The Effect of Allopurinol and Acetylsalicylate on Ischemia-Reperfusion Related Injury of Liver

KARACİĞER İSKEMİ-REPERFÜZYON HASARINA ASETİLSALİSİLAT VE ALLOPURİNOL'UN ETKİSİ

Mehmet AKÖZ*, Alaaddin DİLSİZ**, Mehmet GÜRBİLEK*, Aytekin KAYMAKÇI**, Mahmut AY*, Fatih GÜLTEKİN*

Departments of *Biochemistry, **Pediatric Surgery Faculty of Medicine, Selçuk University, KONYA, TURKEY

Summary

It is assumed that, the injury which occurs in tissues during ischemia period increases during reperfusion period, and this increase is due to lipid peroxidation caused by oxygen derived free radicals in biomembranes. Free radicals are formed through various mechanisms and two of them are xanthine oxidase system and arachidonic acid pathway. The aim of the present study is to evaluate the effect of these systems on free oxygen radicals production and lipid peroxidation by using allo-purinol (a xanthine oxidase inhibitor) and acetylsalicylate (an inhibitor of arachidonic acid metabolism) in the rat model of liver ischemia - reperfusion.

For that purpose, 28 Wistar albino rats were divided into five groups. No operation was performed on the rats in the control group. Ischemia was induced by clamping right upper branches of hepatic artery and portal vein for 90 minutes in the second group. In the third group, after 90 minutes of ischemia period, reperfusion was achieved by opening the clamps for 30 minutes. The same ischemia and reperfusion procedures were performed on the allopurinol pretreated rats in the fourth group and on the acetylsalicylate pretreated rats in the fifth group. Allopurinol and acetylsalicylate were administered by oral route for three days before the procedures. In all groups, lipid peroxidation was evaluated by measuring malondialdehyde-thiobarbituric acid (MDA-TBA) levels using thiobarbiturate method. It was observed that ischemia increased lipid peroxidation and this increase was enhanced during reperfusion period. In the allopurinol group, lipid peroxidation significantly diminished compared with reperfusion group ($p<0.001$) while no change was observed in the acetylsalicylate group.

Our findings suggest that xanthine oxidase system is more effective than arachidonic acid pathway on ischemia-reperfusion related injury of liver and, allopurinol has a protective effect on ischemia - reperfusion injury.

Key Words: Ischemia, Reperfusion, Allopurinol, Acetylsalicylate, Liver injury

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Özet

Dokularda iskemi sırasında meydana gelen hasarın reperfüzyon sırasında arttığı, bu artışın da biyomembranlarda oksijen kaynaklı serbest radikallerin yol açtığı lipid peroksidasyonundan kaynaklandığı kabul edilir. Serbest radikaller çeşitli kaynaklardan oluşurlar. Bu kaynaklardan ikisi; ksantin oksidaz sistemi ve araşidonik asit yoludur. Bu çalışmanın amacı, bu sistemlerin lipid peroksidasyonu ve serbest oksijen radikalleri üretimi üzerine olan etkisini karaciğer iskemi-reperfüzyonu rat modeliyle değerlendirmektir. Bu maksat için 28 Wistar albino rat 5 gruba ayrıldı. Kontrol grubundaki ratlar üzerinde operasyon yapılmadı. İkinci grupta hepatik arterin sağ üst kolu ve portal ven 90 dakika süre ile klempe edilmek suretiyle iskemi oluşturuldu. Üçüncü grupta 90 dakikalık iskemi periyodu sonunda klampları açmak suretiyle 30 dakika süre ile reperfüzyon sağlandı. Aynı iskemi ve reperfüzyon işlemleri, allopurinol verilen dördüncü grup ve asetilsalisilat verilen beşinci grup ratlar üzerine uygulandı. Allopurinol ve asetilsalisilat oral yoldan işlemlerden önce üç gün süre ile verildi. Bütün gruplarda tiobarbitürat metodu kullanarak malondialdehit-tiobarbiturik asid (MDA-TBA) seviyelerini ölçmek suretiyle lipid peroksidasyonu değerlendirildi. İskeminin lipid peroksidasyonunu arttırdığı, bu artışın reperfüzyon sırasında fazlalaştığı görüldü. Lipid peroksidasyonu reperfüzyon grubu ile karşılaştırıldığında allopurinol grubunda anlamlı bir şekilde azaldığı ($p<0.001$) görülürken asetilsalisilat grubunda değişmediği gözlendi.

