

## Can Oxidative Stress Induced by Obstructive Jaundice be Improved by Iloprost? An Experimental Study in Rats

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### Abstract

**Background/Aims:** Iloprost is a synthetic analogue of epoprostenol which is synthesized by endothelial cells from arachidonic acid. We aimed to evaluate presumed hepatoprotective and antioxidant effects of iloprost in experimentally created obstructive jaundice in rats.

**Materials and Methods:** Thirty-two adult male Wistar albino rats were randomly divided into four groups: sham group (S) (n=8); after sham operation, iloprost administered (SI) group (n = 8); obstructive jaundice (OJ) group (n=8); iloprost administered and obstructive jaundice group (OJI) (n=8). The rats were sacrificed ten days after the induction of obstructive jaundice. Blood levels of isoprostane, protein carbonyl, lipid hydroperoxide and 8-hydroxy-2-deoxyguanosine (8-OHdG) were measured.

**Results:** Levels of the oxidative stress products including protein carbonyl [mean, (40.46 versus 24.7 nmol/ml, p=0.005)], lipid hydroperoxide [mean, (5.11 versus 1.85 Mmol/ml,p=0.001)] and 8-OHdG[mean, (1.53 versus 1.13 ng/ml, p=0.03)] in the OJI group were significantly lower than the OJ group.

**Conclusion:** Iloprost supports antioxidant activity in obstructive jaundice without causing further liver damage in rats.

**Keywords:** Antioxidants, bile duct ligation, experimental study, iloprost, obstructive jaundice, oxidative stress.

### I. Introduction

Obstructive jaundice is a clinical entity occurring after blockage of bile flow from the liver to the small intestine. Direct hyperbilirubinemia developed after obstruction of bile flow causes several disruptions including immune suppression, liver toxicity, coagulation disorders, intestinal barrier damage, increased oxidative stress, bacteremia and endotoxemia (Guicciardi et al. 2013). Oxidative stress is an imbalance between the production and degradation of reactive oxygen species *in vivo*. It damages the hepatocytes and intestinal cells in obstructive jaundice (Liu et al. 2001, Assimakopoulos et al. 2004a, Vendemiale et al. 2002, Assimakopoulos et al. 2004b, Assimakopoulos et al. 2006). Patients with obstructive jaundice are prone to develop serious complications during treatment process such as sepsis, renal complications and even death (Pain et al. 1985). Oxidative stress has a major role in the pathogenesis of hepatic injury and sepsis (Vendemiale et al. 2002, Allemeh et al. 2012). New treatment alternatives reducing oxidative stress may be effective to prevent complications associated with obstructive jaundice.

Iloprost is a synthetic analogue of epoprostenol (prostaglandin I<sub>2</sub>, prostacyclin) which is synthesized by endothelial cells from arachidonic acid. Beside the vasodilatory, antiplatelet and cytoprotective effects of the iloprost, it has fibrinolytic activity and it increases red cell elasticity, increases smooth muscle proliferation and supports microcirculation (Della Bella et al. 2001, Grant and Goa 1992). Considering its beneficial effects, iloprost seems as a good candidate to be evaluated experimentally against oxidative stress induced by obstructive jaundice. In this study, the effects of systematic application of iloprost on oxidative stress parameters and liver histopathology were assessed in rats with obstructive jaundice. This is the first study showing antioxidant effects of iloprost against oxidative stress induced by obstructive jaundice.

## II. Materials and Methods

The Ethics Committee of Experimental Animal Research of our university approved the study protocol. Thirty two Wistar-Albino male rats, weighing 250-300g, were used. The rats were housed four in per cage, under constant temperature (20- 22 °C) and humidity (50F) with an artificial 12 hour light-dark cycle and randomized into four groups. Obstructive jaundice was induced by ligation of the common bile duct. The rats were fed *ad libitum* with standardized industrial rat prey during the study period except 12 hours prior to operation. Twelve hours before the operation feeding was stopped. The rats were divided randomly into four equal weight-matched groups containing eight rats each. The study groups were sham (S) group; after sham operation, intraperitoneal iloprost applied (SI) group; obstructive jaundiced (OJ) group (the control group); after obstructive jaundice induction, intraperitoneal iloprost applied (OJI) group. A daily dose of 2 mcg/kg iloprost was given to the rats in the SI and OJI groups. Beneficial effects of the same dose iloprost in Wistar rats were shown previously (Vasiliadis et al. 2007). The S and OJ groups had been administered daily same volume 0.9 % NaCl solution intraperitoneally, while the others receiving iloprost injections. Ten days after the induction of obstructive jaundice, blood samples were taken with intra cardiac puncture under general anesthesia. A cervical dislocation was performed before liver tissue sampling.

