OPEN ACCESS Freely available online

Journal of Perioperative Medicine

Research Article

The Effect of Preoperative Simvastatin on Heme Oxygenase-1 (HO-1), Plasma NGAL and the Incidence of Post-Operative Acute Kidney Injury in Patient Undergoing Exploratory Laparotomy Surgery

Erwin Pradian*

Department of Anesthesiology and Intensive Care, Dr. Hasan Sadikin Central General Hospital, Padjadjaran University, Bandung, Indonesia

ABSTRACT

Background: Stress due to surgical laparotomy causes the release of cytokine and inflammatory mediators which may increase the formation of Reactive Oxygen Species (ROS) and free heme plasma. Free heme plasma acts as a strong oxidant and may damage cells including the kidneys tubules causing Acute Kidney Injury (AKI) marked by an increase of Neutrophil Gelatinase Associated Lipocalin (NGAL). This heme oxidant is neutralized by Heme Oxygenase-1 (HO-1). Simvastatin is known to increase HO-1 gene expression and plasma level. The aim of this research is to determine the effect of preoperative simvastatin on HO-1 and NGAL plasma levels, and the AKI incidents on exploratory laparotomy surgery patients.

Methods: This research is a randomized control trial on patients undergoing laparotomy. Following approval from the ethics committee, patients was randomized into two groups, the control/placebo (C) group and trial group that were given Simvastatin 40 mg, with 16 patients in each group. NGAL dans HO-1 were evaluated before and after surgery, while creatinine and total urine output were measured before and 24 hours post-surgery. All patients were given a routine induction and general anesthesia protocol. NGAL, HO-1, creatinine and total urine were analyzed using multivariable statistical analysis.

Results: This article reports significant differences of NGAL and HO-1 between the two groups. In the control group, there was an increase of NGAL and decrease of HO-1. On the trial group, there was a no significant correlation with AKI, plasma creatinine and urine production 24 hour.

Conclusion: Simvastatin 40 mg has a protective effect on cell with increased plasma HO-1 in patients undergoing laparotomy.

Keywords: AKI; HO-1; Laparotomy; NGAL; Simvastatin

INTRODUCTION

Surgery may result in surgical stress that causes disadvantages on normal body homeostasis. Reactive Oxygen Species (ROS) is a key metabolite that plays in the role of biological process disruption in many pathologic conditions. ROS involvement in surgical stress has been described before in invasive surgeries, such as open-heart surgeries, organ transplant surgeries, and other major surgeries that lasted long in duration or caused massive hemorrhage. This will surely have impacts on post-surgery outcomes [1].

Laparotomy surgeries have been reported to cause intestinal damage due to oxidative stress on test animals. Ischemic damage due to ROS may occur during ischemic process or during reperfusion. Surgical stress involves cytokine and inflammatory mediators release, such as TNF-α, IL-1, and IL-6. These mediators may cause a systemic inflammatory response syndrome, knowns as SIRS, and may produce more ROS. These

Correspondence to: Erwin Pradian, Department of Anesthesiology and Intensive Care, Dr. Hasan Sadikin Central General Hospital, Padjadjaran University, Bandung, Indonesia, E-mail: erwin.pradian@unpad.ac.id

Received: 12-Apr-2022, Manuscript No. JPME-22-16745; **Editor assigned:** 18-Apr-2022, PreQC No. JPME-22-16745 (PQ); **Reviewed:** 02-May-2022, QC No. JPME-22-16745; **Revised:** 06-May-2022, Manuscript No. JPME-22-16745 (R); **Published:** 19-May-2022, DOI:10.35248/2684-1290.22.5.123.

Citation: Pradian E (2022) The Effect of Preoperative Simvastatin to Heme Oxygenase-1 (HO-1), Plasma NGAL and The Incidence of Post-Operative Acute Kidney Injury in Patient Undergoing Exploratory Laparotomy Surgery. J Perioper Med. 5:123.

ROS will cause endothelial dysfunction, especially in microcirculations, henceforth causes disruption of blood flow. Splanchnic blood flow is one of the first to be sacrificed and the last to be functionally restored under stress condition. Renal blood flow, included in the splanchnic area, oftentimes undergo ischemic-hypoxia under stress or shock condition [2].

There are many oxidants known inside the body, such as: superoxide radical (O_2^-), hydroxyl radical (OH^-), peroxyl radical (ROO^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$), hypochlorous acid ($HOCl^-$), and heme. While the known body antioxidants are: Superoxide Dismutase (SOD), catalase, glutathione reductase, glutathione peroxide and Heme Oxidase (HO) [3].

Heme is a ferrous portophyrin-IX that is included in the hemoprotein group, alongside with hemoglobin, myoglobin, and cytochrome. While free heme is an unbound heme protein that is synthesized by the body but does not have a part in the hemoprotein metabolism. Free heme is released from hemoprotein under oxidative stress condition, which is very toxic, and its toxicity increases when this heme binds to other oxidants such as peroxides. Hence, hemoprotein and free heme have significant roles under oxidative stress [4,5].

Under normal condition, the level of free heme is around 100 nm [6,7]. Heme can induce capase-3 expression and may result in apoptosis process [8]. In the serum, heme is regulated by cell-cycle inhibitor p21 that can induce cell death cycle and hamper cell proliferation. Heme's antiproliferative effect has been studied in heme protein such as myoglobin [9]. Heme's nephrotoxicity has also been clinically observed with administration of large amounts of hematin to suppress porphyrin synthesis in patients with acute intermittent porphyria whom progressed to oliguric acute renal failure [10].

Inside the body, heme is neutralized by Heme Oxygenase-1 (HO-1) into bilirubin, biliverdin, and carbon monoxide (CO). HO-1 activity in neutralizing heme has been observed by using glycerol as a protein heme model that induce renal failure. Intramuscular injection of hypertonic glycerol in mice resulted in myolysis and hemolysis which increases heme protein sharply. This heme protein will then be distributed to the kidneys and damage the kidneys by the mechanism of vasoconstriction, kidney stone formation and direct cytotoxic effects that cause Acute Kidney Injury (AKI). At the same time, HO-1 level increased and if these test animals were given a substance that inhibits HO-1 activity, the kidney damage that occurs will get worse. This proves that HO-1 is protective against kidney damage that is caused by heme protein [11]. Another study that proved increased HO-1 level, namely by stimulating the kidneys to use small amounts of hemoglobin, endotoxin, nephrotoxic substances have protective properties against glycerol injection which causes kidney failure [10,11].

