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HISTOPATHOLOGY AND RENAL FUNCTION IN WISTAR RATS AFTER INTRAVASCULAR INJECTION OF IODINATED CONTRAST MEDIA IOHEXOL AND IOPAMIDOL

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Abstract

This research aimed to analyze the histopathology (tubular necrosis and proteinaceous casts) and renal function (SCr and BUN) differences of male Wistar strain white rats (*Rattus norvegicus*) after intravascular injection of iodinated contrast media Iohexol and Iopamidol. This research is an experimental laboratory with a post-test only control group design. Male Wistar rats that fit the criteria were divided into three groups by random sampling technique: Control (K), Treatment 1 (P1, Iohexol 350 mg iodine/mL), and Treatment 2 (P2, Iopamidol 370 mg iodine/mL). Iohexol and Iopamidol were injected at a dose of 1600 mg iodine/kg BW. The histopathology differences were observed under a light microscope with a magnification of 400x, which were analyzed semi-quantitatively through slides formed by the paraffin method and H&E staining. SCr and BUN levels were checked using an automatic analysis machine with blood samples taken through the cardiac ventricle. Kruskal-Wallis test ($\alpha= 0.05$) on renal histopathology scores, both tubular necrosis and protein casts showed Asymp. Sig. value > 0.05 , which means there is no significant difference between the groups (K, P1, and P2). Kruskal-Wallis test ($\alpha= 0.05$) on SCr levels also showed the Asymp. Sig. value > 0.05 and One-Way ANOVA Comparative Test on BUN levels showed the Sig. value > 0.05 which means there is no significant difference in renal function between the groups. This study proved no difference in histopathology and renal function in Wistar rats after injection of iodinated contrast media Iohexol and Iopamidol.

Keywords: Iohexol; Iopamidol; intravascular; intravenous; tubular necrosis; proteinaceous casts; serum creatinine (SCr); blood urea nitrogen (BUN); contrast-induced acute kidney injury (CI-AKI)

Introduction

Contrast media is a group of medical drugs used to increase the visibility of internal organ structures by X-ray based imaging techniques, such as radiography and computed tomography (CT). This contrast media is indispensable in radiology practice

for diagnostic and therapeutic (Andreucci et al., 2014). The most commonly used contrast media in Indonesia according to Formularium Nasional (Fornas) and Daftar Obat Esensial Nasional (DOEN) are iodinated contrast media, namely Iohexol and Iopamidol. Radiologists and other medical personnel involved with iodinated contrast media should be aware of the risk factors for a reaction to the contrast media. It needs vigilance and strategies to minimize, recognize, and manage the emergence of unwanted effects (Singh & Daftary, 2008).

The injected contrast media dilutes in the bloodstream and is immediately distributed throughout the extracellular fluid. This contrast media is not well bound to serum albumin, is freely filtered by the glomeruli, and excreted by the kidneys (Andreucci et al., 2017). The flow of this contrast media allows for side effects. Although it is generally safe to use, severe, life-threatening reactions can occur (Thomson & Varma, 2010). The most important unwanted side effect of using contrast media is acute kidney injury (AKI), a sudden decrease in kidney function due to kidney damage (Dirkes, 2011). AKI that occurs after administration of contrast media is called contrast-induced AKI (CI-AKI) or Contrast-Induced Nephropathy (CIN). The incidence of CI-AKI is about 1-2% in low-risk patients with normal renal function but increases to 25% in high-risk patients with chronic kidney disease or diabetes mellitus. CI-AKI, especially in patients with chronic kidney disease, will increase long-term mortality and morbidity (Tasanarong et al., 2014).

