The Effects of Platelet Activating Factor and Hyperbaric Oxygenation on The Isolated Duodenal Contractility

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✔ Platelet-activating factor has potent effects on gastrointestinal tract, and oxidative stress appears to play a role in the pathogenesis of some gastrointestinal tract diseases. We aimed to investigate the effects of hyperbaric oxygenation on the duodenal contractility of rats pretreated with platelet-activating factor or isotonic sodium chloride solution. Rats were exposed to hyperbaric oxygenation following platelet-activating factor or isotonic sodium chloride solution administration. The isometric contractions of isolated duodenal segments were measured. We observed that acetylcholine-induced contractions were more effective in the platelet-activating factor pretreated group diltiazem inhibited the contractility, and the contractile responses decreased in calcium-free medium. Histologically, we observed that pretreatment with platelet-activating factor caused inflamatory changes, and exposure to hyperbaric oxygenation exaggerated these effects. These results support the hypothesis which assumes that platelet-activating factor acts via free radical production or via prostaglandin formation and/or directly by increasing the contractility of smooth muscle, and these effects are exaggerated by hyperbaric oxygenation.

Key words: *Platelet-activating factor, hyperbaric oxygenation, oxidative stress, smooth muscle contractility, duodenum*

✔ **Trombosit Aktive Edici Faktör ve Hiperbarik Oksijenasyonun İzole Duodenum Kontraktilitesi Üzerine Etkileri**

Trombosit aktive edici faktör, sindirim kanalında proinflamatuvar etkiye sahip güçlü bir mediyatördür. Bazı sindirim kanalı hastalıklarının patogenezinde oksidatif stresin rolü olabileceği bildirilmektedir.

Çalışmada izotonik sodyum klorür solüsyonu veya trombosit aktive edici faktör ön uygulaması yapılan sıçanlarda duodenal kontraktilite üzerine hiperoksijenasyonun etkilerini incelemeyi amaçladık. Sıçanlar trombosit aktive edici faktör veya izotonik sodyum klorür solüsyonu uygulandıktan sonra hiperoksijenasyona maruz bırakıldılar.

İzole duodenal segmentlerin izometrik kontraksiyon yükseklikleri kaydedildi. Önce trombosit aktive edici faktör uygulaması yapılan grupta kontrole göre asetilkolinle indüklenen kontraksiyon yüksekliklerinin daha yüksek olduğu, Diltiazemin bu kontraksiyonları inhibe ettiği, kalsiyumsuz ortamda kontraktil cevapların azaldığı görüldü. Histolojik olarak, trombosit aktive edici faktör uygulamasının duodenumda inflamasyon cevapları oluşturduğu, hiperoksijenasyonun bu etkileri daha da arttırdığı gözlendi.

Bu sonuçlar, trombosit aktive edici faktörün serbest radikal oluşumu veya prostaglandin formasyonu aracılığı ile etkili olabileceğini ve/veya duodenum düz kas kontraksiyonlarını direkt etki ile arttırabileceğini, bu etkilerin hiperbarik oksijenasyonla daha da artabileceğini düşündürdü.

Anahtar kelimeler: *Trombosit aktive edici faktör, hiperbarik-oksijenasyon, oksidan stres, düz kas kontraktilitesi, duodenum*

INTRODUCTION

Platelet activating factor (PAF) is a glycerophospholipid, identified as a potential mediator of allergic and inflammatory reactions[1]. Cells which are able to synthesize and release PAF comprise white blood cells, platelets, monocytes, macrophages and endothelial cells. PAF induces severe pathological changes in various organs and has numerous potent effects. It causes gastric ulcerations and damages of small bowel[2]. Recent studies have shown that PAF is involved in the pathophysiology of various diseases of gastrointestinal tract in humans. The biopsies from colonic and ileal mucosa revealad increased amounts of PAF in Crohn's disease. PAF is also present at higher levels in stools of patients with Crohn's disease or ulcerative colitis than in those of healty individuals[2].