Simvastatin, like other statins, has pleiotropic and LDL-lowering effects. Simvastatin also increased gene expression of HO-1, especially in Vascular Smooth Muscle Cells (VSMCs). Intraperitoneal injection of Simvastatin in mice can increase HO-1 expression for more than 24 hours. Administration of statin therapy prior to Percutaneous Coronary Intervention (PCI) significantly reduced the mortality of patients whose C-Reactive Protein (CRP) which is a marker of inflammation increased. Studies using NF-κB and nitric oxide production in VSMCs as markers of inflammation have shown that the anti-inflammatory effect of simvastatin is mainly through increased HO-1 gene expression [12].

AKI is a clinical problem that occurred in 7% of hospitalized patients. In the Intensive Care Unit (ICU), the prevalence of AKI patients reached up to 67%. One study stated that 6% of AKI patients admitted to the ICU eventually required haemodialysis, and the mortality rate of these patients was 60% [13]. The incidence of AKI in patients who underwent exploratory laparotomy of the digestive tract admitted to the ICU in Hasan Sadikin General Hospital in 2008 was 21.1%. AKI is an independent predictor of mortality and prolonged hospital stay [14]. AKI can occur in a variety of clinical conditions with mild manifestations such as an increase in serum creatinine, to severe conditions, such as kidney failure. Severe cases require expensive therapy so delaying therapy will exacerbate kidney damage and increase the need for human and financial resources [13,14].

The term AKI was introduced in 2005 by the Acute Kidney Injury Network (AKIN) to describe the spectrum of acute kidney injury, which is a process that causes kidney damage within 48 hours and is defined as an increase in serum creatinine level 0.3 mg/dL (50% increase) or decreased urine output (oliguric state with urine output <0.5 mL/kg/hour for more than 6 hours) [15]. To diagnose AKI, the clinical criteria recommended by the Acute Dialysis Quality Initiative (ADQI) in 2002 were used, namely the RIFLE criteria (risk, injury, failure, loss and end stage renal disease). These criteria are based on an increase in serum creatinine levels, a decrease in the glomerular filtration rate (GFR) and a decrease in the amount of Urine Output (UO) [16]. GFR is influenced by several prerenal factors such as reduced circulating volume and changes in afferent arterioles that cause a decrease in GFR and an increase in creatinine without damage to the kidney structure. Serum creatinine is influenced by muscle mass and metabolism, race, age, sex and protein intake. This makes serum creatinine less sensitive as a biomarker of AKI. The lack of biomarkers that can detect AKI at an early stage hinders interventions that should be carried out to prevent further kidney damage [17].

Examination of levels of Neutrophil Gelatinase Associated Lipocalin (NGAL) can be used as an early marker of the occurrence of ischemic processes in the kidney because its levels increase at 1 to 2 hours in the urine and plasma of AKI patients. In a prospective study conducted by Dent, et al. in children undergoing Cardiopulmonary Bypass (CPB), AKI (defined as a 50% increase in serum creatinine) occurred in 28% of subjects, but diagnosis using serum creatinine can only be done on 1–3 days after surgery. In stark contrast to the assessment of AKI using the NGAL examination, which shows a tenfold or more increased only in 2–6 hours after surgery [18]. Zappitelli, et al. studied 106 paediatric patients admitted to the ICU and found that NGAL levels could detect AKI with a sensitivity of 54% and specificity of 97% [19]. Research on NGAL levels in adults treated in the ICU found an increase in plasma NGAL levels,

especially in the first 48 hours of treatment, which was significantly associated with the incidence of AKI. From this study, it was stated that NGAL levels could detect the occurrence of AKI with a sensitivity of 73% and specificity of 81% [18-20].

Several studies have shown that both urine and plasma NGAL levels can be early markers of AKI, especially when the timing of the onset of renal impairment is known, for example after cardiac surgery and after radio contrast use [21,22]. Its use in patients whose time of onset of renal impairment is unclear, such as in patients with intraoperative open bowel surgery, needs further investigation. To date, there have been no studies linking the effect of preoperative simvastatin administration to a reduction in the incidence of AKI following exploratory laparotomy. Thus, the author is interested in investigating the effect of preoperative simvastatin on the incidence of postoperative AKI in patients undergoing exploratory laparotomy of the digestive tract.

MATERIALS AND METHODS

Study setting and design

This research design is an experimental research design. The study was conducted prospectively by conducting a double-blind randomized controlled trial.

Eligibility

The subjects of this study were patients treated at Hasan Sadikin General Hospital who were about to undergo open bowel surgery under general anaesthesia who had met the research criteria and had been given an explanation about the study and willingness to participate in the study, and given informed consent.

Inclusion criteria

1. Elective exploratory laparotomy digestive surgery
2. Adult subject
3. Physical status ASA II-III

Exclusion criteria

1. Patients with a history of chronic kidney disease
2. Use of statin drugs 3 days before surgery.

Drop-out criteria

Subjects in whom intraoperative shock (MAP<65 mmHg) cannot be managed with fluids or vasopressor drugs.

Sample size

The sample size was determined based on the research objectives, namely as a clinical trial and correlation. The sample size in each group was calculated using the Pocock formula [23]. The treatment groups in this study consisted of two groups, namely the group that was given Simvastatin 40 mg orally preoperatively and the control group (placebo).

$n= 2\sigma/(\mu 2-\mu 1)\ f(\alpha,\beta)$

n=number of samples

σ=standard deviation

1=percentage increase in HO-1 control group

2=percentage increase in HO-1 in simvastatin group

μ=error rate I (set 0.05), significance level (1-α)=0.95

μ=error rate II (set 0.1)

f(α,β)=the value in the Pocock table, the value is 10.5.