The most common current definition of CI-AKI is an increase in serum creatinine (SCr) level 25% or an absolute increase of 0.5 mg/dl (44.2 μ mol/L) from initial values occurring 48-72 hours after exposure to contrast media (Mehran & Nikolsky, 2006). SCr is elevated when there is a significant decrease in the glomerular filtrate rate (GFR) or when urinary elimination is impeded. An increase in creatinine can be detected after a decline in renal function of 50%. Based on this, serum creatinine is an advanced indicator of AKI. In addition to serum creatinine, another elevated marker in acute and chronic kidney disease is serum urea or blood urea nitrogen (BUN). Creatinine and urea are metabolites that are excreted through the kidneys so that an increase in serum creatinine and BUN can signify impaired renal function, including CI-AKI (Gounden & Jialal, 2020). The term CI-AKI is thought to reflect the presence of an early stage of kidney damage. When the contrast media is injected intravenously or intraarterially, it is almost completely filtered by the glomerulus and concentrated in the tubular lumen during the primary urine production process, resulting in exposure of the tubular epithelial cells to an increase in the concentration of radiographic contrast media. As a result, tubular epithelial cell damage occurs, a histopathological sign in CI-AKI (Andreucci et al., 2014).

CI-AKI is a common iatrogenic cause of AKI, so a particular study is needed to discuss the side effects of contrast media (Tasanarong et al., 2014). CI-AKI is the subject of intense research (Kiss & Hamar, 2016). There are still no studies that specifically address the differences in side effects of using Iohexol and Iopamidol. In this case, analysis is needed to see the histopathological differences and renal function

(SCr and BUN) after intravascular injection of iodinated contrast media Iohexol and Iopamidol.

Method

This research has received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, with letter number: 263/EC/KEPK/FKUA/2020.

This research is an experimental laboratory with a post-test only control group design. The sample in this study was a white rat (*Rattus norvegicus*) male Wistar strain obtained from the Department of Anatomy, Histology and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya. Determination of the sample size using the Lemeshow formula taken by the random sampling method. Wistar rats that met the criteria were divided into three groups, namely Control (K), Treatment 1 (P1), and Treatment 2 (P2). Group P1 will be injected with Iohexol (Omnipaque 350 mg Iodine/mL), while Group P2 will be injected with Iopamidol (Iopamiro 370 mg Iodine/mL). Iohexol and Iopamidol are injected at a dose of 1600 mg Iodine/KgBW. This quantity is the standard dose of contrast media for clinical use and other relevant experiments in rat models (Tasanarong et al., 2014). Forty-eight hours after the injection of iodinated contrast media, all experimental animals will be terminated using ether anaesthesia by inhalation.

Histopathology examinations were carried out by looking at the entire field of view of the slides in each kidney from each group. The slides were made using the paraffin method with H&E staining. The slides were analyzed semi-quantitatively with the help of a pathologist. The magnification used for examination is 400x. Histopathology changes analyzed were tubular necrosis and proteinaceous casts. Tubular necrosis and proteinaceous casts were graded as 0 = no damage, 1 = mild (unicellular, isolated damage), 2 = moderate (damage <25%), 3 = severe (damage 25-50%), and 4 = very severe (damage >50%) (Yamasowa et al., 2005).

Blood samples as much as ± 3 mL were taken through the ventricles of the cardiac (Intracardial). The blood samples taken were put into SST tubes then examined for SCr using the modified Jaffe's kinetic method and BUN using the enzymatic UV. Method test by an automated analysis machine.

This research used primary data, which were analyzed using IBM SPSS Statistics 26 software. Renal histopathology scores, SCr, and BUN level are variables with ratio and interval data scales, so it is necessary to test for normality and homogeneity first as a condition for the one-way ANOVA test. If the results of the normality test show that the distribution is not normal and homogeneous or normal and not homogeneous, then a different test is carried out with the Kruskal-Wallis test ($\alpha = 0.05$). If there is a difference, it is continued with the Independent T-test or the Mann-Whitney test ($\alpha = 0.05$).

