1.44 ± 0.81	2.51 ± 0.98 *	1.66 ± 0.71	1.95 ± 1.27	2.14 ± 0.99 *
M1 5 sec [AU]	2.07 ± 0.95	2.88 ± 1.15 *	2.82 ± 1.84	4.14 ± 1.17 *	2.31 ± 0.6
M 10 sec [AU]	4.31 ± 1.73	6.98 ± 2.35 *	6.02 ± 3.16 *	5.34 ± 3.56	7.14 ± 2.52 *
M1 10 sec [AU]	5.12 ± 2.15	8.85 ± 3.84 *	7.14 ± 3.75 *	11.33 ± 2.18*#	5.95 ± 1.68*

means ± S.D.; * p<0.05 vs. Control, # vs. other groups

AI = aggregation index; AU = arbitrary unit

9. Figure captions

Figure 1.

Blood flux units measured on the surface of tongue (**A**), small intestine (**B**), liver (**C**) and pancreas (**D**) in Control group, acute pancreatitis group (AP), acute pancreatitis + flunixin group (AP+Fl), acute pancreatitis + pentoxifylline group (AP+Pe) and in acute pancreatitis + clexane group (AP+Cle).

means ± S.D.; * $p<0.05$ vs. Control

Figure 2.

Blood pH (**A**) and lactate concentration [mmol/l] (**B**) in Control group, acute pancreatitis group (AP), acute pancreatitis + flunixin group (AP+Fl), acute pancreatitis + pentoxifylline group (AP+Pe) and in acute pancreatitis + clexane group (AP+Cle).

means ± S.D.; * $p<0.05$ vs. Control; # $p<0.05$ vs. other groups

Figure 3.

Calculated EI_{max} (**A**) and shear stress values at half-EI_{max} [Pa] (**B**) in Control group, acute pancreatitis group (AP), acute pancreatitis + flunixin group (AP+Fl), acute pancreatitis + pentoxifylline group (AP+Pe) and in acute pancreatitis + clexane group (AP+Cle).