PAF also induces the contraction of intestinal smooth muscle, and tracheal strips from guinea pig, and rat colon[1]. The observation that many of the features of septic shock such as systemic hypotension, hemoconcentration and gastrointestinal ulceration, could be mimicked by intravenous administration of PAF, led to the hypothesis that PAF might be an important mediator of septic shock[3]. It was demonstrated that hypoxia induced ischemic bowel necrosis was mediated by PAF[4]. The mediators involved in PAF-induced mucosal dysfunction remained unclear. Some reports indicated that reactive oxygen metabolites, including superoxide (O_2.-) and hydrogen peroxide (H_2O_2), had a role in PAF induced mucosal ulceration[5]. Furthermore, it has been suggested that PAF-induced oxidants production may result from the activation of parenchymal cell-associated xanthine oxidase and/or granulocyte-associated NADPH oxidase[5]. It was also reported that oxidative stress altered intestinal motility in gastrointestinal tract[6]. On the other hand, it is known that generation of the reactive oxygen species (ROS) increases under hyperbaric conditions[7,8] and ROS play important roles in tissue injury[9]. However, there are some contradictory papers in literature. Some of them state that oxidants induce the contractility of gastrointestinal system[10], while others say that hypoxia causes the dismotility on small bowel[2].

Therefore, we aimed to investigate the effects of hyperbaric oxygenation (HBO) on the isolated duodenal contractility of rats which were pretreated with PAF.

MATERIALS AND METHODS

Healthy adult Wistar rats (n=24) of either sex, weighting 120-200 g were obtained from the Animal Care and Research Center of Faculty of Medicine, University of Ankara. Animals were housed in standard environmental conditions, and were deprived of food but not water for 16-18 hours before the experiments. Two experimental sets were designed. Both experimental sets comprised two groups and each group consisted of six animals. The first group (n=6) of the first experimental set to which only isotonic NaCl solution (serum physiologic, SP) were injected (i.p.) was defined as the control group. The second group (n=6) of the first experimental set to which PAF (40 g kg-1) was administered (i.p.) was defined as the PAF group. Two hours later, the animals were killed by decapitation and the pieces of the duodenum segments were removed rapidly. In the second experimental set (n=12), PAF administered (n=6) and SP administered (n=6) animals were both exposed to HBO. The rats in the HBO exposured groups were subjected to 2.5 ATA HBO for 90 minutes in

the Bethlehem Hyperbaric Chamber. During the HBO treatment in each session, a maximum of six rats were accommodated in the chamber and approximately 1.5 L minute-1 of ventilation was provided for each rat in order to prevent the accumulation of CO2 in the chamber. Immediately after moderate decompression, the animals were killed by decapitation and the pieces of the duodenum segments were removed rapidly. These group were defined as "PAF + HBO group" and "SP + HBO group" respectively.

Duodenum segments of approximateley 10 mm. long were cleaned from the adherent connective tissue and their contents were flushed with ice-cold Tyrode. In order to investigate the histopathology of duodenal tissue, at the same time, another duodenum segments of 1 cm. long were removed and fixed into 10% formalin buffered by 0.067 M phosphate pH 7.25. The solution was reneved two times a day and paraffin was embedded for histological sectioning and staining with hematoxilen eosin. The histopathology of the tissue was investigated by using light microscopy. In order to measure the isometric contractions of duodenum, the tissue segments were suspended in the isolated tissue bath containing 10 ml standard Tyrode solution (10^{-3} M; NaCl 136.9, KCl 2.68, $CaCl_2$ 1.80, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42 and Glucose 5.5) and bubbled with 95% O_2/5% CO_2 mixture at 37 °C, pH=7.4. For recording of tension, the samples were mounted vertically between a fixed holder and a force transducer (Ugo Basile isometric transducer No. 7003), and the tissues were brought into equilibrium in 30 minutes under optimal resting tension of 0.3 g. The isometric tension was recorded by Ugo Basile No: 7050 recorder. Contraction of the duodenum segments was induced by adding acetylcholine (ACh, $2.7X10^{-6}$ M) into the bath solution. In order to investigate the involvement of the Ca^{2+} channels in the response, we used diltiazem ($5.5X10^{-7}$ M), the selective antagonist of L-type Ca^{2+} channels which are the most abundant channels. In order to examine the effects of the blocker, the samples were pre-incubated with diltiazem for 3 minutes prior to testing. The same procedure was repeated in the Ca^{2+}-free medium. Ca^{2+}-free solution was prepared by equimolar substitution of $CaCl_2$ with NaCl in Tyrode solution.