Result and Discussions

1. Effect of Iohexol and Iopamidol on Renal Histopathology

The scores for tubular necrosis in each group with minimum and maximum values were K (1; 1), P1 (1; 2), and P2 (1; 2), while proteinaceous casts were K (1; 2), P1 (2; 2), and P2 (1; 2). All of these data were tested for normality by Shapiro-Wilk ($\alpha = 0.05$) and the Sig. value was obtained <0.05 , which means that the data is not normally distributed. Data that were not normally distributed did not meet the requirements of the One-Way ANOVA test, so the Kruskal-Wallis test ($\alpha = 0.05$) was performed to see if there were differences between groups. Kruskal-Wallis test ($\alpha = 0.05$) on the scores of tubular necrosis and proteinaceous casts showed the Asymp. Sig. value > 0.05 , which means there is no significant difference between the groups (Table 1).

Table 1
Kruskal-Wallis test on renal histopathology score

Renal histopathology	Group	N	Average \pm SD	Minimum	Maximum	Asymp. Sig. Kruskal-Wallis test (α)
Tubular Necrosis	K	6	1,00 \pm 0,00	1	1	0,636
	P1	7	1,14 \pm 0,38	1	2	
	P2	7	1,14 \pm 0,38	1	2	
Proteinaceous Casts	K	6	1,67 \pm 0,52	1	2	0,272
	P1	7	2,00 \pm 0,00	2	2	
	P2	7	1,71 \pm 0,49	1	2	

Note: Asymp. Sig. value showed no significant difference ($\alpha >0.05$)

This research found no significant renal histopathology differences in both tubular necrosis and proteinaceous casts between the groups. These results mean that intravascular injection of iodinated contrast media Iohexol and Iopamidol did not improve renal histopathology in the treatment group (P1 and P2) compared to the control group (K) (Figure 1). One of the factors that influenced the results of the study was the use of white rats (*Rattus norvegicus*) male Wistar strain as a control variable in the study. Kiss and Hamar (2016) said that the difference in sensitivity between species and the ability of high kidney concentrations could protect experimental animals from CI-AKI, including severe histopathology damage. Rats and mice have a higher concentration ability than rabbits. A single injection of contrast media (5 g Iodine/KgBW Ioxilan) induced CI-AKI only in rabbits. However, it did not cause significant kidney damage in rats and mice. Besides that, direct toxic effects of contrast media on tubular necrosis or proteinaceous casts are unlikely in healthy kidneys until hypoxia sensitizes the tubular to contrast media toxicity. Tubular epithelial cells are the most sensitive part of the kidney to hypoxia because of their high metabolic demands,

so many studies have described hypoxia as an important contributor to CI-AKI (Kiss & Hamar, 2016).

