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ORIGINAL ARTICLE

Mesenchymal stem cell-derived secretome may attenuate nephrotoxicity by modulating cyclooxygenase-2 and caspase-3 levels in Wistar rats: an ELISA-based analysis

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ABSTRACT

Aim Nephrotoxicity is a major clinical concern because it can impair kidney function through inflammation and apoptosis. This study aimed to evaluate the effects of mesenchymal stem cell (MSC)-derived secretome on cyclooxygenase-2 (COX-2) and caspase-3 levels in a rat model of doxorubicin-induced nephrotoxicity.

Methods A total of 24 male Wistar rats were randomly allocated into four groups: normal control (NC), nephrotoxicity control (TC), early MSC-derived secretome treatment (P1), and delayed MSC-derived secretome treatment (P2). Nephrotoxicity was induced using a single intraperitoneal injection of doxorubicin (10 mg/kg body weight). MSC-derived secretome was administered intravenously (0.15 mL) immediately after doxorubicin injection in the P1 group, and administered to the P2 group on day 5 after doxorubicin injection. All rats were euthanised on day 12. COX-2 and caspase-3 levels were measured using enzyme-linked immunosorbent assay (ELISA).

Results A significant overall difference in COX-2 levels was observed among groups ($p = 0.035$). A strong, statistically significant positive correlation was observed between COX-2 and caspase-3 levels ($r = 0.684$, $p = 0.002$). The lowest COX-2 levels were found in the P1 group. No significant differences in caspase-3 levels were observed.

Conclusion This study showed a significant overall difference in renal COX-2 levels among groups in a doxorubicin-induced nephrotoxicity rat model; however, Holm-adjusted post hoc comparisons did not confirm significant pairwise differences. Caspase-3 levels did not differ significantly among groups. These findings suggest that MSC-derived secretome may have potential anti-inflammatory effects, particularly when administered early after doxorubicin exposure, but further studies are required to confirm its effects on renal inflammation and apoptosis.

Keywords: apoptosis, doxorubicin, inflammation, kidney injury, regenerative medicine

INTRODUCTION

Kidney injury disrupts the body's homeostatic balance and can arise from exposure to harmful substances, including drugs and chemicals. This condition is commonly referred to as nephrotoxicity. It may present with various clinical manifestations, with acute kidney injury (AKI) as one of its major outcomes. Nephrotoxicity has been reported as the third leading cause of AKI, and its occurrence has increased over recent decades (1–4). In fact, drug-induced nephrotoxicity accounts for approximately 19–26% of AKI cases in hospitalised patients. For instance, doxorubicin, a widely used chemotherapeutic agent, has been associated with nephrotoxic effects (5,6).

The increasing burden of kidney injury highlights the urgent need for effective long-term therapeutic strategies. In this context, mesenchymal stem cell (MSC)-derived secretome has emerged as a promising approach in regenerative medicine (7–9). The secretome is a complex mixture of trophic factors released into the culture medium and contains biologically active molecules such as growth factors, cytokines, chemokines, mes-

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senger RNAs (mRNAs), microRNAs (miRNAs), and proteins. These elements act synergistically to foster an environment supportive of tissue repair and regeneration (8,9).

Previous studies have demonstrated that MSC-derived secretome can reduce tubular injury and improve renal function in ischaemia-reperfusion injury (IRI)-induced AKI rat models (7,8). However, evidence regarding the potential of MSC-derived secretome as an early or delayed therapeutic intervention in drug-induced nephrotoxicity remains limited. Therefore, this study aimed to evaluate the effects of MSC-derived secretome on inflammatory and apoptotic-related markers, specifically cyclooxygenase-2 (COX-2) and caspase-3, in a doxorubicin-induced nephrotoxicity rat model.

The aim of this study was to evaluate the effects of MSC-derived secretome on renal inflammatory and apoptotic-related responses in a doxorubicin-induced nephrotoxicity rat model by measuring COX-2 and caspase-3 concentrations.

MATERIALS AND METHODS

Subjects and study design

Male Wistar rats aged 10–12 weeks and weighing 200–250 g were obtained from a licensed experimental facility in Bandung, Indonesia. The animals were housed under controlled conditions with stable temperature and humidity, a 12-hour light/dark cycle, and free access to standard chow and water. Before the intervention, the rats were fasted for 8 hours. All procedures were conducted in accordance with the established Animal Welfare Act and relevant ethical guidelines.

