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Antioxidant Activity of Apocynin against Intestinal Ischemia-Reperfusion Induced Oxidative Damage in Lung and Intestinal Tissues

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Abstract; Potential effects of Apocynin against intestinal ischemia reperfusion-related oxidative damage was investigated in intestinal and lung tissues. Intestinal ischemia-reperfusion model was established. The superior mesenteric artery was occluded via an atraumatic clamp for 1 hour. Following ischemia, reperfusion was allowed for 2 hours. After the experimental processes, the animals were immolated, intestinal and lung tissue samples were removed. MPO activity and TOS, MDA, OSI, TNF-α, IL-1β levels raised in the ischemia-reperfusion group while TAS level and SOD activity diminished. TAS value and SOD activity elevated while MPO activity, OSI, TOS, TNF-α, IL-1β, and MDA levels decreased in low and high doses of Apocynin (20 mg/kg and 50 mg/kg) groups. Different doses of Apocynin administration demonstrated beneficial effects against intestinal ischemia reperfusion-induced oxidative damage in intestinal and lung tissues.

INTRODUCTION

Intestinal ischemia-reperfusion (I/R) mostly occurs due to a decrease in blood flow and perfusion [1]. It can be observed during trauma, major abdominal and vascular interventions, and sepsis [2]. A collapse in systemic circulation may lead to I/R injury [3]. Intestinal I/R injuries are related to intestinal obstruction, necrotizing colitis, and similar health conditions [4, 5]. Intestinal I/R is detrimental to the intestine and other tissues [6]. Intestinal I/R may lead to organ injuries, including liver, kidneys, heart, and lungs [7]. Acute lung injury (ALI) is an intestinal I/R complication which may induce acute respiratory distress syndrome (ARDS) [8]. Reactive oxygen species (ROS) have essential roles in ALI formation [9, 10]. Intestinal I/R may also damage remote organs through cytokines, ROS, and inflammatory cells. Excessive ROS levels and low antioxidant system activity may result in an I/R injury. The lung is a sensitive organ against ROS [11]. ROS injure the endothelial and epithelial parts of lung tissues by inducing proinflammatory cytokines [12]. It has been mentioned I/R-induced intestinal damage through ROS in previous studies [13-15].

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) is considered as the main source of ROS formation in ALI [16]. Apocynin (Apoc) (4-hydroxy-3-methoxy-acetophenone) is a catechol that inhibits NOX. It is the primary enzyme responsible for generating the initial ROS molecule superoxide in activated leukocytes [17]. Apoc has been reported to be a powerful antioxidant, and anti-inflammatory molecule in studies conducted to date [18, 19]. Apoc alleviates cerebral infarction through declining superoxide formation [20]. It also eases brain injury via diminishing inflammation [21]. In another study, Apoc demonstrated therapeutic effects against severe acute pancreatitis-induced lung injury [22]. Here, Apoc was examined against intestinal and lung injuries induced by intestinal I/R in rats.

MATERIAL and METHODS

Ethical approval and drugs

Permission for the current study was obtained from our University Experimental Animals Local Ethics Committee (protocol no:30.03.2018/63), and the experimental steps were carried out at our University Experimental Animals Research

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and Application Center, where also the animals were procured. Standard laboratory conditions were provided, including appropriate temperature, moisture, and light/night cycle.

Xylazine (Alfazyne %2 Injectable, Ege Vet. Hayvancılık San. ve Tic. Ltd. Şti. İzmir, Turkey) and Ketamine (Ketalar 500 Mg Injectable Flacon, Pfizer İlaçları Ltd. Şti. İstanbul, Turkey) were preferred for anesthesia. Apoc was purchased from Sigma Aldrich USA. Apoc doses and the method of administration used in the study were made according to previous studies [23, 24].

Experimental design, drugs, and groups

Thirty-two healthy Wistar Albino female rats were weighed (250-300 gr) and randomized to 4 groups (n=8). All rats were fixed on board in the supine position. Surgical areas were shaved firstly. Then, the area was disinfected with 10% povidone-iodine. Anesthesia (ketamine/xylazine 60/10 mg/kg, intraperitoneally, i.p.) was applied during all experimental steps.

Sham group: Following the animals' preparation, abdominal midlines were incised for 2 cm and sutured with 3/0 silk suture.

I/R group: After incision, the superior mesenteric artery was exposed to microvascular clip occlusion for 1 hour to create ischemia for each rat. Then, the clips were released, incision fields were sutured, and intestinal reperfusion was allowed for 2 hours as described in previous studies [(25, 26)].

Apoc 20 mg/kg group: All steps were performed as the same with I/R group. However, 20 mg/kg of Apoc was administered i.p. just before the reperfusion phase.

Apoc 50 mg/kg group: All steps were the same as Apoc 20 mg/kg group. But 50 mg/kg Apoc was administered i.p. just before the reperfusion phase.

In the end, rats were immolated by high-dose anesthesia. Intestinal and lung tissue samples were collected.