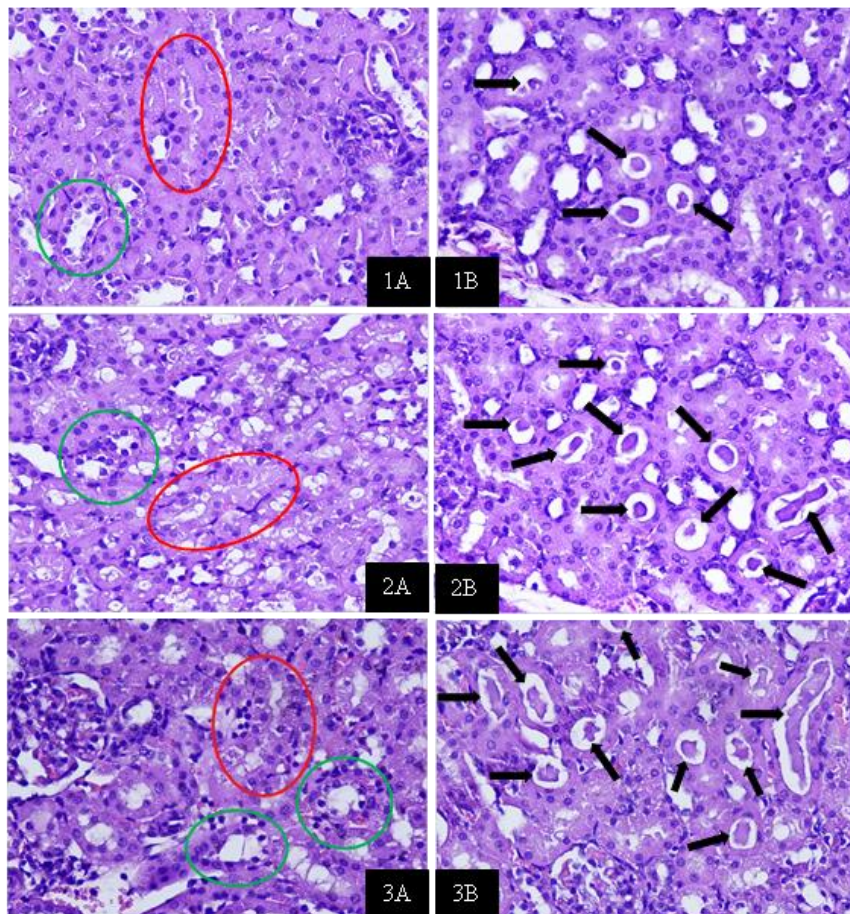


Figure 1

Renal histopathology in Wistar rat with 400x magnification and H&E staining
1: Control group, 2: Iohexol injected group, 3: Iopamidol injected group; A: tubular necrosis (red circle), normal tubular (green circle); B: proteinaceous casts (black arrow)

In differ to the results of research by [Tasanarong et al. \(2014\)](#), which proved that the histopathology examinations of the kidneys in the treatment group injected with Iopromid showed significant severe damage consisting of tubular necrosis, proteinaceous casts, PTC congestion, and interstitial oedema when compared to the control group. Another study by [Liu et al. \(2018\)](#) also proved that Iohexol injection gave severe tubular necrosis, medullary congestion, and proteinaceous casts in the treatment group compared to the control group. However, in both studies, the contrast media Iopromid/Iohexol was administered concurrently with Indomethacin and N -nitro-L-Arginine methyl esterase (L-NAME) did not give an idea of the effect of the contrast media itself. That is in accordance with the statement of [Kiss and Hamar \(2016\)](#), which said that necrosis in rodents only appears if other hypoxic triggers are also applied as part of the model.

2. Effect of Iohexol and Iopamidol on Renal Function

The results of the SCr examination in each group with the minimum and maximum values were K (0.5; 0.6), P1 (0.5; 0.7), and P2 (0.5; 0.7), while BUN are K (13; 18), P1 (15; 19), and P2 (10; 20). All of these data were tested for normality by Shapiro-Wilk ($\alpha = 0.05$), the Sig. value was obtained <0.05 on SCr and Sig value. > 0.05 on BUN. Value of Sig. <0.05 means that the data is not normally distributed, while the value of Sig. > 0.05 means the data is normally distributed. Data that were not normally distributed did not meet the requirements of the One-Way ANOVA test, so the Kruskal-Wallis test ($\alpha = 0.05$) was performed to see if there were differences between groups. The Kruskal-Wallis test ($\alpha = 0.05$) on the results of the SCr showed the Asymp. Sig. value > 0.05 , which means there is no significant difference between the groups (Table 2). The BUN examination data, which were normally distributed, were subjected to a One-Way ANOVA comparative test ($\alpha = 0.05$) with the condition that the homogeneity of the data was met. The significance value of the homogeneity test of the data is 0.087 (Sig. > 0.05) so that the One-Way ANOVA comparative test can be carried out and the Sig. value is obtained > 0.05 , which means that the BUN results between groups were not significantly different (Table 3).