The sample size was calculated using the Federer formula: $(n-1)(t-1) \geq 15$, where n represents the number of animals per group and t represents the number of treatment groups (4 groups). Based on the formula above, the minimum required sample size was 6 animals per group. A total of 24 rats were used for the in vivo study and were randomly assigned to four groups: normal control (NC), nephrotoxicity control (TC), early MSC-derived secretome treatment group (P1), receiving secretome immediately after doxorubicin injection, and delayed MSC-derived secretome treatment group (P2), receiving secretome 5 days after doxorubicin injection. Simple randomisation was used for group allocation. Group allocation was determined before the intervention, and each animal was assigned a unique identification code. Kidney tissue samples were labelled using these codes before enzyme-linked immunosorbent assay (ELISA) analysis. The investigator performing the ELISA measurements was blinded to the treatment group allocation during sample processing and outcome measurements.

Methods

Doxorubicin (10 mg/5 mL, manufactured by PT. Global Onkolab Farma, PT Kalbe Farma, Indonesia) was administered intraperitoneally at a dose of 10 mg/kg body weight as a single injection. In the P1 and P2 groups, rats received 0.15 mL of MSC-derived secretome (USEPro 06 batch/lot number UCD18110102 260324 supplied by PT. Prodia Stemcell Indonesia), which was sourced from healthy human umbilical cord tissue and administered intravenously as a single dose. The characterisation of the MSC-derived secretome used in this study is presented in Table 1.

Table 1. Characterisation of mesenchymal stem cell (MSC)-derived secretome

Parameter	Result	Reference
GM-CSF (pg/mL)	3.3	5.8–12515.7
IL-10 (pg/mL)	26.5	7.7–13771.7
IL-6 (pg/mL)	67.7	3.6–10521.0
IL-12p70 (pg/mL)	61.6	0.8–10437.2
IL-17A (pg/mL)	12.3	3.5–10929.3
IL-9 (pg/mL)	21.2	5.2–10632.6
IL-4 (pg/mL)	22.9	3.6–12292.7
IFN-α (pg/mL)	8.1	1.8–16948.0
TNF-α (pg/mL)	15.6	4.8–11260.3
Glutamate (mg/L)	39.2	32.9–51.1
Tryptophan (mg/L)	8.8	5.8–407.8
Cystine (mg/L)	<144.9	398.2–888.6
Histidine (mg/L)	19.8	18.1–88.5
Threonine (mg/L)	73.0	70.9–89.5
Tyrosine (mg/L)	47.1	44.3–182.4
Serine (mg/L)	18.2	11.5–44.9
Phenylalanine (mg/L)	45.4	43.1–142.8
Isoleucine (mg/L)	68.7	66.0–88.5
Valine (mg/L)	67.4	64.7–85.0
Alanine (mg/L)	177.0	136.9–236.3
Arginine (mg/L)	49.1	45.5–271.8
Glycine (mg/L)	27.6	26.2–42.3
Lysine (mg/L)	105.3	80.2–105.3
Aspartate (mg/L)	2.0	1.5–3.5
Leucine (mg/L)	68.3	66.1–98.5
Proline (mg/L)	4.8	4.3–38.5
Methionine (mg/L)	<1.5	1.5–15.5

GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; MSC, mesenchymal stem cell; TNF, tumour necrosis factor.

A preliminary dose-finding test was conducted before the main experiment to determine a tolerable intravenous dose of MSC-derived secretome in Wistar rats. Several doses were tested, including 1.0, 0.5, 0.25, and 0.15 mL. Doses higher than 0.15 mL were associated with acute mortality after administration and were therefore considered unsuitable for the definitive experiment. Based on this preliminary observation, 0.15 mL was selected as the highest tolerated dose for the main study. The animals used in the preliminary dose-finding test were not included in the final analysis of the present study.

The use of human-derived secretome in rats was intended to support future translational research towards clinical applications.