### Bile duct ligation

The rats were anesthetized by intraperitoneal administration of 100 mg/kg ketamine hydrochloride (Ketalar; Parke- Davis, Istanbul, Turkey) and 5 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey). A 2 cm midline incision was made. In the Sham groups, the gastroduodenal ligament was isolated and the common bile duct was mobilized without any ligation or division. In the obstructive jaundice groups, the common bile ducts were ligated from 2 different points in middle with 4/0 silk sutures after common bile duct mobilization and divided. The abdominal incisions were closed in two layers with continuous 4/0 polyglactin and 4/0 silk sutures respectively. After the procedure, the rats were put into the cages.

### 2.2. Biochemical Analyses

The blood samples were stored in silicone tubes. The blood samples were centrifuged for five minutes at 6500 rpm. The serums were then separated.

The analysis of plasma 8-hydroxy-2-deoxyguanosine (8-OHdG), an indicator of oxidative damage on DNA, was made with competitive ELISA method using ELISA kits (NWLSS<sup>TM</sup> 8-OHdG ELISA, Northwest Vancouver, Canada). The concentration of samples was calculated using the standard calibration curve. The standard concentration ranges were 0.125 ng/ml, 0.250 ng/ml, 0.5 ng/ml, 1 ng/ml, 4 ng/ml and 10 ng/ml respectively.

The plasma isoprostane possesses potent biological activity and are released from tissues in a number of disease states, such as during ischemic injury and neurodegenerative brain disease. The measurement of isoprostane is considered to be the «gold standard» to assess oxidative stress in various human diseases. The analysis of isoprostane was made with competitive ELISA method using ELISA kits (NWLSS<sup>TM</sup> 8-OHdG ELISA, Northwest, Vancouver, Canada). The concentration of samples was calculated using the standard calibration curve, and the concentration ranges were 0.005 ng/ml, 0.1 ng/ml, 1 ng/ml, 5 ng/ml, 10 ng/ml, 50 ng/ml, 100 ng/ml respectively.

The plasma protein carbonyl, which reveals after the oxidation of amino-acid chains, analysis was made using a commercial kit (NWLSS<sup>TM</sup> Protein Carbonyl Assay, Northwest, Vancouver, Canada) based on spectrophotometric principles. Absorbance of the samples was measured at a wavelength of 320nm. Concentrations of the samples were measured by using molar absorptivity coefficient ( $22.000 \text{ M}^{-1}, \text{cm}^{-1}$ ) of protein carbonyl.

The plasma lipid hydroperoxide (LOOH), an indicator of the peroxidation of lipids and damage of the cell membrane, analysis was made using a commercial kit (NWLSS<sup>TM</sup> Protein Carbonyl Assay, Northwest, Vancouver Canada) based on spectrophotometric principles. Absorbance of the samples and calibrator was measured at a wavelength of 560 nm. Concentrations of the samples were measured by using molar absorptivity coefficient ( $33.700 \text{ M}^{-1}, \text{cm}^{-1}$ ) of protein carbonyl.

### 2.3. Histopathologic Examination

The liver samples were placed in 10% buffered formaline solution for 3 hours. After alcohol, acetone, xylene and paraffin procedures, the blocks were prepared. Then four microns cuts were obtained from the blocks and they were stained with hematoxylin and eosin (H&E) stain. Knodell index was used for histologic grading and staging (Knodell et al. 1981). The prepared slides were evaluated under light microscope with magnification of 40x and 100x. Their photos were taken by the camera connected to the microscope. The slides were assessed between - (0) and +++ (3) according to adiposity, hydropic degeneration, Kupffer cell proliferation/ sinusoidal cell proliferation, bile stasis, inflammatory activity in portal space (mononuclear cell infiltration), bile stasis, cholangitis (neutrophil and mononuclear cell infiltration to bile ducts), micro abscess formation.