From previous study [12], the obtained value for μ2=77.4%, μ1=25%, hence the sample size based on the above formula is n=13.9 (rounded to 14). To avoid dropout, 15% was added so that the sample became 16 per group. Hence, the required sample in each group is 16 samples. Subject selection was carried out by consecutive admission, namely based on the order of the operating schedule of subjects who met the inclusion criteria and randomized and divided into 2 groups.

Data collection

After obtaining approval from the Research Ethics Committee of Hasan Sadikin General Hospital/Faculty of Medicine, University of Padjadjaran, Subjects who have entered the inclusion criteria and excluded the exclusions and agreed to participate in the study by signing the Informed Consent.

A pre-operative examination was conducted the day before and the subjects were asked if they were willing to participate in the study. Then the subject's blood was taken to evaluate the levels of pre-operative HO-1, NGAL, and creatinine. Then the subjects were grouped into Simvastatin group and control group. In the Simvastatin group, subjects were given simvastatin 40 mg orally while the control group was given a placebo.

When the subject enters the operating room, blood pressure monitor, ECG, pulse oximeter, central venous catheter, and urinary catheter were installed. Hemodynamic data, bleeding, fluid intake, urine output and plasma creatinine levels were recorded. Furthermore, a preoperative evaluation was carried out according to standard procedures for anaesthesia and surgery until the operation was completed. Then the subject's blood was taken again for examination of HO-1 and NGAL levels, plasma creatinine levels. The subject was then awakened and moved to the recovery room. Then, the 24-hour urine output was recorded and the data obtained were then carried out statistical tests.

Blood samples were taken by taking 5 cc of venous blood, and then inserted into a tube containing anticoagulant. Stored for 30-60 minutes at room temperature, then centrifuged at 3000 rpm for 15 minutes. Separate in aliquots of 500 μL–1,000 μL, then stored at -20°C or -80°C, until the number of all samples is met. The reagents used to evaluate HO-1 level are anti-human HO-1 (detector A) and anti-rabbit IgG (detector B).

Data analysis

Data were analyzed to assess differences in plasma HO-1 levels, plasma NGAL, plasma creatinine and 24-hour urine volume between the group of subjects who were given Simvastatin 40 mg orally preoperatively and the control group, using the SPSS version 21 software program, taking into account the time of measurement.

The analysis used is as follows:

1. Data normality test was performed for each numerical variable. The results of the normality test have an effect on determining the comparison test method and the correlation test to be carried out.
2. Normality test: If n<50 use the Shapiro Wilk test, if n>50 use the Kolmogorov Smirnov test. The data is normally distributed if p>0.05.
3. Numerical comparison test: If the two groups of data are normally distributed, use the independent t test, whereas if one or both groups of data are not normally distributed, the Mann Whitney test is used. The difference is significant if p<0.05, very significant if p<0.01.
4. Comparison test (relationship) on both categorical variables: Using Chi Square test. The difference is significant if p<0.05, very significant if p<0.01.
5. Correlation test using Pearson product moment correlation analysis and multiple linear regression.

Operational definitions

Plasma NGAL: The NGAL level obtained in plasma by examination using the NGAL (human) ELISA kit from Enzo (unit of measurement is pg/mL).

Plasma HO-1: Heme Oxygenase-1 is a form of Heme oxygenase which is an antioxidant in breaking down free heme into bilirubin, biliverdin and CO, measured by the HO-1 (human) ELISA kit from Enzo (unit of measurement is ng/mL).

Plasma creatinine: The level of creatinine in plasma as measured from pre-operative and post-operative blood sampling (pre-operative is the day before surgery and before giving simvastatin or placebo, post-operative is one hour after surgery is completed) (unit of measurement is mg/dL).

24-hour urine output: Postoperative urine production measured from urine bag starting one hour postoperatively until 24 hours later (unit of measurement is mL).

Intraoperative urine output: The hourly urine production from the urine bag during surgery. Urine output is maintained >0.5 mL/kgBW/hour.

Blood pressure: The result of measuring systolic and diastolic pressure with a non-invasive blood pressure measuring device, the Nihon Kohden brand.

Mean Arterial Pressure (MAP): The mean value of blood pressure obtained from non-invasive blood pressure measurements with the Nihon Kohden brand<the value is maintained between 65-90 mmHg.

Pulse rate: The number of pulses in one minute using a Nihon Kohden pulse oximeter.

Central Venous Pressure (CVP): The venous pressure in the v. subclavia obtained from the results of pressure measurements using a central venous catheter and a nihon kohden brand measurement tool. The pressure is maintained between 8-12 mmHg.

Intraoperative bleeding: The amount of bleeding that occurs during the operation which is calculated based on the estimated bleeding that occurs from the suction bottle and gauze.

Acute Kidney Injury: A sudden decrease in kidney function after surgery which results in the kidneys not being able to excrete nitrogen and other waste substances which manifests in the accumulation of creatinine and blood urea and is often followed by a decrease in urine production. Diagnosed using the following RIFLE criteria (Table 1).

Table 1: RIFLE criteria for acute kidney injury.

	GFR criteria	Urine output criteria
Risk	SCr 1.5 times elevated	UO<0,5 mL/kg/hour
	Or decrease in GFR>25%	(for 6 hours)
Injury	SCr 2 times elevated	UO<0,5 mL/kg/hour
	Or decrease in GFR>50%	(for 12 hours)
Failure	SCr 3 times elevated	UO<0.3 mL/kg/hour
	Or decrease in GFR>75%	(for 24 hours)
	Or SCr ≥ 4 mg/dL	or anuria within 12 hours
Loss	Loss of kidney function>4 weeks	
ESRD	There is a need for RRT>3 months	

Note: SCr: Creatinine Serum Level; UO: Urine Output; GFR: Glomerular Filtration Rate; RRT: Renal Replacement Therapy

Simvastatin: An oral drug of the statin class for lowering cholesterol levels made by Dexa Medica, batch no. GKL9805024217A1, 20 mg pack.

Placebo: An oral medication containing saccharum lactis which is made to resemble simvastatin in terms of shape and size by PT Dexa Medica.