PA (1-0-Hexadecyl-2-acetyl-sn-glycero-3-phosphocholine, PAFC16), diltiazem and ACh were obtained from Sigma Chemical Co., Sigma-Aldrich Chemical, Gmbh, Steinheim, Germany. Agents were dissolved in distilled water.

Statistical analyses were evaluated by using Mann-Whitney-U and Wilcoxon tests. Results were expressed as means S.E. The level of statistical significance p was less than 0.05.

RESULTS

We observed that ACh-induced contractions in standart Tyrode medium, were more effective in the PAF group than those in the SP group ($p<0.05$, Table I), whereas ACh-induced contractile responses in standart Tyrode medium were significantly higher in the PAF + HBO and the SP + HBO groups ($p<0.05$, Table). Potent Ca^{2+} channel blocker diltiazem decreased the contractility significantly in all groups ($p<0.05$, Table), and also contractile activities decreased significantly in Ca^{2+} free medium ($p<0.05$, Table).

In histological investigation of duodenal tissue, light microscopic studies have shown that there was no change in duodenal muscle but submucosa and mucosa was edematous and neutrophilic and eosinophilic

accumulation was observed with PAF administration (Figure 1). Exposure to HBO exaggerated this effects. Increased neutrophilic accumulation and a marked edema were seen in most of the superficial portions of mucosa (Figure 2).

Table. ACh-Induced Contractions and Effects of Diltiazem on The Contractility (mm) in Both Standart Tyrode and Ca^{2+}-Free Solutions. Values are given as mean S.E

	In standart tyrode medium		In Ca^{2+}-Free medium	
Groups	ACh-induced contraction	Diltiazem added contraction	ACh-induced contraction	Diltiazem added contraction
Control (SP) (n=6)	15.60 ± 1.79	3.20 ± 0.21	8.00 ± 2.06	2.00 ± 0.33*
PAF (n=6)	20.33 ± 0.86	5.66 ± 1.30*	10.66 ± 1.10	1.83 ± 0.18*
SP + HBO (n=6)	25.14 ± 3.13	4.57 ± 1.78*	12.00 ± 2.92	2.66 ± 0.63*
PAF + HBO (n=6)	33.18 ± 3.79	6.90 ± 2.32*	18.63 ± 3.87	4.27 ± 1.14*

*p<0.05 statistically significant with respect to the ACh-induced contractions.
p<0.05 statistically significant with respect to the control group.
p<0.05 statistically significant with respect to the PAF administered group.