At the end of the experimental period on day 12, all rats were humanely euthanised via cervical dislocation and kidney tissues were collected for further analysis. The harvested kidney tissue samples were weighed, and 100 mg of each sample was homogenised in 1 mL of phosphate-buffered saline (PBS) using a homogeniser, with the process carried out on ice to maintain protein stability. Following homogenisation, the samples were centrifuged at 14,000 × g for 15 minutes at 4 °C. The supernatant was collected and stored at −20 °C until analysis. COX-2 and caspase-3 levels were quantified using ELISA kits (Rat PTGS2/COX2 ELISA kit, catalogue ELK2681; Rat CASP3 ELISA, catalogue ELK1528, PT Alpha Science Innolab) according to the manufacturer's sandwich immunoassay protocol.

Statistical analysis

Data normality was assessed using the Shapiro–Wilk test. Because the data were not normally distributed, continuous vari-

Table 2. Kidney weight (g), cyclooxygenase-2 (COX-2) and caspase-3 concentrations (ng/mL) in a nephrotoxicity rat model according to mesenchymal stem cell (MSC)-derived secretome administration

Variable	NC (n = 6)	TC (n = 6)	P1 (n = 6)	P2 (n = 6)	p^*
		Median (Minimum–Maximum)			
Kidney weight (g)	0.94 (0.69–1.74)	0.80 (0.72–0.91)	0.93 (0.80–1.11)	0.70 (0.46–1.07)	0.581
COX-2 (ng/mL)	68.94 (61.62–75.59)	76.45 (76.03–77.58)	52.58 (51.14–60.46)	66.72 (47.11–79.02)	0.035
Caspase-3 (ng/mL)	77.05 (22.00–134.40)	135.40 (127.60–136.50)	22.80 (17.30–127.50)	110.40 (14.90–119.50)	0.064

* Kruskal–Wallis test. Post-hoc Mann–Whitney U-tests with Holm adjustment showed no statistically significant pairwise differences. NC, normal control; TC, nephrotoxicity control; P1, early MSC-derived secretome treatment group; P2, delayed MSC-derived secretome treatment group; COX-2, cyclooxygenase-2; MSC, mesenchymal stem cell.

Table 3. Correlation matrix of observed variables

Variable Pair	Correlation (r)	p
Kidney weight vs COX-2	0.034	0.893
Kidney weight vs caspase-3	−0.212	0.399
COX-2 vs caspase-3	0.684	0.002

COX-2, cyclooxygenase-2; r, Spearman correlation coefficient.

ables are presented as median with minimum and maximum values. Differences among the groups were analysed using the Kruskal–Wallis test. When significant differences were identified, post hoc pairwise comparisons were performed using the Mann–Whitney U test with Holm adjustment for multiple comparisons. Spearman's rank correlation test was used to assess the strength and direction of relationships among variables, with the correlation coefficient reported as r. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Median kidney weight was highest in the NC group at 0.94 g, followed by the P1 group at 0.93 g, TC group at 0.80 g, and P2 group at 0.70 g, respectively (p = 0.581) (Table 2). The highest median COX-2 concentration was observed in the TC group at 76.45 ng/mL, followed by the NC group at 68.94 ng/mL and P2 group at 66.72 ng/mL, whereas the lowest value was observed in the P1 group at 52.58 ng/mL, with a significant overall difference among groups (p = 0.035); however, Holm-adjusted post hoc comparisons showed no significant pairwise differences (all p > 0.05) (Table 2). The highest median caspase-3 concentration was observed in the TC group at 135.40 ng/mL, followed by the P2 group at 110.40 ng/mL and NC group at 77.05 ng/mL, whereas the lowest value was observed in the P1 group at 22.80 ng/mL, without significant differences among groups (p = 0.064) (Table 2). Spearman correlation analysis showed no significant correlation between kidney weight and COX-2, r = 0.034 (p = 0.893), or between kidney weight and caspase-3, r = −0.212 (p = 0.399). A strong positive correlation was observed between COX-2 and caspase-3, r = 0.684 (p = 0.002) (Table 3).

DISCUSSION

This study evaluated the effects of MSC-derived secretome on renal inflammatory and apoptotic-related markers in a Wistar rat model of doxorubicin-induced nephrotoxicity. COX-2 concentrations differed significantly among the groups, with the highest median value in the nephrotoxicity control group and the lowest value in the early treatment group. However, Holm-adjusted post hoc comparisons did not confirm significant pairwise differences; therefore, this finding should be interpreted as preliminary rather than definitive evidence of a treatment effect.