Bulgularımız, iskemi-reperfüzyon ile ilgili karaciğer hasarı üzerinde ksantin oksidaz sisteminin araşidonik asit yolundan daha etkili olduğunu, allopurinolün iskemi-reperfüzyon hasarı üzerine koruyucu bir etkiye sahip olduğunu göstermektedir.

Anahtar Kelimeler: İskemi-reperfüzyon, Allopurinol, Asetilsalisilat, Karaciğer hasarı

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Yazışma Adresi: Dr.Mehmet AKÖZ
Selçuk Üniversitesi Tıp Fakültesi
Biyokimya AD, KONYA

Ischemia is often considered to be a case which is caused by reduction in the blood flow. Hypoxia, on the other hand, is a term used to point out the fact that inadequate oxygen is obtained for the re-

quirement of the tissues. In ischemic hypoxia, a retention of metabolites in the bloodstream occurs and that leads to the lack of oxygen in tissues. Together with a reduced blood flow during ischemia, ATP consumption rate increases. One of the inevitable results of ischemia is the lactic acid production caused by the stimulation of anaerobic metabolism and the other is a decrease in venous oxygen saturation (1,2).

When ischemia-reperfusion periods are assessed, release of intracellular enzymes and free oxygen radicals in many tissues, calcium influx, destruction of cell membrane, and finally, necrosis of the tissues are observed to a certain extent. Free radicals are highly reactive substances and many of these having half life of only a fraction of a second. However, they might initiate chain reactions and thus an explosive production of free radicals can be generated if the process has been initiated. Free oxygen radicals react with lipids to produce lipid peroxide products. Although free oxygen radicals are capable of damaging all biological macromolecules, polyunsaturated fatty acids are especially vulnerable to free-radical-induced lipid peroxidation. If a molecule of polyunsaturated fatty acids reacts with a free radical, a lipid radical is formed which can be oxidized to a hydroperoxide radical which can react with a new polyunsaturated fatty acid molecule to form a new lipid radical. Free radicals not only injure polyunsaturated fatty acids in membranes, they may degrade structural proteins, inactivate enzymes and even destroy DNA. Since these findings were believed to be the result of the phenomena occurring during reperfusion, rather than the destruction caused by biochemical changes during ischemia, this injury has been called as 'reperfusion injury' (3-7).

Measurement of lipid peroxide concentrations by the malondialdehide-thiobarbituric acid (MDA-TBA) assay is the most widely recognized measure of free radical activity used in current clinical research, because MDA is the stable product of lipid peroxidation (8).

Activated neutrophils, the leak of electrons through electron transport system, xanthine oxidase system, arachidonic acid pathway, autooxidation the other compounds are considered as the sources of free oxygen radicals which play a role on ischemia - reperfusion injury (9,10). But it remains to be investigated what the main source or sources of these free oxygen radicals are.

In this study, we aimed to determine whether the xanthine oxidase system or arachidonic acid pathway are more effective on the production of free oxygen radicals. For this purpose, we used a xanthine oxidase inhibitor (allopurinol) and an inhibitor of cyclooxygenase (acetylsalicylate) in arachidonic acid pathway and evaluated their effects on the production of free oxygen radicals during ischemia-reperfusion period.