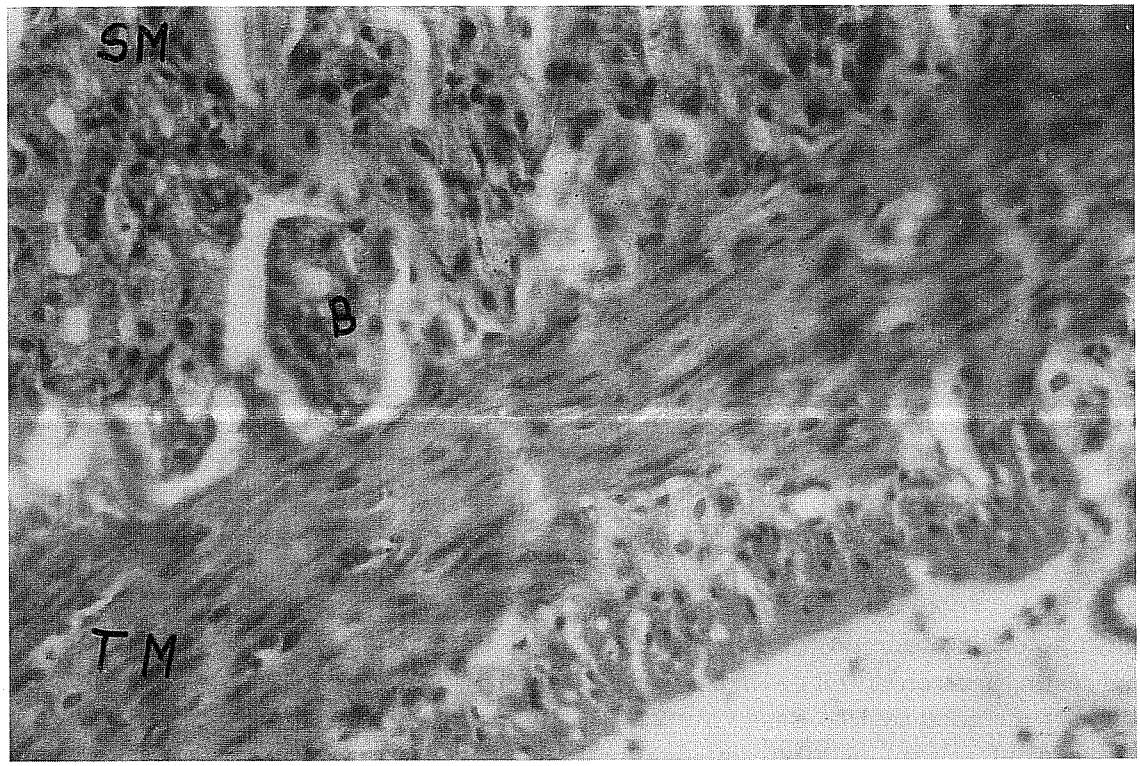

Figure 1. The Effects of PAF Administration on Rat Duodenal Tissue. **SM:** Submucosa, **B:** Brunner's Glands in Submucosa, **TM:** Tunica Muscularis (H.E. X 100)

