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Effect of Splenectomy and Autologous Spleen Transplantation on the Serum Platelet-Activating Factor Acetylhydrolase (PAF-AH) Activity and Acute Phase Response (APR) in a Porcine Model

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Abstract—The aim of this study was to evaluate the inflammatory response after total splenectomy and spleen autotransplantation in a porcine model by measuring serum platelet-activating factor acetylhydrolase activity, C-reactive protein and albumin concentrations. Nineteen piglets were used in the experiment. After induction of anesthesia, animals were randomly divided into three groups: sham-operation with spleens intact (n=6), total splenectomy (n=6), and splenic autotransplantation (n=7) with small fragments of the spleen autotransplanted into the greater omentum. The blood samples were taken just before surgery and on day 1st, 5th, 12th, 26th and 40th postoperatively. PAF-AH activity, CRP and albumin concentrations were assayed in the sera. After total splenectomy, PAF-AH activity was significantly increased on day 5th, while there was no significant increase after spleen autotransplantation or the sham-operation. CRP was significantly increased after surgery in all experimental groups. Albumin was significantly decreased after surgery from day 5th until day 40th in splenectomized and autotransplanted pigs. Increased PAF-AH activity after splenectomy and spleen autotransplantation might be attributed to inflammatory conditions due to the loss of splenic tissue and trauma. Time-course increase of CRP, in all groups after surgery suggests post-injury inflammatory response due to tissue lesion during operation.

KEY WORDS: splenectomy; spleen autotransplantation; inflammation; acute phase response; platelet activating factor acetylhydrolase; C-reactive protein; albumin; porcine model.

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INTRODUCTION

For many years, splenectomy was regarded as a routine treatment for splenic injury in man without harming the patient. However, subsequent papers reported overwhelming post-splenectomy infection and severe immunological dysfunction with a risk of life-threatening sepsis [1]. This highlighted the importance of spleen functions in many specific and unspecific immune reactions, in particular in cases of infectious diseases and inflammation [2]. Therefore, great efforts have been done to establish the methods for the preservation of splenic tissue following splenic trauma or hematological diseases [3–5]. Autologous splenic

transplantation appears to be the only possible spleen-saving technique after total splenectomy [6]. The capability of regenerated splenic autotransplants of recovering its primary immune function is still controversial. Although many studies reported immunoprotective effects of implanted autologous splenic tissue against infection in rats [7–11] as well as in man [12, 13], a few other studies observed no beneficial effects [14–16].

Platelet-activating factor (PAF) is one of the most potent inflammatory mediators synthesized by a variety of mammalian cells after stimulation with either endotoxin or cytokines which induces and mediates acute phase response (APR). Among a variety of physiological actions, PAF activates polymorphonuclear leukocytes and monocytes thus being a well established activator of the immune system [17]. Platelet-activating factor acetylhydrolase (PAF-AH, E.C. 3.1.1.47) was identified to tightly regulate the degradation of PAF to avoid its inappropriately high level. Plasma PAF-AH is primarily secreted by tissue macrophages and it is considered as a potentially anti-inflammatory enzyme [18]. There are many evidences that plasma PAF-AH plays an anti-inflammatory role in the pathogenesis of human diseases associated with inflammation and an elevation of plasma PAF-AH activity could be a physiological response to inflammatory stimuli [19]. Pro-inflammatory cytokines that regulate immune response also induce the biosynthesis of positive acute phase proteins such as the C-reactive protein (CRP) [20].

The purpose of this study was to evaluate the serum PAF-AH activity after total splenectomy and autologous spleen transplantation in pigs in order to assess possible beneficial effect of splenic autotransplants concerning inflammation. CRP, as a widely used unspecific marker of inflammatory response, and albumin concentrations were also determined.

MATERIALS AND METHODS

Animals

The experimental protocol was approved by the Department of Veterinary Science, Ministry of Agriculture, Republic of Croatia and was conducted in accordance with the guidelines for the treatment of laboratory animals. Emotional reassurance (gentle restraint, petting and talking) was provided by the handler. Nineteen piglets of either sex, aged 3 months, weighing 19–26 kg were used in the experiment. In each animal food was withheld for 12 h and water for 2 h before the experiment.

Anesthesia and Surgery

The animals were premedicated with 2 mg kg^{-1} i.m. of xylazine, and left auricular vein was catheterized percutaneously for continuous infusion of lactated Ringer's solution at a rate of 10 ml kg^{-1} h^{-1} during surgical procedures and for administration of drugs. Anesthesia was induced with 5 mg kg^{-1} i.v. of ketamine and 10 μg kg^{-1} i.v. fentanyl, and animals were intubated, connected to the circle system and maintained on spontaneous ventilation. Anesthesia was maintained with 1.5% isoflurane and continuous intravenous infusion of fentanyl in a dose of 0.8 μg kg^{-1} min^{-1}. Supplemental doses of ketamine were given during surgery to maintain sufficient anesthesia depth. Perioperative antibiotic prophylaxis was administered using 20 mg kg^{-1} ampicillin and sulbactam i.v.