Materials and Methods

In this study, a total of 28 Wistar albino rats were used and divided in to 5 groups consisting of control (n=5), ischemia (n=5), reperfusion (n=4), allopurinol (n=8), and acetylsalicylate (n=6) groups. All animals were kept in standard chow and tap water, at 21^{o}C, light/dark cycle, 12 h. Additionally, rats were fed with allopurinol (20 mg/kg/day,orally); in allopurinol group, with acetylsalicylate (300 mg/kg/day,orally) in acetylsalicylate group, for three days before ischemia-reperfusion procedure. No operation was performed on the rats in control group, only blood samples were taken. Under light ether anesthesia, median laparotomy was performed on the other rats. The right upper branches of hepatic artery and portal vein were clamped to induce ischemia. After an ischemic period of 90 minutes, reperfusion was achieved by opening the clamped vessels. Blood samples were taken from dorsal vein at the end of 90 min ischemia period in Ischemia group, and at the end of 90 min ischemia plus 30 min reperfusion periods in the other groups. Plasma lipid peroxide concentrations was assessed by measuring the malondialdehide-thiobarbituric acid (MDA-TBA) level using thiobarbiturate method (8,11).

The statistical analysis was performed using the analysis of variance (ANOVA), followed by tukey HSD procedure.

Results

Lipid peroxidation levels were found higher in ischemia group (5.69± 1.37) compared with control group (0.84±0.19) ($p<0.001$), but lower compared with reperfusion group (7.04±1.85) ($p<0.001$). In allopurinol group, a significant decrease was ob-

Table 1. Lipid peroxidation levels

		Ischemia (Group 2) 5.69±1.37 nmol/ml	Reperfusion (Group 3) 7.04±1.85 nmol/ml	Allopurinol (Group 4) 1.19±0.51 nmol/ml	Acetylsalicylate (Group 5) 6.80±2.01 nmol/ml
Groups	n	5	4	8	6
Control (Group 1) (0.84± 0.19) nmol/ml	5	p<0.001	p<0.001	p>0.05	p<0.001
Ischemia (Group 2) (5.69±1.37) nmol/ml	5	-	p<0.001	p<0.01	p>0.05
Reperfusion (Group 3) (7.04±1.85) nmol/ml	4	-	-	p<0.001	p>0.05
Allopurinol (Group 4) (1.19±0.51) nmol/ml	8	-	-	-	p<0.01

served in lipid peroxidation levels (1.19±0.51) compared with ischemia and with reperfusion groups (p<0.001). There was no difference in lipid peroxidation levels between acetylsalicylate group (6.80±2.01) and reperfusion group (p>0.05). Lipid peroxidation levels (measured as MDA) of all groups are shown in Table 1.

Discussion

In fact, the field of medicine has lately experienced an explosive interest in the study of effects of free radicals. It has been suggested that free radicals are involved in a series of human diseases such as cancer, atherosclerosis, rheumatoid arthritis, and so forth. Today it is clear that tissue injury associated with hypoxia occurs to a large extent in the post-hypoxic reoxygenation period (4) that hypoxia would lead to excess oxygen free radical production (12). It is of interest in a number of clinical conditions: when a patient is resuscitated with oxygen after hypoxia like the newborn baby who suffers from hypoxia during delivery and is resuscitated with high concentrations of oxygen; the preterm baby with pulmonary insufficiency who is ventilated with high concentrations of oxygen; patients resuscitated after cardiac arrest; resuscitation of organs after hypoxia or ischemia e.g. the myocard after cardiac surgery or myocardial infarction; and perfusion of organs like kidneys, liver, heart and lung in connection with transplantation (4).

In the present study, we used an experimental model which proposed for studies on the liver injury caused by free radicals, studies those are made in order to improve hepatoprotective drugs, and investigations for the liver transplantation (13).

Egashira et al (13) reported an increase in the lipid peroxide of liver tissues after an ischemia of 90 minutes, and this increase was enhanced after enabling reoxygenation. They observed similar findings also in SGOT, SGPT and LDH levels. Our findings are consistent with those of Egashira et al.