means ± S.D.; * $p<0.05$ vs. Control; # $p<0.05$ vs. other groups

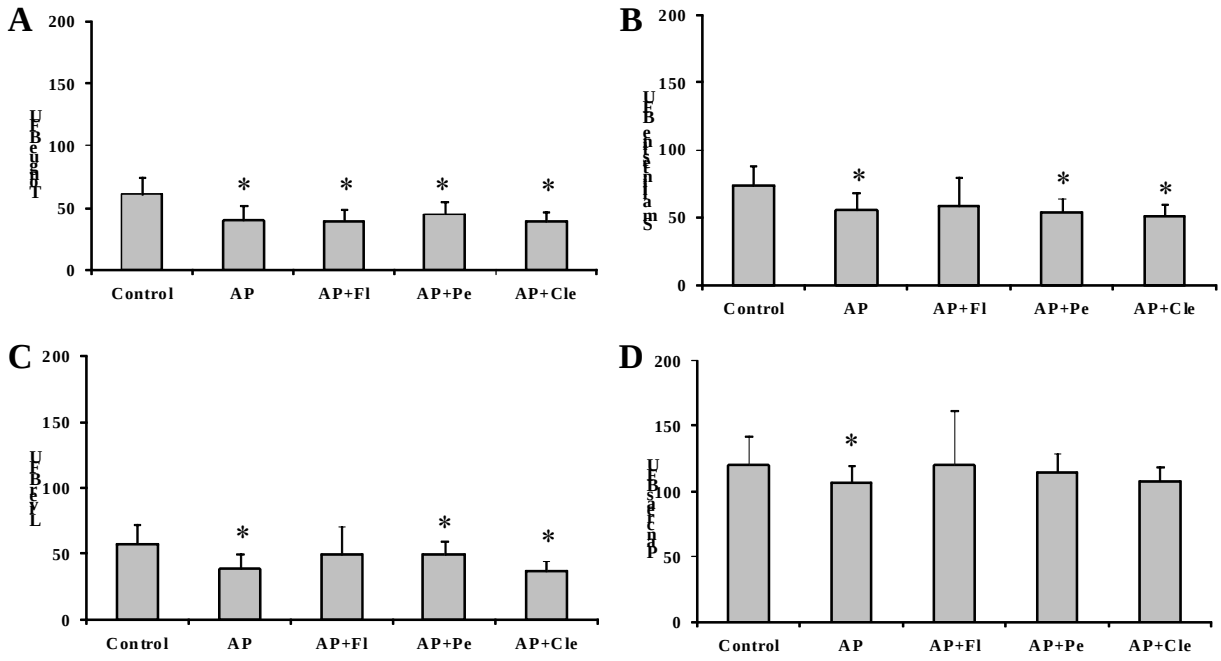


Figure 1.

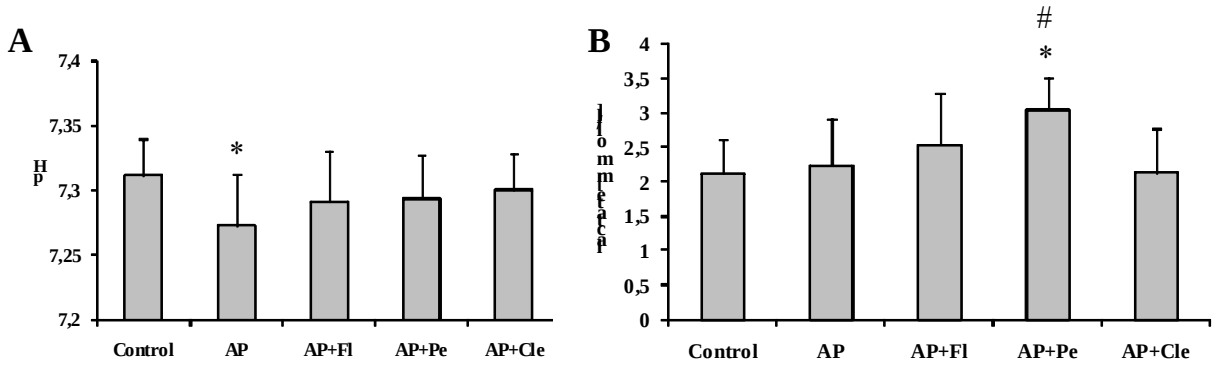
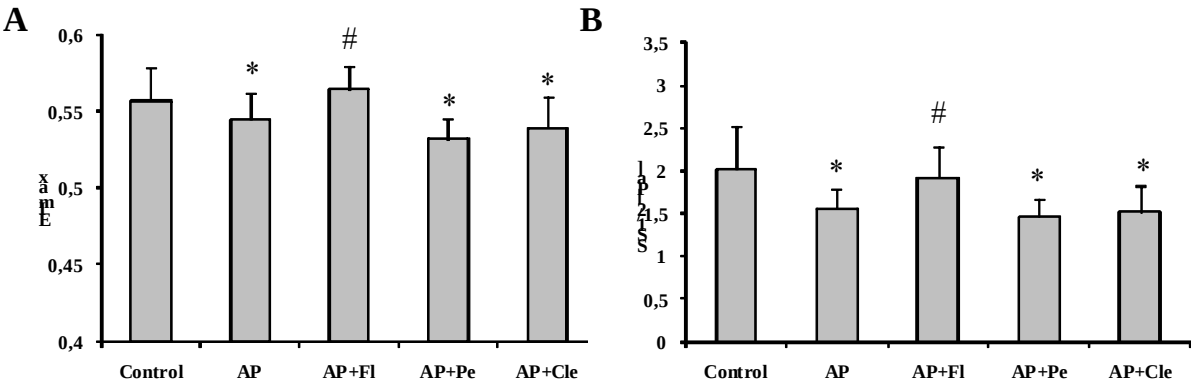


Figure 2.


A
0,6
0,55
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Control
AP
AP+Fl
AP+Pe
AP+Cle
B
3,5
3
2,5
2
1,5
1
0,5
0
Control
AP
AP+Fl
AP+Pe
AP+Cle

Figure 3.