### 2.4. Statistical Analysis

The data were presented as mean  $\pm$  standard deviation. Differences among the groups were evaluated by Kruskal-Wallis test by using SPSS 12 (SPSS Inc. Chicago USA). P values less than 0.05 were considered significant. When statistically significant values were identified, Mann-Whitney U test was used for paired comparisons and Bonferroni correction was used to detect statistical significance. P values less than 0.0083 were considered significant.

## III. Results

No rats died during study period.

### Biochemical parameters

There was no statistically significant difference in biochemical parameters between S and SI groups (Table 1). 8-OHdG (p=0.006), isoprostane (p=0.003), lipid hydroperoxide (p=0.001) parameters were found to be increased in OJ group compared to S group. Protein carbonyl (p=0.005) and lipid hydroperoxide (p=0.001) parameters were found to be decreased in OJI group compared to OJ group. Isoprostane (p=0.003) were found to be increased in OJI group compared to SI group.

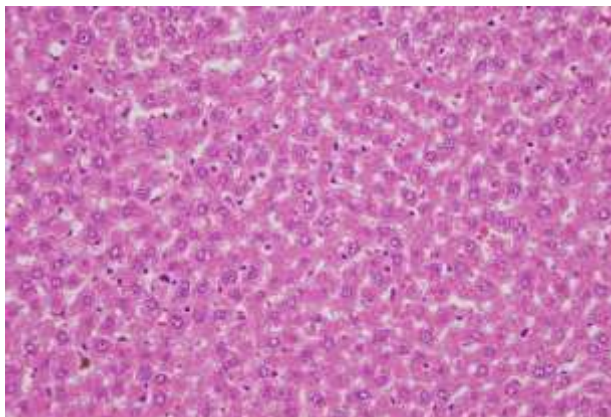
**Table I:** Results of oxidative stress parameters

	Sham	Sham+iloprost	OJ	OJ+iloprost	P value $\ddagger$
8-OHdG (ng/ml)	1.21 $\pm$ 0.1	1.16 $\pm$ 0.2	1.53 $\pm$ 0.22*	1.13 $\pm$ 0.25#	p=0.012
Isoprostane (ng/ml)	1.1 $\pm$ 0.08	1.05 $\pm$ 0.14	2.16 $\pm$ 0.7**	1.85 $\pm$ 0.4	p=0.000
Protein carbonyl (nmol/ml)	30.91 $\pm$ 12.33	23.92 $\pm$ 8.35	40.46 $\pm$ 7.36	24.7 $\pm$ 5.09###	p=0.02
Lipid hydroperoxide (Mmol/ml)	1.96 $\pm$ 0.39	1.82 $\pm$ 1.11	5.11 $\pm$ 1.66§	1.85 $\pm$ 1.01†	p=0.001

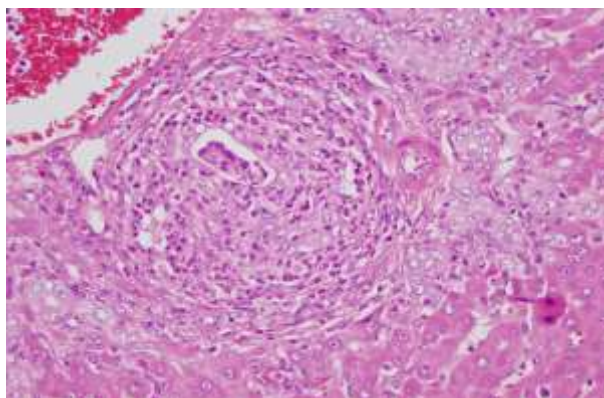
(8-OHdG: 8-Hydroxydeoxyguanosine; OJ: obstructive jaundice),  $\ddagger$ : p value of the Kruskal-Wallis test. All parameters were comparable between the sham versus sham+iloprost groups. The sham group versus OJ group: \*: p=0.006, \*\*: p=0.003, §: p=0.001. The OJ group versus OJ+iloprost: #: p=0.03, ###: p=0.005, †: 0.001.