Free oxygen radicals are formed through various sources. Xanthine oxidase (XO) system (9) and arachidonic acid metabolism (10) are two of these sources. In ischemic cells, during hypoxia, the reduced oxygen supply decreased the production of ATP, which in turn led to an accumulation of ADP and AMP. AMP was subsequently catabolized to adenosine, inosine and then hypoxanthine. Concurrent with these changes in energy status, xanthine dehydrogenase was converted by sulphydryl oxidation or limited proteolysis to xanthine oxidase. Then, following the reintroduction of oxygen (reperfusion), xanthine oxydase in the presence of its substrates, oxygen and hypoxanthine, produced large amounts of superoxide anions and hydrogen peroxide. This burst of free radical production overwhelmed the antioxidant defenses of the endothelial cells and injury occurred. (12)

When ischemic tissue is reperfused, hypoxanthine react with molecular oxygen in order to form xanthine. Superoxide anions are formed when the emerging xanthine is turned into uric acid by XO. Superoxide anions, on the other hand, may form hydroxyl radicals by reducing intracellular Fe^{+3} to Fe^{+2} via Fenton reaction. In endothel cells, the transformation of xanthine dehydrogenase into

xanthine oxidase may be a result of the contact between the cell and tumor necrosis factor-alpha (TNF-α), C5a, or chemotactic oligopeptid N-formil-met-leu-phe as well (9).

Karwinski et al (14) treated rats with allopurinol in order to prevent liver injury related ischemia - reperfusion. Instead of a single dose, they administrated two doses of allopurinol before ischemia and before reperfusion. In this two doses treatment, there was an improvement in the ATP generation and an increase in hypoxanthine concentration.

Yamanoi et al (15) also reported that allopurinol and cyclosporine decreased the ischemia - reperfusion related liver injury. It is known that allopurinol prevents the ischemia-reperfusion injury by inhibiting xanthine oxidase and production of free oxygen radicals.

Arachidonic acid metabolism is also an important source of free oxygen radicals. Separation of arachidonic acid from cell membrane phospholipids and its enzymatic oxidation leads to the formation of various free radical products and cyclic endoperoxides.

It was claimed that non-steroidal anti-inflammatory agents could inhibit the formation of cyclooxygenase products in arachidonic acid metabolism (16). Hayaishi and Sihimizu (17) demonstrated that, after the treatment with acetylsalicylate of the rabbits, there was a significant decrease in plasma total lipid peroxides within a few hours. However similar results were not demonstrated in human.

Simpsa et al (18) reported that, in reperfused myocard, the protective effect of ibuprofen (one of the non-steroid anti-inflammatory agents) is due to inhibition of Indium-111 labelled neutrophiles. Inhibitors of both cycloxygenase and lipoxygenase such as BW-755C and BAY-g-6575 decrease myocardial infarct severity compared with controls.

However in our study, it was observed that administration of acetylsalicylate statistically did not cause a significant decrease in lipid peroxide levels. A possible explanation for this different finding might be that arachidonic acid pathway did not play a role as a source of free oxygen radicals in our model.

On the other hand, during hypoxia, the decrease of electron transport chain components closer to cytochrom C1, allows the formation of superoxide radicals by mitochondrias. Besides, there is a loss in the cell content, of glutation peroxidase and superoxide dismutase which are of the antioxidant systems against the effects of free radicals (19). For this reason, suitable conditions have been available for the formation of H_2O_2 and highly reactive hydroxyl radicals. Inhibition of biochemical pathways which provide all these conditions helps the antioxidant defense.

We can conclude that, administration of allopurinol in ischemia-reperfusion not only inhibits the generation of free oxygen radicals, but also helps to protect hypoxanhtine which can be reused in ATP synthesis. Acetylsalicylate does not seem as effective as allopurinol for prevention of free radicals. Thus it can be suggested that the hypoxanthine-xanthine oxidase system is more effective on ischemia-reperfusion related liver injury than the arachidonic acid pathway.

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