Biochemical Analysis of Intestinal and Lung Tissues

Intestinal and lung tissues were homogenized using 1 ml cold phosphate buffer per 100 mg of tissue. The homogenized tissues were centrifuged at 9000g at +4 ° C for 10 minutes, and their supernatants were obtained. After the homogenization of tissues, all biochemical analyses were performed via supernatants. Malondialdehyde (MDA) value was gauged with the method by Ohkawa et al. [27]. Superoxide dismutase (SOD) [28] and Myeloperoxidase (MPO) [29] activities were evaluated. The measurements of total oxidant status (TOS) (Rel Assay Diagnostics, Gaziantep, Turkey) and total antioxidant status (TAS) (Rel Assay Diagnostics, Gaziantep, Turkey) were carried out with applicable kits. Oxidative stress index (OSI) is the rate of TOS to TAS. TNF-α and IL-1β values were examined with appropriate Elisa kits (Elabscience, Wuhan, China).

Statistical analysis

All data were shown as mean±SD and determined by One-way ANOVA. Tukey test was used for pairwise comparisons of the groups. The differences were approved significantly in case of $p<0.05$.

RESULTS

SOD and TAS levels declined while MPO, TOS, OSI, and MDA values elevated in the I/R group compared to the sham group in intestinal tissues,. When Apoc 20 mg/kg group and I/R group were compared, all parameters (except SOD) demonstrated a statistically significant change, and oxidative markers decreased while the TAS level increased. All values changed significantly in Apoc 50 mg/kg group compared to the I/R group (table 1).

Remote tissue damage was evaluated for the lung tissues. When the I/R group was compared to the sham group, SOD and TAS values declined, but TOS, MDA, OSI, and MPO levels elevated. When Apoc 20 mg/kg and I/R groups were compared, TOS, MDA, MPO, and OSI levels diminished significantly, but the increase in TAS and SOD values was not significant. When the Apo 50 mg/kg group is compared to the I/R group, TOS, MDA, MPO, and OSI values declined while SOD and TAS values were raised statistically significantly (table 2).

Table 1 The effects of Apoc treatment in I/R-induced intestinal injury

Experimental Groups (n=8)	TAS (mmol/L)	TOS (µmol/L)	OSI	SOD (U/mg protein)	MPO (U/g protein)	MDA (µmol/g tissue)
Sham	1,50±0,24	4,31±0,41	0,29±0,02	309,26±85,91	343583,30±188185,50	61,85±8,89
I/R	0,52±0,06[a]	6,86±1,34[a]	1,34±0,34[a]	183,09±31,67[c]	585283,71±99139,59[a]	99,35±21,57[a]
Apoc 20 mg/kg	0,98±0,08[b]	4,87±0,56[b]	0,49±0,03[b]	277,02±86,11	416053,60±80234,94[d]	71,73±11,45[b]
Apoc 50 mg/kg	1,43±0,22[b]	4,55±0,54[b]	0,31±0,05[b]	284,31±41,70[d]	352168,94±76521,13[b]	64,58±8,90[b]

[a]$p<0.001$ and [c]$p<0.05$ compared to sham group. [b]$p<0.001$ and [d]$p<0.05$ compared to I/R group

When IL-1β and TNF-α values of intestinal and lung tissues were evaluated, there was an increase in both tissues in the I/R group compared to the sham group. With Apoc treatment, IL-1β and TNF-α values declined significantly in both tissue samples of Apoc 20 mg/kg and Apoc 50 mg/kg groups (figure 1 and figure 2).

Table 2. Effects of Apoc treatment in I/R-induced lung injury

Experimental Groups (n=8)	TAS (mmol/L)	TOS (μmol/L)	OSI	SOD (U/mg protein)	MPO (U/g protein)	MDA (μmol/g tissue)
Sham	1,16±0,21	8,56±1,28	0,76±0,22	180,66±31,56	326543,26±37695,22	69,27±2,71
I/R	0,79±0,07[a]	12,10±1,62[a]	1,51±0,18[a]	125,88±32,84[a]	475788,7±62770,03[a]	108,46±30,99[a]
Apoc 20 mg/kg	0,99±0,16	9,30±0,85[b]	0,96±0,20[b]	163,09±27,16	335486,44±23297,06[b]	77,81±12,37[b]
Apoc 50 mg/kg	1,19±0,21[b]	8,70±0,65[b]	0,74±0,08[b]	176,52±54,17[b]	331418,75±68529,04[b]	75,26±8,90[b]

[a]p<0.001 compared to sham group. [b]p<0.001 compared to I/R group

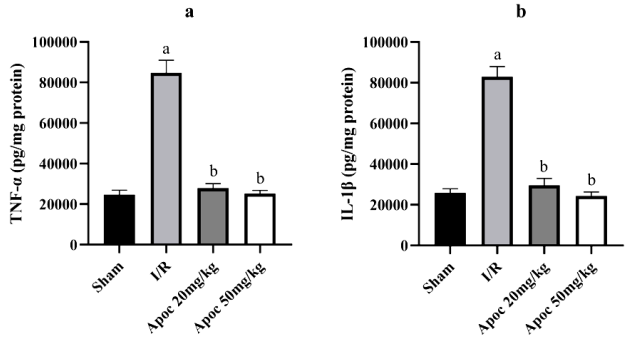

Figure 1. The results of **(a)** TNF-α and **(b)** IL-1β levels in I/R-induced intestinal injury.