RESULTS

This study was conducted on 32 subjects with physical status ASA II and III who underwent digestive explorative laparotomy

surgery in the operating room of Hasan Sadikin General Hospital, Bandung. Subjects were double-blinded randomly assigned into 2 groups, namely the control group and the simvastatin group. Each group consisted of 16 research subjects. General characteristics of research subjects (Table 2).

Table 2: General characteristics of the subjects of this study.

General Characteristics	Control (n=16)	Simvastatin (n=16)	P value
Age (years old)	42.13 (12.2)	42.63 (18.11)	0.928[a]
Body weight (kg)	53.44 (4.88)	57.5 (7.38)	0.078[a]
Sex			
Male	8 (50%)	9 (56.3%)	1.000[b]
Female	8 (50%)	7 (43.8%)	
ASA			
II	12 (75%)	12 (75%)	1.000[b]
III	4 (25%)	4 (25%)	
Amount of fluid given intraoperatively			
Crystalloid	1112.5 (236.29)	1006.25 (220.51)	0.210[c]
Colloid	768.75 (185.18)	725 (249)	0.491[c]
Blood	312.5 (252.65)	350 (258.2)	0.752[c]

Note: a: Based on unpaired T-test; b: Chi-square test; c: Mann-Whitney test; Significant difference if $p<0.05$.

Based on Table 2, all the general characteristics of the research object were stated to be homogeneous or not significantly different between the control group and the Simvastatin group, which was indicated by the p-value of the test results ($p>0.05$). Thus, it can be concluded that the characteristics of the research object were homogeneous, so that they can be compared objectively.

Table 3: Control variables comparison.

Variables	Control (n=16)	Simvastatin (n=16)	P value
Systolic blood pressure (mmHg)	125.2 (7.1)	124.9 (5.5)	0.821
Diastolic blood pressure (mmHg)	77.0 (8/2)	76.3 (6.4)	0.604
Mean blood pressure (mmHg)	93.0 (8.5)	92.8 (5.8)	0.865
Heart rate (beats per minute)	82.4 (7.2)	82,1 (6.8)	0.86
Bleeding volume (mL)	862.5 (328.38)	1018.75 (379.86)	0.223
Surgery duration (minutes)	183.75 (52.39)	181.56 (64.9)	0.752
Intraoperative diuresis (mL/hour)	56.3 (5.1)	54.2 (2.3)	0.732

Note: Calculated based on unpaired T-test, except for bleeding and surgery duration using Mann-Whitney test. The p value is significant if $p<0.05$.

We compared the control variables of this study: Mean systolic and diastolic blood pressure, heart rate, bleeding volume, duration of operation, and intraoperative diuresis volume per hour. The comparison results between the control and simvastatin group are described (Table 3). The statistical analysis showed the control variables on both groups were significantly not different. With the homogeneity of these control variables, we determined that they are not confounding variables. Hemodynamic variables (blood pressure, pulse rate, bleeding and diuresis) were used as control variables in this study because changes in these hemodynamic parameters can affect perfusion to the splanchnic area including the kidneys which can have an impact on kidney function up to the occurrence of Acute Kidney Injury (AKI). In addition, hemodynamic parameters can also provide a rough description of the depth of anaesthesia or the level of surgical stress that occurs. Observations of control variables were carried out on the sample from just before the induction until the operation ended and all anaesthetic drugs had been discontinued, their progress was recorded every 5 minutes up to 120 minutes thereafter. From the results of blood pressure observations, there were 8 samples whose average blood pressure fell below 65 mmHg but can be overcome by giving fluids and vasopressors (ephedrine) so that blood pressure returns to normal limits and does not fall into the drop out criteria.

Table 4: Diagnosis and surgery types.

Diagnosis	Surgery Type	Control (n=16)	Simvastatin (n=16)
Malignancy			
Colorectal cancer	EL +Anastomosis	6	10
Pancreatic cancer	Bypass	6	5
Gastric cancer	EL +Anastomosis	1	1
Infective			

Cholangitis	Bypass	2	-
Peritonitis	EL +Anastomosis	1	-

Abbrevation: EL:Explorative Laparotomy.

In this study, we randomly collected data from elective digestive laparotomy surgery subjects with ASA status of II and III. The diagnosis and said type of surgeries on both groups are listed in Table 4. Based on the diagnosis and the most types of surgery included in the sample of this study, we found that 90.6% were subjects with malignancy, where 50% of the sample in this study was subjects with colorectal malignancy and exploratory laparotomy surgery was performed with bowel anastomosis or colostomy. Although the diagnosis and type of surgery in the study subjects were different; the duration of surgery, the amount of bleeding, the amount of fluid administered and the intraoperative urine production in the two groups were not statistically significant, so it was worth comparing.

Plasma HO-1 Levels

HO-1 is an HO isoenzyme, located on chromosome 22 (22q12), released when stress occurs to organs and tissues due to hypoxia, ischemia, hyperoxia, oxidative stress, proinflammatory and the presence of heavy metals. Under normal conditions, HO-1 levels are minimal in the renal tubules, but when the kidneys are stressed, these HO-1 levels can increase greatly in the renal tubules and arterioles [10]. HO-1 under normal conditions is also produced by the stomach, intestines and colonic mucosa, and its production will increase when these organs are inflamed. There are several studies that show HO-1 acts as a regulator in cellular protective mechanisms and referred to as stress responsive protein [24]. In a study using cobalt-protoporphyrin to induce HO-1 prior to intestinal ischemia-reperfusion, intestinal microvascular damage was significantly reduced [25].

The mechanism of protection of HO-1 against ischemic-reperfusion injury in the intestine is through 4 mechanisms: namely degradation of free heme formation of CO, formation of biliverdin/bilirubin and binding of Fe^{2+}. Free heme produced during tissue damage can be derived from heme proteins such as hemoglobin, myoglobin, cytochromes, and enzymes such as Nitric Oxide Synthase (NOS), myeloperoxidase and catalase which are highly toxic to cells. CO produced from the degradation of free heme by HO-1 also has a protective effect on cells where this CO functions as a strong anti-inflammatory, anti-apoptotic, vasodilator, inhibits platelet aggregation and has a bactericidal effect. Bilirubin and biliverdin produced from heme degradation by HO-1 also have anti-peroxy radical scavengers properties and can inhibit complement activation, while Fe released from heme degradation reactions although can increase the damaging effects of oxidative stress but isneutralized by ferritin and NGAL for storage in cells where the reaction is induced by HO-1 [26].