### Histopathologic parameters

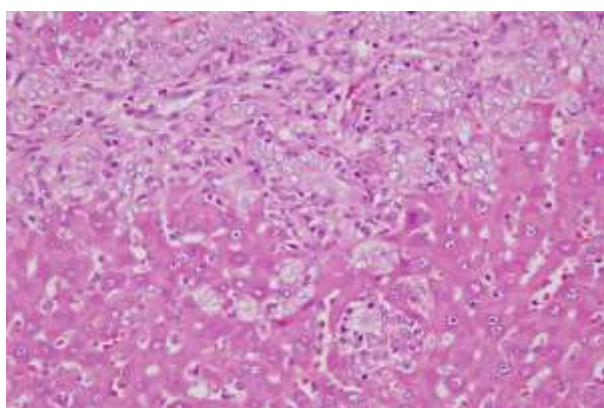
There were no statistically significant differences in histologic parameters between S (Figure 1) and SI (Figure 2) groups. Bile stasis (p=0.001), portal inflammation (p=0.001), bile ductus proliferation (p=0.001) and cholangitis (p=0.001) parameters were found to be increased in OJ (Figure 3) group compared to S group.



**Figure 1:** Control group. It's seen almost normal looking hepatocytes except mild hydropic changes. (HE x 400)



**Figure 2.** Iloprost group: Mixt inflammatory cells infiltrating portal area is associated with ductular proliferation.



**Figure 3.** Cholangitis group: Prominent ductular proliferation and steatosis is seen

Hydropic degeneration ( $p=0.006$ ), Kupffer cell proliferation ( $p=0.004$ ), bile stasis ( $p=0.000$ ), portal inflammation ( $p=0.001$ ), bile ductus proliferation ( $p=0.000$ ) and cholangitis ( $p=0.001$ ) parameters were found to be increased in OJI group compared to SI group. No statistically significant differences were observed between OJ and OJI groups in histologic evaluation. Microabscess formation in liver were observed in one rat ( $p=0.37$ ). The histopathologic results between of the S versus SI groups and OJ versus OJI groups were given (*Table 2*).

**Table II:** The results of histopathological evaluation.

	Sham	Sham+iloprost	OJ	OJ+iloprost	P value●
Adiposity	0	0	0	0	$p=0.089$
Hydropic degeneration	0	$0.25\pm 0.16$	$1.13\pm 0.13$	$1.29\pm 0.18\ddagger$	$p=0.002$
Kupffer cell proliferation	0	$0.25\pm 0.16$	$1.13\pm 0.13$	$1.43\pm 0.2\ddagger\ddagger$	$p=0.002$
Bile stasis	0	0	1*	1‡	$p=0.000$
Inflammatory activity in the portal space	0	$0.25\pm 0.16$	2**	$1.86\pm 0.14\ddagger\ddagger\ddagger$	$p=0.000$
Bile duct proliferation	0	$1.13\pm 0.13$	$2.89\pm 0.13\§$	$2.86\pm 0.14\diamond$	$p=0.000$
Cholangitis	0	$1.13\pm 0.13$	$1.63\pm 0.26\§\§$	$2\pm 0.22$	$p=0.000$
Microabscess formation	0	0	0	$0.29\pm 0.28$	$p=0.37$

(OJ: obstructive jaundice), ●: p value of the Kruskal-Wallis test. All parameters were comparable between the sham versus sham+iloprost groups and OJ versus OJ+iloprost groups. The sham group versus OJ group: \*:  $p=0.001$ , \*\*:  $p=0.001$ , §:  $p=0.001$ , §§:  $p=0.001$ . The sham+iloprost versus OJ+iloprost groups: †:  $p=0.006$ , ††:  $p=0.004$ , ‡:  $p=0.000$ , ‡‡:  $p=0.001$ , ‡‡‡:  $p=0.000$ .