Tabel 2
Kruskal-Wallis test on SCr

Renal function	Group	N	Average \pm SD	Minimum	Maximum	Asymp. Sig. Kruskal-Wallis test (α)
Serum Creatinine (SCr)	K	6	0,57 \pm 0,52	0,5	0,6	0,230
	P1	7	0,60 \pm 0,58	0,5	0,7	
	P2	7	0,63 \pm 0,76	0,5	0,7	

Note: Asymp. Sig. value showed no significant difference ($\alpha > 0.05$).

Table 3
One-Way ANOVA test on BUN

Renal function	Group	N	Average \pm SD	Minimum	Maximum	Sig. One-Way ANOVA test (α)
Blood Urea Nitrogen (BUN)	K	6	15,67 \pm 1,87	13	18	0,513
	P1	7	17,00 \pm 1,41	15	19	
	P2	7	15,58 \pm 3,60	10	20	

Histopathology and Renal Function in Wistar Rats after Intravascular Injection of Iodinated Contrast Media Iohexol and Iopamidol

Note: Sig. value showed no significant difference ($\alpha > 0.05$)

Based on the results of this study, there were no significant differences in kidney function on both SCr and BUN examinations between the study groups. These results mean that intravascular injection of iodinated contrast media Iohexol and Iopamidol did not decrease kidney function in the treatment group (P1 and P2) compared to the control group (K). In addition to the experimental animal factors discussed previously, the type of contrast media is suspected to be a risk factor for CI-AKI. Contrast media are generally classified by osmolality, determined by the number of osmotically active particles per kilogram of solvent (Stacul, 2001). Iohexol and Iopamidol are non-ionic monomers LOCM (low-osmolality contrast media) with an osmolality of 600-850 mOsm/Kg (Detrenis et al., 2005). However, the osmolality of the contrast media previously thought to be responsible for CI-AKI was not proven in Bucher et al. (2014) study because HOCM (high-osmolality contrast media) withdrawal did not reduce the incidence of CI-AKI. In addition, the clinical study by Moore et al. (1992) found no difference in the incidence of CI-AKI or renal safety profile between LOCM and HOCM.

Suppose there is an increase in SCr and BUN levels. In that case, renal hypoxia becomes an important factor in the pathogenesis of CI-AKI. Contrast media injection causes an initial increase in RBF (renal blood flow) followed by a prolonged decrease in RBF with GFR. Meanwhile, extrarenal blood vessels experience temporary vasoconstriction and are followed by a decrease in peripheral resistance (Detrenis et al., 2005). Reduction of renal plasmatic flow mainly occurs in the renal medulla because of the medullary perfusion and the much lower partial pressure of O₂ (PO₂) than the renal cortex. That impacts on the ascending limb of the loop of Henle, which is located in the medulla which has metabolic activity and increased demand for O₂ due to active ion transport through its membrane (Deray, 1999). This hemodynamic effect causes renal ischemia, which is responsible for the occurrence of CI-AKI (Detrenis et al., 2005). However, Kiss and Hamar (2016) stated that injection of contrast media alone would not cause obvious AKI in rodents, requiring additional substances to induce histopathology and decreased renal function. That is in accordance with Tasanarong, Kongham and Itharat (2014) and Liu et al. (2018) studies which found a significant increase in SCr and BUN in a mouse model added with Indomethacin and L-NAME in addition to the injection of the contrast media itself.

Based on the above discussion, in addition to the ability of high renal concentration in rats, no increase in SCr and BUN level was found in this study, it could also be caused by the use of contrast media without additional substances or other CI-AKI induction agents which are responsible for renal hypoxia as an important factor in the pathogenesis of CI-AKI.

Conclusions

Based on this study, it can be concluded that there is no difference in histopathology and renal function of Wistar rats after intravascular injection of iodinated contrast media Iohexol and Iopamidol so that their use is safe for renal. If further research is carried out, it is recommended to use urine output monitoring indicators or new markers in plasma/urine for early diagnosis of CI-AKI as well as immunohistochemical staining and evaluation of protein/gene expression in apoptosis or inflammatory pathways to confirm CI-AKI.

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