Simvastatin, aside f having an inhibitory effect on the HMG-CoA enzyme which is responsible for sterol biosynthesis, also has a pleiotropic effect that functions to reduce oxidative stress and vascular inflammation. The mechanism by which simvastatin reduces infarct size and the no-reflow state in ischemia reperfusion states is thought to be via the PI3K/Akt/eNOS pathway. In animal studies using a single dose of simvastatin and creating ischemic reperfusion conditions, it was found that there was a reduction in infarct size and inhibition of the no-reflow phenomenon compared to the control group, and this did not occur when the PI3K/Akt/eNOS pathway was inhibited [27].

Simvastatin increases gene expression of HO-1, especially in vascular smooth muscle cells. Simvastatin administration can increase HO-1 expression for more than 24 hours. Administration of statin therapy before PCI significantly reduced Subject mortality where CRP, a marker of inflammation, was increased [28]. Research using NF-κB and NO production in VSMCs as markers of inflammation showed that the anti-inflammatory effect of simvastatin is mainly through HO-1, while the anti-apoptotic and anti-inflammatory effect of HO-1 is mainly through its degradation product, namely CO [29].

Plasma HO-1 levels in both groups (Table 5). From the results of the study, in the simvastatin group there was an increase in the level of HO-1 from the initial median of 13.7 ng/ml to 17.1 ng/ml postoperatively, and this difference was statistically very significant ($p=0.007$). Different results in the control group a decrease from the median 16.7 ng/ml to 12.45 ng/ml. If the percentage increase is calculated, the simvastatin treatment group's plasma HO-1 level increased by 18.6%, while the control group decreased by 19.4%; and this difference was statistically significant with the Mann-Whitney test ($p<0.01$). Hence in conclusion, it was found that plasma HO-1 levels in the simvastatin group increased significantly postoperatively and supported previous studies which stated that simvastatin increased gene expression of HO-1.

Postoperative HO-1 levels in the control group experienced a very significant decrease, presumably due to tissue hypoxia after regional reperfusion ischemia occurred during surgery, where from previous studies it was said that hypoxia can suppress gene expression of HO-1 [30]. This is supported by increased NGAL levels. Postoperative control group which is thought to be due to tissue damage in ischemic reperfusion during surgery compared to the simvastatin group. The results of this study also corroborate the results of previous studies which state the pleiotropic effect of simvastatin, where in this group simvastatin can reduce ischemic reperfusion and reduce ROS formation, reduce tissue damage and reduce plasma NGAL formation [27].

In the study of the function of NGAL as a protective substance against cell damage, it was found that the nature of NGAL as a substance that protects new cells will function if there is an adequate amount of HO-1 in plasma [31]. From this study, it is clear that the administration of simvastatin provides benefits in addition to its own protective properties against cells, it also directly increases plasma HO-1 levels which can degrade the strong oxidant heme and its metabolite products in the form of CO, bilirubin and biliverdin which are also anti-oxidants.

Plasma NGAL Levels

Surgery can result in ischemia and reperfusion injury after

major operations e.g. aortic, cardiac, digestive sugeries where there is a period of ischemia during surgery. Although restoration of blood flow to an ischemic area is important to prevent irreversible ischemic damage, this reperfusion of blood flow alone can induce a local and systemic inflammatory response that results in tissue damage beyond the ischemic damage. This ischemia event will then cause a series of biomolecular changes in the body, starting with a lack of oxygen forces in the cells causing the failure of the formation of energy Adenosine Triphosphate (ATP) which causes the cells to fail to maintain their integrity. The next process is the lysis of the cell membrane and the formation of oxygen and nitrogen radicals (ROS and RNS). When the systemic circulation improves, the ROS and RNS that are formed will then be carried to more distant sites which then damage other cells and organs [24,25].

Due to the lack of oxygen, the cell membrane is in a depolarized state where Na^+ enters the cell and K^+ leaves the cell. Under normal circumstances, the cell will undergo repolarization by pumping Na^+ from the cell and the entry of K^+ back into the cell. This process requires the enzyme Na-K-ATP-ase and sufficient ATP. In the hypoxic state of the cell, insufficient ATP is formed for this process so that the cell is continuously in a depolarized state. In addition, due to the entry of Na^+, water passively enters the cell causing cell oedema. Lactate formed from anaerobic glycolysis causes hyperlactatemia. Hyperkalaemia also occurs in this state of hypoxia and acidosis, which describes the magnitude of the degree of cell damage and is more pronounced when accompanied by impaired urine production by the kidneys. As a result of continuous depolarization of the cell membrane, it will open Ca^{2+} channels resulting in the entry of Ca^{2+} into the cell. In addition, membrane depolarization causes the release of excitatory amino acid neurotransmitters (glutamate) which will bind to the NMDA receptor (N-Methyl D-Aspartate) and subsequently activate NMDA to open Ca^{2+} channels with the result that more Ca^{2+} enters the cell. Cell hypoxia also causes the endoplasmic reticulum to release Ca^{2+} into the cytoplasm. The accumulation of Ca^{2+} in the cytoplasm will activate a series of enzymatic processes in the cell (lipid peroxidase) which will lyse the cell membrane. The return of blood flow to the area causes a reperfusion injury cascade. The formation of ROS, RNS and free heme are highly toxic to cells. The intestine is said to be composed of labile cells that are highly susceptible to reperfusion injury. Intestinal reperfusion injury causes intestinal mucosal damage, villous damage, impaired tissue oxygenation and decreased perfusion in the intestinal mucosa. In some studies, it is stated that the incidence of reperfusion injury to the intestine occurs in two phases, the first is within 2 hours after reperfusion and the second, occurs from 6-24 hours after reperfusion. Capillary no-reflow and leukocyte-endothelial interactions are the hallmarks of intestinal reperfusion injury. As a result of reperfusion injury, many cells are damaged where erythrocytes are one of the targets most often affected by the formation of this ROS. Further damage to erythrocytes will cause the release of free heme into the circulation which will further exacerbate reperfusion injury [24,25,32].