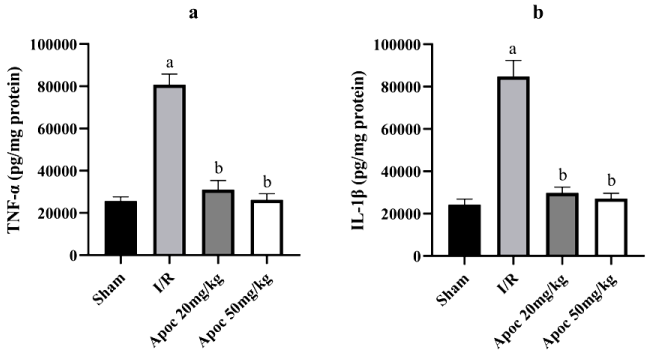

Figure 2. The results of **(a)** TNF-α and **(b)** IL-1β levels in I/R-induced lung injury.

DISCUSSION

Intestinal tissues are highly vulnerable to I/R [30]. Various conditions, including acute mesenteric ischemia, sepsis may lead to intestinal I/R [31]. Interruption of blood supply is the main reason for intestinal ischemia [32]. During surgical interventions, reperfusion, which follows elongated ischemia, leads to oxidative stress [3, 33, 34]. Intestinal I/R causes primary (intestinal tissues) and secondary (remote organ tissues) organ damage such as lungs, which are exposed to ALI and ARDS [35, 36]. Intestinal I/R may result in ALI and ARDS, which increase endothelial leakage and accumulation of inflammatory cells [37]. Inflammation and oxidative stress induce proinflammatory response activation and play a role in I/R pathogenesis [38]. ROS also play a part in I/R injury pathogenesis [39-42]. Oxidative stress, which plays a significant role in the formation of I/R damage, continues a process leading to lipid peroxidation, oxidation of cell proteins, DNA helix damage, and death [3, 41, 43]. The prolongation of the reperfusion period initiates an inflammatory cascade that causes irreversible tissue damage [44].

Several therapies have been tried to reverse cellular injury after I/R. Studies about antioxidant agents about this health condition have been increasing recently [45, 46]. TAS and TOS are preferred in I/R injury-related studies for the biochemical analysis to evaluate oxidative balance [47]. OSI, TOS, and TAS ratio plays a role in the follow-up of therapy besides reflecting the oxidant and antioxidant status [48]. Besides, powerful antioxidant enzymes such as SOD perform cellular defense [49]. Oxidant molecules such as MDA occur during I/R and increase the formation of free radicals that cause damage in cell membranes [50]. NOX is an enzyme whose physiological function is to generate ROS. Possible NOX-derived ROS cellular sources occur during I/R in the lung including leukocytes, endothelial cells, epithelial cells, and dendritic cells [51]. Apoc has been shown to stimulate the synthesis of some antioxidant enzymes and inhibition of ROS formation through NOX inhibition and eliminate various molecules that cause oxidation of proteins such as MDA [23, 52-54]. Our results show that high dose Apoc administration contributed significantly to increased antioxidant enzyme levels, and both Apoc doses administration decreased oxidant molecule level.

MPO is a specific oxidase in polymorphonuclear leukocytes (PMNL). It is used to predict MPO activity, PMNL chemotaxis, and infiltration in tissues [29]. PMNL infiltration during the reperfusion period can result in the generation and release of oxidants that aggravate this harmful cascade [55]. Apoc acts on both MPO and NOX [56]. The importance of Apoc in the MPO inhibition process has been reported [57]. In our study, the MPO level increased in the I/R group while approaching sham group's value in Apoc applied groups. MPO level decreased significantly in the Apoc 50 mg/kg group, particularly. Our results are compatible with various I/R models in the literature [23, 58].

I/R damage is associated with the coordinated activation of several cytokines and adhesion molecules. Apoc has been reported to cause NF-κB inhibition in various diseases [58]. NF-κB inhibition suppresses levels of proinflammatory cytokines such as TNF-α and IL-1β. This study measured serum TNF-α and IL-1β levels to evaluate intestinal and lung inflammation activity by the ELISA method. We found that Apoc therapy inhibits the I/R induced inflammatory response. Following the literature, we have again shown that Apoc is a potent anti-inflammatory molecule.

CONCLUSION

Apoc administration demonstrated protective effects on alleviating I/R injury triggered by intestinal I/R in intestinal and lung tissues. Current data may be new hope for intestinal and even other I/R injuries in further studies.

Conflict of interest

The authors declare that they have no conflict of interest

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