#### **IV. Discussion**

Internal or external biliary drainage is the cure for obstructive jaundice. Our experimental obstructive jaundice model does not reflect real clinical scenario in general. Palliative drainage or definitive restoration bile flow can be achieved in majority of the patients with obstructive jaundice. However, high bilirubin levels may maintain in certain time period even after bile drainage or attempts to eliminate bile flow obstruction may fail. Under these circumstances prevention from the harmful effects of hyperbilirubinemia is needed until the definitive treatment. Since extrahepatic bile drainage obstructed permanently, severity of obstructive jaundice increased gradually during study period. Nevertheless, iloprost supplementation reduced DNA oxidation under gradually increasing hyperbilirubinemia. Since the liver has a high regeneration capacity and capable to regain its functions, protecting integrity of DNA may be beneficial during management of obstructive jaundice. Hyperbilirubinemia may be genotoxic via its oxidant effects (Basu et al. 2014). In addition to its use to monitor DNA damage, 8-OHdG has been suggested as a factor initiating carcinogenic pathways (Valavanidis 2009). An anti-metastatic effect of iloprost was shown decades ago (Sava et al. 1989, Honn et al. 1981). Prostacyclin analogues may reduce risk of metastasis by interacting between tumor cell and host (Schneider et al. 1994). It has been shown that iloprost can be used in cancer patients safely for treatment of systemic sclerosis (Pehlivan et al. 2012). Iloprost may prevent genotoxicity under hyperbilirubinemic condition. However, there is no clinical evidence supporting anti-neoplastic effects of iloprost. We observed beneficial effects of iloprost on oxidative stress parameters, but there was no improvement in histological evaluation of the liver. As we mentioned above, we blocked the bile flow permanently and created a gradually increasing hyperbilirubinemic condition as an experimental model. This may be the major factor for not observing any favorable histologic results. Combination of antioxidants is proposed to provide synergistic effects and increase each others activity, and therefore combination of iloprost with other antioxidants may be analyzed to obtain better results on histological evaluation of the liver (Isik et al. 2015).

Obstructive jaundice complicates many vital functions maintaining homeostasis (Scott-Conner and Grogan 1994). High bile acid levels, systemic endotoxemia and associated inflammatory response, over expression of inducible nitric oxide synthase, increased neutrophil chemotaxis, superoxide anion production and decreased levels of vitamin E augment oxidative damage in obstructive jaundice (Sakaguchi et al. 1996, Tsai et al. 1997). Oxidative stress is hepatotoxic (Liu et al. 2001, Vendemiale et al. 2002). Biliary cholestasis induces lipid peroxidation by reducing antioxidant capacity (Pastor et al. 1997). Obstructive jaundice caused oxidative damage in our control group. Since our surgeons are experienced on obstructive jaundice model in rats with the same technique and we carry out continuous research on obstructive jaundice (Alturfan et al. 2014, Aydin et al. 2010) we did not measure blood bilirubin, aspartate aminotransferase and alanine aminotransferase to prove hyperbilirubinemia and associated liver damage biochemically in the rats which were included to this study for minimizing expenses of the biochemical analyses. Although there is some data regarding to antioxidant effect of iloprost (Erre and Passiu 2009), use of iloprost in OJ induced oxidative stress has been poorly studied. Considering the significant decrease in oxidative stress parameters in OJI group compared to OJ group, our results may reveal that iloprost decrease the oxidative damage caused by oxidative jaundice.

Nowadays, a growing number of studies have suggested that reduction of oxidative stress is considered to have an important role for the prevention of liver damage due to cholestasis (Shiesh et al. 2000, Padillo et al. 2004). The antioxidant mechanisms of iloprost are under investigation (Aytac et al. 2008, Aytac et al. 2006). Atherosclerosis, senescence and oxidative stress reduces prostacyclin production by inhibiting cyclooxygenase which is the major enzyme in prostanooids synthesis (Dembinska-Kiec et al. 1979, Whorton et al. 1985). Since oxidative stress causes endothelial dysfunction, prostacyclin production decreases (Giugliano et al. 1996). Lack of prostacyclin may worsen microcirculation and subsequently augment production of oxidants as a vicious cycle. Toxic effects of oxidants on endothelium well known for decades and obstructive jaundice complicates hepatic microcirculation (Sacks et al. 1978, Okava et al. 2008). Iloprost supplementation may ameliorate microcirculation, improve tissue oxygenation and reduce production of oxidants in obstructive jaundice. It has been clinically and experimentally shown that iloprost improves microvascular functions (Ciuffetti et al. 2003, Rasmussen et al. 1992). In our OJ group, iloprost may have decreased the oxidation of the proteins and lipids with its effect on hepatic microcirculation.

In conclusion, iloprost supports antioxidant activity in obstructive jaundice without causing further liver damage in rats. New studies to explain molecular pathways of antioxidant activities supported by iloprost are needed.

The authors have nothing to disclose.

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