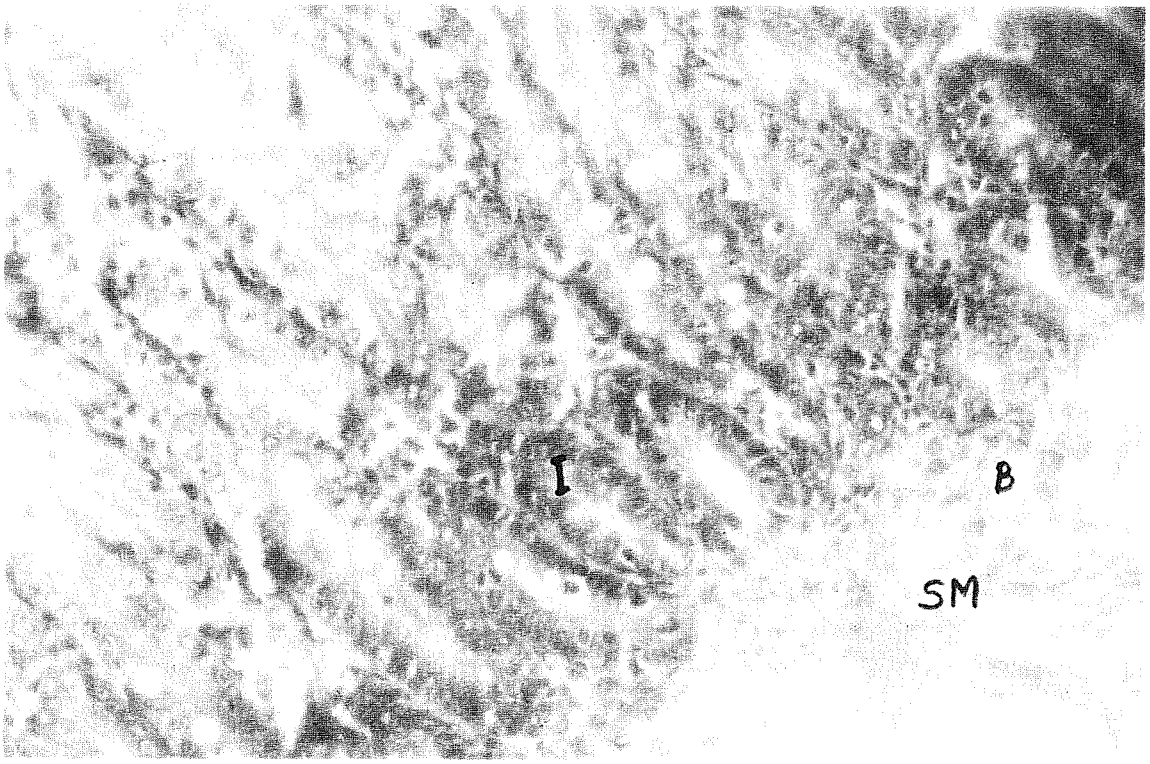

Figure 2. The Effects of PAF and HBO Administration on Rat Duodenal Tissue. **I:** Intestinal glands in mucosa, **B:** Brunner's Glands in Submucosa, **SM:** Submucosa (H.E. X 100)

DISCUSSION

Many investigators have been studying the effects of PAF on smooth muscle contractility since its first description in 1972 [11]. Extensive studies, have revealed that PAF increases the smooth muscle contractility in various tissues[1,2,12]. In the present study, the effect of invivo PAF administration on duodenal contraction by ACh was investigated in isolated rat duodenal segments. We found that pretreatment with PAF increased the contractile response to ACh in standart Tyrode, whereas the response decreased in Ca^{2+}-free medium. The ACh induced contractions in standart Tyrode in Ca^{2+}-free solution were decreased significantly by diltiazem. There was still a contraction in the Ca^{2+} free medium with diltiazem. There are not only one single type Ca^{2+} channel in smooth muscles. We blocked only L-type Ca^{2+} channels which are the most abundant channels. Other types of channels such as T-type (intermediate type) were still effective. Moreover, there was also some Ca^{2+} in the intracellular storage[13]. And probably, the Ca^{2+} in the intracellular storage might cause the contractions via small amount of other types of channels. This observation indicates that PAF exerts its effect by increasing the Ca^{2+} content of the cells. PAF might do this, by increasing the influx of Ca^{2+} directly or indirectly. Some investigators postulated that there was a spesific interaction between PAF and PGE_2 [14]. It is well known that prostaglandins are synthesized in large amounts and are widely distributed throughout the gastrointestinal tract and PGE_2 has been shown to cause the

contraction of intestinal smooth muscle[15]. But contrary to this suggestion, Jeanneton et al. (1993), presumed that, the effects of PAF were not mediated through the release of PGE_2. Thus, one can say that PAF stimulates the contractions directly via its spesific receptors on smooth muscle cells.

In this study, after exposure to HBO, contractile responses to ACh increased in the PAF and SP administrated animals. However, the exact mechanisms of the effects of HBO on the contractile machinery are still unclear. It is well documented that exposure to HBO produces the oxygen free radicals[12,16]. Hess et al. have reported that the oxidants might induce changes in the plasma membrane ion transport systems e.g. alteration of Ca^{2+}-ATPase activity. This could allow a massive influx of extracellular calcium. They suggested that, the oxidants did not alter the functional structure of the smooth muscle membrane. Also, it was demonstrated that, $O^{2\cdot-}$stimulates the D-myo-inositol 1,4,5-triphosphate (IP3)-induced Ca^{2+} release from the vascular smooth muscle.

There are some findings in the literature which indicate that PAF increases the size of the paracellular pathways between epithelial cells and/or the paracellular permeability. Similarly to our histological findings, it was shown that PAF did not cause damage in the epithelium of guinea-pig tracheae even though there was a large increase in permeability[14].

In our study, histological evaluation confirmed the suggestion that, neither PAF nor HBO administration altered the functional structure of smooth muscle cells. Duodenal segments exposed to HBO might have increased the influx of Ca^{2+} without damaging the cell membrane and this fenomena may be the cause of increased contractile response to HBO.

As a conclusion PAF acts via free radical production or via prostaglandin formation or directly by increasing the contractility of smooth muscle and this effect is exagerated by HBO.

Geliş tarihi : 20.02.2001
Yayına kabul tarihi : 04.06.2001

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