After induction of anesthesia, animals were randomly divided into three groups: sham-operated pigs with spleens intact (control group, $n=6$), splenectomized pigs ($n=6$), and splenectomized pigs with small fragments of 20% mass of the spleen autotransplanted into the greater omentum ($n=7$).

Blood Sampling and Experimental Protocol

Blood samples were taken from *v. jugularis* into the Vacutainer® tubes containing clot activator (Becton Dickinson & Company, Plymouth, UK) just before surgery and on day 1st, 5th, 12th, 26th and 40th postoperatively. After clotting for 2 h at room temperature, blood samples were centrifuged at 3,000 rpm for 15 min and serum samples were stored at −70°C until analysis.

PAF-AH Activity Assay. Platelet-activating factor acetylhydrolase (PAF-AH) activity was determined by the spectrophotometric assay described by Kosaka *et al.* [21]. Briefly, 2 μl serum was added to 240 μl of 200 mmol/l HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) buffer (Reagent 1), pH 7.6 and pre-incubated at 37°C for 5 min. The reaction was started by adding 80 μl of 20 mmol/l citric acid monohydrate buffer, pH 4.5 containing 90 mmol/l 1-myristoyl-2-(4-nitrophenylsuccinyl)phosphatidylcholine (Reagent 2), (Azwell Inc., Osaka, Japan). The liberation of p-nitrophenol was monitored at 405 nm and 505 nm at 1 and 3 min after the addition of Reagent 2 using the automatic

Table 1. PAF-AH Activity (U/l) in Sham-Operated Pigs (Control Group), Splenectomized Pigs and in Pigs with Autologous Splenic Transplants

		Days after surgery				
	Before surgery	1st	5th	12th	26th	40th
Sham-operation	483±28	449±17	446±25	426±16	405±12[a]	390±13[a]
Splenectomy	472±15	431±34	589±41[a,b]	527±54	428±38	426±26
Autotransplantation	525±63	455±39	519±27	471±28	580±57[b]	467±21[b]

All values are presented as mean±SEM
[a] $P<0.05$ statistical difference with respect to the value before surgery within each experimental group
[b] $P<0.05$ statistical difference with respect to the value in sham-operated pigs on the same day of the experiment

biochemical analyzer Olympus AU 600 (Olympus Optical Co., LTDL, Tokyo, Japan). The assay is linear up to 5 min of incubation. Linearity up to 1,500 U/l of the enzyme activity was observed as well [21]. PAF-AH activity in serum is expressed in international units (U/l) as the amount of substrate hydrolyzed per minute and per liter of serum (μmol min^{-1}/l).

C-reactive Protein (CRP) and Albumin Concentrations Assays. The concentration of C-reactive protein was determined by the latex-enhanced immunoturbidimetric assay kit (Full range CRP, Randox Laboratories Ltd., Ardmore, UK). Albumin concentration was measured by standard commercially available kit (Olympus Diagnostica GmbH, Hamburg, Germany). CRP concentration in serum is expressed as milligrams per liter (mg/l) while albumin concentration is expressed as grams per liter (g/l).All measurements were performed using automatic analyzer Olympus AU 600 (Olympus Optical Co., LTDL, Tokyo, Japan).

Statistical Analysis

Normality and equal variance were tested by using the Kolmogorov–Smirnov test and Leven's test. In order to assess significant differences between investigated groups the ANOVA was applied using SPSS software (SPSS Inc., Chicago, Illinois, USA). Pearson correlation coefficient was used to examine the correlation between two parameters. Statistical significance was based on values $P<0.05$.