NGAL, also known as human neutrophil lipocalin or lipocalin-2, originates from the granules of activated neutrophil cells. First identified in 1990 using western blot analysis, there are 3 different forms of the NGAL molecule, namely 25-kDa monomeric, 45-kDa disulphide-linked homodimer and 135-kDa heterodimeric bound to MMP-9. NGAL is synthesized in the bone marrow during myelopoiesis and then stored in neutrophil granules. In addition to the bone marrow, NGAL is also produced by the colon, trachea, lungs and the epithelium and renal tubules. Interleukin-1 which is an inflammatory mediator can also stimulate NGAL synthesis, so that an increase in plasma NGAL levels can occur in conditions of acute peritonitis, acute exacerbation of chronic obstructive pulmonary disease and acute bacterial infections. This strengthens the suspicion that NGAL expression will increase in inflammatory conditions. In studies examining NGAL from various tissues in vitro, showing that activated neutrophils release homodimeric NGAL, renal epithelium releases monomeric NGAL while renal tubules release very low concentrations of heterodimeric NGAL. NGAL levels in the circulation will be freely filtered by the glomerulus and will be effectively reabsorbed in the proximal tubule by megalin through endocytosis. If there is damage or disruption to the proximal renal tubule, NGAL reabsorption will be inhibited resulting in the excretion of NGAL into urine and plasma [33,34].

Table 5: Dependent variables comparison.

Variables	Control (n=16)	Simvastatin (n=16)	
HO-1 (ng/mL)			**p value***
Pre-operative			
Median	16.7	13.7	0.138
Range	7.3-68.7	3.5-45.8	
Post-operative			
Median	12.45	17.1	0.956
Range	6.8-40.8	4.6-28.4	
Pre-op and post-op comparison (p-value)**	0.001	0.007	
Increase of percentage (median)	-19.40%	18.60%	<0.001
NGAL (pg/mL)			**p value*****
Pre-operative			
Median	344806.25 (108277.72)	339318.75 (119970.36)	0.893
Range	157250–549000	149400–597000	
Post-operative			

Median	465996.88 (90686.59)	341006.25 (149103.96)	0.008****
Range	299900–600900	111250–576000	
Pre-op and post-op comparison (p-value)**	<0.001****	0.944	
Increase of percentage (median)	44.76%	0.78%	0.001
Creatinine (mg/dL)			**p value*** **
Pre-operative			
Median	0.85 (0.33)	0.84 (0.29)	0.933
Range	0.39 – 1.54	0.34 – 1.40	
Post-operative			
Median	0.78 (0.31)	0.86 (0.32)	0.482
Range	0.40–1.64	0.36–1.65	
Pre-op and post-op comparison (p-value)**	0.135	0.726	
Increase of creatinine (mean)	-6.30%	6.20%	0.159
Urine volume (mL)			**p value*** **
24 hours	1218.75	1212.5	0.933
Mean per hour	50.52	50.78	0.933

Note: *Based on Mann-Whitney test; **Based on Wilcoxon test; ***P value is calculated using unpaired T-test; ****P value is calculated using paired T-test.

Plasma NGAL levels in both groups (Table 5). From the results of the study as shown in Table 5, it was found that in the simvastatin group there was an increase in postoperative plasma NGAL levels (the average increased by 0.78%) but the increase was not statistically significant (p=0.944), while in the control group there was an increase in plasma NGAL levels (the average increased 44.76%) which was very significant (p<0.001). Whencompared to the percentage increase in the two research groups based on the unpaired t test, the p value=0.001 (very significant).

In this study, it was found that the plasma NGAL level before surgery was quite high even though the plasma creatinine level was within normal limits and the subjects did not have impaired renal function. This is probably because the study subjects were diagnosed with malignancy and inflammation of the intestines, where the production of NGAL under these conditions was greatly increased. The results of this study are consistent with studies measuring NGAL levels in colorectal malignancy subjects, where more than 92% of these cancer cells produced significant amounts of NGAL [34]. The increase in postoperative plasma NGAL levels in this study was due to surgical stress followed by oxidative stress and caused damage to cells thereby stimulating the production of NGAL. This is supported by studies of plasma NGAL levels which increase up to 10 times in conditions of oxidative stress [2].

Although in this study the free heme formation was not directly evaluated, however, an increase in NGAL levels in the control group of more than 10 times normal after surgery confirmed the suspicion of reperfusion injury and free heme formation during surgery, while in the simvastatin group an increase in postoperative NGAL levels. Surgery is not significant, it is suspected that the effect of simvastatin can increase HO-1 levels and neutralize the free heme formed so that it can protect cell damage due to reperfusion injury.

Plasma creatinine levels

Plasma creatinine levels in both groups are described in Table 5. The measurement of creatinine levels both pre-operatively and post-operatively, as well as the percentage increase in the two study groups statistically did not show any significant difference (p<0.05).

Diuresis

The mean and standard deviation of the amount of urine per hour for 24 hours postoperatively in the two treatment groups can be seen in Table 5. We found that there was no significant difference in the amount of urine 24 hours post-surgery in the two groups. During the 24-hour postoperative observation, there were no subjects whose urine amount was less than 0.5 cc/KgBW per hour.

DISCUSSION

In this study, there were no study subjects diagnosed with postoperative AKI in the two study groups. Both use the criteria for increased creatinine levels and the amount of urine according to RIFLE or AKIN. None of the subjects met the criteria for AKI even though there was a very significant increase in NGAL levels, especially in the control group, so the statement that NGAL is a strong predictor for the diagnosis of AKI is questionable. The results of this study are in accordance with studies in Subjects who underwent CPB surgery where plasma and urine NGAL levels were increased without the incidence of postoperative AKI [35].

There are several things that can explain the results of this study, namely, monitoring of hemodynamics and urine levels during surgery and intervention with fluid administration and vasopressors can prevent the occurrence of AKI, examination of plasma NGAL levels carried out in this study is not specific for NGAL released by the renal tubules, namely monomeric NGAL and until now there is no NGAL test kit available in the market that can distinguish the form of NGAL itself. The increase in

serum creatinine levels started 24 hours to 48 hours post-injury while in this study it was taken at 24 hours postoperatively.

Table 6: Correlation with simvastatin administration.

Correlation between	Correlation coefficient (RPBI)	P value
Simvastatin administration with the percentage increase of NGAL	-0.506	0.003
Simvastatin administration with the percentage increase of HO-1	0.727	<0.001
Simvastatin administration with the percentage increase of creatinine level	0.255	0.159

Note: RPBI: Biserial Point Correlation; simvastatin administration (1=not administered; 2=administered)

Based on Table 6, we found the administration of simvastatin was associated with a very significant increase in NGAL and HO-1 ($p<0.01$). The percentage of simvastatin increase in NGAL treatment was lower when compared to the control group ($r=-0.506$); while the administration of simvastatin with an increase in HO-1 was positively correlated, meaning that the percentage increase in HO-1 in the simvastatin group was higher when compared to the control group.

CONCLUSION

The author found an increase in HO-1 levels from 14.08 ng/mL to 15.35 ng/mL postoperatively and it was statistically very significant ($p=0.007$) in the simvastatin group. The results were different in the control group where the level of HO-1 actually decreased from 19.53 ng/mL to 14.01 ng/mL postoperatively and it was statistically very significant ($p=0.00$). An increase in postoperative plasma NGAL levels in the simvastatin group was not statistically significant ($p=0.944$), whereas in the control group there was a very significant increase in postoperative plasma NGAL levels ($p<0.0001$), based on the results of measurements of creatinine levels both pre-operatively and post-operatively, as well as the percentage increase in the two study groups, there is no statistically significant difference ($p<0.05$), as well as from the measurement of urine production every hour for 24 hours postoperatively in the two groups there was no significant difference. And there were no study subjects whose urine production was less than 0.5 cc/KgBW per hour.

Based on the results of this study, the author concluded that: preoperative administration of Simvastatin 40 mg orally increased postoperative plasma HO-1 levels in Subjects undergoing digestive surgery exploratory laparotomy, preoperative administration of Simvastatin 40 mg orally reduces the postoperative increase in plasma NGAL levels in subjects undergoing digestive surgery exploratory laparotomy, and we did not find any positive correlation between the administration of simvastatin and the criteria for Acute Kidney Injury (AKI) according to the RIFLE criteria, HO-1 levels and plasma NGAL.

LIMITATION OF THE STUDY

In this study, the selected subjects were Subjects with bowel disorders in which more than 90% of cases were intestinal malignancies. We know that NGAL is produced apart from the renal tubular epithelium but also by neutrophils and other organs such as the gut. This led to a bias as to whether the elevated NGAL levels were due to damage to the renal tubular epithelium or the increased production of NGAL by activated neutrophils due to surgical stress. From the results of the study, none of the Subjects with increased NGAL accompanied by the incidence of postoperative AKI, so that the limitation of this study was the selection of samples, preferably not in Subjects with bowel disorders or other NGAL-producing organs if we want to see the relationship between increased NGAL and increased incidence of AKI.

Another limitation of this study is the examination of serum creatinine levels performed 24 hours postoperatively. From previous studies, it was found that the increase in creatinine levels began 24-48 hours after injury to the kidney, so there is a suspicion that the samples taken in this study had not experienced a significant increase in creatinine levels. Although creatinine levels were not rechecked after 24 hours, in this study urine production was monitored for 3 days postoperatively, and it was found that none of the urine production met the criteria for AKI according to RIFLE.

The kit for plasma NGAL examination currently available in the market has not been able to distinguish whether the NGAL examined is homodimer, monomeric or heterodimeric which is typical for certain organs so that the increase in NGAL that occurs does not specifically indicate which organ is affected.

ETHICAL CONSIDERATIONS

This research was carried out after receiving a research ethic eligibility letter from the Health Ethics Commission, Faculty of Medicine, University of Padjadjaran. Each subject who participated in this study was given an explanation of the course of the research. An informed consent sheet was given so that the subject could know the aims and objectives of this research.

ACKNOWLEDGMENT

None

CONFLICT OF INTEREST

Author declares no conflict of interest.

REFERENCES

1. Ochoa JJ, Vilchez MJ, Mataix J, Ibáñez-Quiles S, Palacios MA, Muñoz-Hoyos A, et al. Oxidative stress in patients undergoing

cardiac surgery: Comparative study of revascularization and valve replacement procedures. J Surg Res. 2003;111(2):248-254.