RESULTS

PAF-AH Activity

The PAF-AH activity in sham-operated pigs (control group), splenectomized pigs and in pigs with autologous splenic transplants was given in Table 1. There were no statistical differences in PAF-AH activity in sham-operated pigs before surgery and day 1st, 5th and 12th postoperatively, but the activity was significantly lower on day 26th and 40th postoperatively compared to the value before surgery ($P<0.05$). In splenectomized pigs the PAF-AH activity was significantly higher on day 5th after surgery compared to the value before surgery ($P<0.05$). Two of six animals with total splenectomy died after day 5th postoperatively caused by severe postsplenectomy infection and sepsis. In pigs with autologous splenic transplants the PAF-AH activity was not significantly changed postoperatively compared to the value before surgery. Comparing PAF-AH activity in splenectomized and autotransplanted pigs with the values in the sham-operated on the same day of the experiment, PAF-AH activity was significantly higher in splenectomized pigs on day 5th, while in autotransplanted pigs, PAF-AH activity was

Table 2. CRP Concentration (mg/l) in Sham-Operated Pigs (Control Group), Splenectomized Pigs and in Pigs with Autologous Splenic Transplants

		Days after surgery				
	Before surgery	1st	5th	12th	26th	40th
Sham-operation	0.46±0.17	2.68±0.28[a]	3.85±0.34[a]	2.34±0.48[a]	2.18±0.40[a]	1.18±0.35
Splenectomy	0.66±0.22	2.80±0.32[a]	3.03±0.38[a]	1.72±0.50[a]	1.15±0.29	1.30±0.07
Autotransplantation	1.24±0.42	2.75±0.20[a]	3.46±0.30[a]	2.38±0.53	2.07±0.38	1.38±0.33

All values are presented as mean±SEM
[a] $P<0.05$ statistical difference with respect to the value before surgery within each experimental group
[b] $P<0.05$ statistical difference with respect to the value in sham-operated pigs on the same day of the experiment

Table 3. Albumin Concentration (g/l) in Sham-Operated Pigs (Control Group), Splenectomized Pigs and in Pigs with Autologous Splenic Transplants

		Days after surgery				
	Before surgery	1st	5th	12th	26th	40th
Sham-operation	42.1±0.4	38.8±0.8	34.7±1.5	37.6±2.4	39.1±0.9	37.9±1.2
Splenectomy	38.7±0.6	36.2±1.2	31.6±0.9[a]	29.1±1.1[a,b]	33.8±1.2[a,b]	30.6±1.2[a,b]
Autotransplantation	39.4±0.9	36.2±1.4	31.7±1.5[a]	30.7±1.9[a]	32.1±1.8[a,b]	31.6±2.0[a,b]

All values are presented as mean±SEM
[a] $P<0.05$ statistical difference with respect to the value before surgery within each experimental group
[b] $P<0.05$ statistical difference with respect to the value in sham-operated pigs on the same day of the experiment

significantly higher on day 26th and 40th compared to the sham-operated.

CRP Concentration

CRP concentration in sham-operated pigs (control group), splenectomized pigs and in pigs with autologous splenic transplants was shown in Table 2. In sham-operated pigs CRP was significantly increased on day 1st and 5th ($P<0.05$), as well as on day 12th and 26th ($P<0.05$) postoperatively than before surgery with the pick value on day 5th and has been gradually decreasing until day 40th after surgery. In splenectomized pigs CRP concentration was significantly higher on day 1st, 5th ($P<0.05$, respectively) and 12th ($P<0.05$) postoperatively than before surgery with the maximum level on day 5th. In pigs with autologous splenic transplants CRP concentration was significantly higher on day 1st ($P<0.05$) and 5th ($P<0.05$) postoperatively than before surgery. Compared to sham-operated pigs on the same day, there were no differences between sham-operated and pig with splenectomy or autotransplantation ($P>0.05$; Table 2).

Albumin Concentration

Table 3 presents the albumin concentration in all investigated groups. In sham-operated pigs there were no significant changes in albumin concentration between value before surgery and values postoperatively. In splenectomized pigs albumin concentration was significantly lower on day 5th, 12th ($P<0.001$, respectively), 26th ($P<0.05$) and 40th ($P<0.001$) compared to the value before surgery. In pigs with splenic transplants albumin concentration was significantly lower on day 5th, 12th, 26th and 40th ($P<0.05$) than before surgery.

No significant correlation between PAF-AH activity and the concentrations of CRP and albumin in any investigated group were found. Correlation coefficient (r) between PAF-AH and CRP in control group was 0.192 ($P>0.05$), in splenectomized pigs was 0.222 ($P>0.05$) and in pigs with autologous splenic transplants was 0.029 ($P>0.05$). Correlation coefficient between PAF-AH and albumin in control group was 0.274 ($P>0.05$), in splenectomized pigs was −0.267 ($P>0.05$) and in pigs with autologous splenic transplants was 0.176 ($P>0.05$). Significant negative correlation ($P<0.05$) was found between CRP and albumin in sham-operated pigs ($r=-0.438$; Fig. 1). Negative correlation between CRP and albumin concentration was also obtained in splenectomized pigs ($r=-0.326$), although the correlation was non-significant ($P=0.096$). There was no correlation between CRP and albumin in pigs with autologous splenic transplants ($r=0.044$; $P>0.05$). Compared to sham-operated pigs on the same day, albumin concentration in splenectomized pigs was significantly lower on day 12th, 26th and 40th, while in autotransplanted pigs was significantly lower on day 26th and 40th compared to the sham-operated.