2. Tsuchiya M, Sato EF, Inoue M, Asada A. Open abdominal surgery increases intraoperative oxidative stress: Can it be prevented? Anesth Analg. 2008;107(6):1946-1952.
3. Paparella D. Cardiopulmonary bypass induced inflammation: Pathophysiology and treatment. An update. Eur J Cardio Thoracic Surg. 2002;21(2):232–244.
4. Wagener FADTG, Eggert A, Boerman OC, Oyen WJG, Verhofstad A, Abraham NG, et al. Heme is a potent inducer of inflammation in mice and is counteracted by heme oxygenase. Blood. 2001;98(6):1802–1811.
5. Larsen R, Gouveia Z, Soares MP, Gozzelino R. Heme cytotoxicity and the pathogenesis of immune-mediated inflammatory diseases. Front Pharmacol. 2012;3(S):77.
6. Abraham NG, Drummond GS, Lutton JD, Kappas A. The biological significance and physiological role of heme oxygenase. Cell Physiol Biochem. 1996;6(3):129–168.
7. Tsiftsoglou AS, Tsamadou AI, Papadopoulou LC. Heme as key regulator of major mammalian cellular functions: Molecular, cellular, and pharmacological aspects. Pharmacol Ther. 2006;111(2):327–345.
8. Gonzalez-Michaca L, Farrugia G, Croatt AJ, Alam J, Nath KA, et al. Heme: A determinant of life and death in renal tubular epithelial cells. Am J Physiol Physiol. 2004;286(2):F370–F377.
9. Ye W, Zhang L. Heme controls the expression of cell cycle regulators and cell growth in HeLa cells. Biochem Biophys Res Commun. 2004;315(3):546–554.
10. Tracz MJ, Alam J, Nath KA. Physiology and pathophysiology of heme: Implications for kidney disease. J Am Soc Nephrol. 2007;18(2):414–420.
11. Zager RA, Johnson ACM, Becker K. Plasma and urinary heme oxygenase-1 in AKI. J Am Soc Nephrol. 2012;23(6):1048–1057.
12. Lee TS, Chang CC, Zhu Y, Shyy JYJ. Simvastatin induces heme oxygenase-1: A novel mechanism of vessel protection. Circulation. 2004;110(10):1296–1302.
13. Hoste EAJ, Clermont G, Kersten A, Venkataraman R, Angus DC, De Bacquer D, et al. RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: A cohort analysis. Crit Care. 2006;10(3):01–10.
14. Purwodarminto D. Angka kejadian acute kidney injury pascabedah laparotomi eksplorasi eksplorasi pada traktus digestivus berdasarkan kriteria RIFLE dan identifikasi faktor resiko di ICU RS Hasan Sadikin Bandung tahun 2008. Padjadjaran; 2011.
15. Ricci Z, Cruz DN, Ronco C. Classification and staging of acute kidney injury: Beyond the RIFLE and AKIN criteria. Nat Rev Nephrol. 2011;7(4):201–208.
16. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure–definition, outcome measures, animal models, fluid therapy and information technology needs: The second international consensus conference of the acute dialysis quality initiative (ADQI) Group. Critical care. 2004;8(4):1-9.
17. Devarajan P. Biomarkers for the early detection of acute kidney injury. Curr Opin Pediatr. 2011;23(2):194–200.
18. Cruz DN, de Cal M, Garzotto F, Perazella MA, Lentini P, Corradi V, et al. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. Intensive Care Med. 2010;36(3):444–451
19. Zappitelli M, Washburn KK, Arikan AA, Loftis L, Ma Q, Devarajan P, et al. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: A prospective cohort study. Crit Care. 2007;11(4):R84.
20. De Geus HRH, Bakker J, Lesaffre EMEH, Le Noble JLML. Neutrophil gelatinase-associated lipocalin at ICU Admission predicts for acute kidney injury in adult patients. Am J Respir Crit Care Med. 2011;183(7):907–914.
21. Makris K, Markou N, Evodia E, Dimopoulou E, Drakopoulos I, Ntetsika K, et al. Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) as an early marker of acute kidney injury in critically ill multiple trauma patients. Clin Chem Lab Med. 2009;47(1):79-82.
22. Núñez K, Baldirà J, Cuartero M, Aguirre H, Rodríguez M, Vera P, et al. Neutrophil gelatinase-associated lipocalin predicts acute kidney injury in critically ill patients. Crit Care. 2010;14(S1):P531
23. Mehta CR, Pocock SJ. Adaptive increase in sample size when interim results are promising: A practical guide with examples. Stat Med. 2011;30(28):3267–384
24. Liao Y-F. Heme oxygenase-1 and gut ischemia/reperfusion injury: A short review. World J Gastroenterol. 2013;19(23):3555.
25. Chi X, Yao W, Xia H, Jin Y, Li X, Cai J, et al. Elevation of HO-1 expression mitigates intestinal ischemia-reperfusion injury and restores tight junction function in a rat liver transplantation model. Oxid Med Cell Longev. 2015;2015:1–12.
26. Agarwal A, Bolisetty S. Adaptive responses to tissue injury: Role of heme oxygenase-1. Trans Am Clin Climatol Assoc. 2013;124:111–22.
27. Li X, Yang Y, Geng Y, Zhao J, Zhang H, Cheng Y, et al. Phosphorylation of endothelial NOS contributes to simvastatin protection against myocardial no-reflow and infarction in reperfused swine hearts: Partially via the PKA signaling pathway. Acta Pharmacol Sin. 2012;33(7):879–87.
28. Plenge JK, Hernandez TL, Weil KM, Poirier P, Grunwald GK, Marcovina SM, et al. Simvastatin lowers C-Reactive protein within 14 days. Circulation. 2002;106(12):1447–52.
29. Indolfi C, Cioppa A, Stabile E, Di Lorenzo E, Esposito G, Pisani A, et al. Effects of hydroxymethylglutaryl coenzyme A reductase inhibitor simvastatin on smooth muscle cell proliferation in vitro and neointimal formation in vivo after vascular injury. J Am Coll Cardiol. 2000;35(1):214–221.
30. Ryter SW, Alam J, Choi AMK. Heme oxygenase-1/carbon monoxide: From basic science to therapeutic applications. Physiol Rev. 2006;86(2):583–650.
31. World Health Organization. Rational use of personal protective equipment for coronavirus disease 2019 (COVID-19) and considerations during severe shortages. World Health Organization. 2020:1–28.
32. Mallick IH, Winslet MC, Seifalian AM. Pyrrolidine dithiocarbamate protects the small bowel from warm ischaemia/reperfusion injury of the intestine: The role of haem oxygenase. Clin Sci. 2006;111(6):373–380.
33. Cai L, Rubin J, Han W, Venge P, Xu S. The origin of multiple molecular forms in urine of HNL/NGAL. Clin J Am Soc Nephrol. 2010;5(12):2229–2235.
34. McLean MH, Thomson AJ, Murray GI, Fyfe N, Hold GL, El-Omar EM, et al. Expression of neutrophil gelatinase-associated lipocalin in colorectal neoplastic progression: a marker of malignant potential? Br J Cancer. 2013;108(12):2537–2541.
35. Cai L, Borowiec J, Xu S, Han W, Venge P. Assays of urine levels of HNL/NGAL in patients undergoing cardiac surgery and the impact of antibody configuration on their clinical performances. Clin Chim Acta. 2009;403(1–2):121–125.