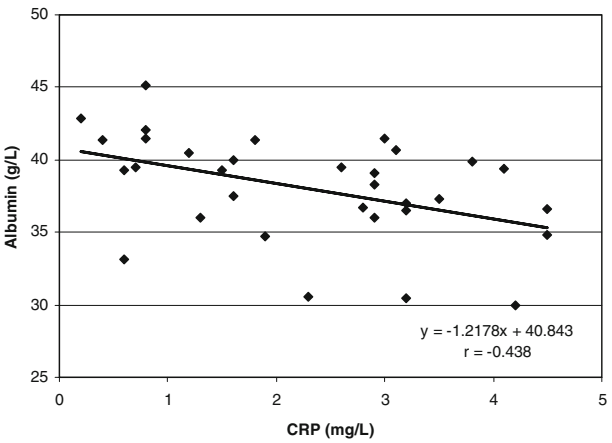

Fig. 1. Correlation between CRP and albumin concentrations in sham-operated pigs (control group). r=-0.438; P<0.05.

DISCUSSION

Conception of the spleen function in immune reactions has supported development of new spleen-saving techniques in cases of spleen injury. Although protective effect of splenic autotransplantation against postsplenectomy infection and inflammation is demonstrated, there is still some controversy on its beneficial effects [22].

In this study we investigated the time-course PAF-AH activity after total splenectomy and spleen autotransplantation. CRP and albumin concentrations were also investigated. Results demonstrate increased PAF-AH activity fifth day after total splenectomy, whereas there were no enzyme activity changes after spleen autotransplantation similar to control group. Compared to sham-operation (control group) PAF-AH activity was significantly higher postoperatively in both splenectomized and autotransplanted pigs. The spleen is effectively involved in many points concerning immune system including phagocytosis, antibodies producing, the liberation of marginated neutrophils, *etc.* [4]. PAF-AH tightly regulates the level of PAF by its degradation and possesses the anti-inflammatory properties [18]. Thus, the PAF-AH activity changes with respect to inflammatory stimuli. Higher PAF-AH activity in splenectomized and autotransplanted pigs might indicate an inflammatory response mediated by elevated PAF level. In addition, two animals died after splenectomy (after fifth day postoperatively) caused by overwhelming postsplenectomy infection and sepsis. Although Van Lenten *et al.* [23] demonstrated decreased PAF-AH activity during the acute phase response (APR) in rabbits, several other studies have found markedly increased PAF-AH activity in animal models during APR as the host response to infection and inflammation [24, 25]. In humans, elevated PAF-AH activity was found in human immunodeficiency virus (HIV) infection [26] and in metabolic diseases such as atherosclerotic diseases, systemic lupus erythematosus, hypothyroidism and acute respiratory distress syndrome [27].

CRP concentration was increased after surgery and peaked 5th day after the operation in all experimental groups regardless of surgical procedure, most likely as a post-injury inflammatory response provoked by tissue lesion during operation. However, the rise was the least in autotransplanted pigs. Recent studies also demonstrated increased CRP level after surgery due to tissue destruction during injury and increased proinflammatory cytokines production [28, 29].

Albumin concentration was significantly decreased after both the total splenectomy and spleen autotransplantation. Hypoalbuminemia is well known to appear in response to various stresses such as inflammation, infection or trauma [30]. However, albumin is multifunctional plasma protein with wide range of properties and activities including ligand-binding and transport properties, antioxidant function and enzymatic activities [31]. Thus, various conditions could influence plasma albumin concentration.

The lack of correlation between PAF-AH activity and CRP concentration was unexpected but it is in accordance with different time-course changes of PAF-AH activity and CRP concentration after surgery. Although both proteins are induced during APR, different mechanisms are probably involved in their up-regulation. In addition, no correlation of albumin with PAF-AH and CRP in most groups could be related to multifunctional role of albumin in the body.

In conclusion, increased PAF-AH activity after total splenectomy and spleen autotransplantation might be attributed to inflammatory conditions due to the loss of splenic tissue and trauma. Time-course changes of CRP concentration after surgery in all experimental groups suggests post-injury inflammatory response due to tissue lesion during surgical procedure. Hypoalbuminemia after total splenectomy and spleen autotransplantation might be also an unspecific response to stress such as inflammation, infection or trauma. Results suggest that, apart from CRP and albumin, PAF-AH is also an unspecific marker of inflammation.

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