The lower median COX-2 concentration observed in the early treatment group is biologically plausible because doxorubicin-induced nephrotoxicity is associated with oxidative stress, inflammatory activation, and renal tissue injury (5,6). COX-2 is an inducible inflammatory enzyme that may increase during tissue injury and inflammatory stimulation (5,6). MSC-derived secretome contains bioactive factors, cytokines, growth factors, and extracellular vesicle-associated molecules that may modulate inflammatory and regenerative pathways (7–10). Previous studies have also suggested that MSC-derived secretome or MSC-based interventions may influence COX-2/prostaglandin E2 (PGE2) signalling, although this effect may depend on the disease model, timing of administration, and inflammatory microenvironment (11–13).

Caspase-3 concentrations did not differ significantly among the groups. Although the nephrotoxicity control group showed the highest median caspase-3 concentration and the early treatment group showed the lowest value, this difference was not statistically significant. Therefore, the present study does not provide conclusive evidence that MSC-derived secretome reduces apoptotic-related activity in this model.

The absence of a significant caspase-3 difference may be related to the timing and scope of apoptosis assessment. Caspase-3 was measured only at one time point, whereas apoptosis is a dynamic process that may vary over time after doxorubicin exposure and secretome administration. Previous studies have shown that doxorubicin can increase apoptotic signalling in renal tissue, including caspase-3 activation and expression of pro-apoptotic proteins (5,6). In addition, MSC-derived secretome has been reported to exert anti-apoptotic effects through growth factors, miRNAs, and modulation of cell-survival pathways (8,14–17). Future studies should therefore include additional apoptotic markers, such as Bax, Bcl-2, cleaved caspase-3, caspase-9, and terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) staining, to provide a more comprehensive assessment of renal apoptotic activity.

A strong positive correlation was observed between COX-2 and caspase-3 concentrations, suggesting an association between inflammatory and apoptotic-related responses in this nephrotoxicity model. This finding is consistent with the biological interaction between inflammation and apoptosis in tissue injury, where inflammatory mediators may contribute to apoptotic signalling and cellular injury may further amplify inflammatory responses (6,18). However, correlation does not establish

causality, and the present findings cannot determine whether modulation of COX-2 directly affects apoptotic pathways.

Kidney weight did not differ significantly among the groups and was not significantly correlated with COX-2 or caspase-3 concentrations. This suggests that changes in inflammatory and apoptotic-related markers were not reflected in kidney mass under the conditions of this study. Kidney weight alone may be insufficient to represent renal structural or functional injury, particularly in an acute experimental model.

This study has several limitations. Renal functional markers, including serum creatinine and blood urea nitrogen, and histopathological assessment were not included. The sample size was limited, and the observation period was restricted to 12 days. In addition, the preliminary dose-finding test was performed to identify a tolerable intravenous dose of MSC-derived secretome, but a formal dose–response and toxicological assessment was not conducted. Future studies with larger sample sizes, renal functional markers, histopathological evaluation, multiple inflammatory and apoptotic biomarkers, and longer follow-up are needed to confirm these preliminary findings.

CONCLUSION

In this doxorubicin-induced nephrotoxicity rat model, MSC-derived secretome was associated with lower median COX-2 concentration when administered early after doxorubicin exposure; however, significant pairwise differences were not confirmed after Holm adjustment. Caspase-3 concentration and kidney weight did not differ significantly among groups, while COX-2 and caspase-3 showed a strong positive correlation. These findings suggest a possible anti-inflammatory effect of MSC-derived secretome, particularly with early administration, but do not provide conclusive evidence of anti-apoptotic or nephroprotective effects. Further studies including larger samples, renal functional markers, histopathological evaluation, and multiple inflammatory and apoptotic biomarkers are needed.

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CONFLICTS OF INTEREST

None to declare.

ETHICS STATEMENT

Ethical clearance for all procedures was granted by the Animal Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia. No. 311/KEPH/VII/2024.

DATA AVAILABILITY STATEMENT

All data underlying the results are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS (CRediT)

Conceptualization: D.S.; **Data curation:** D.S., R.F.; **Formal analysis:** D.S., R.F.; **Funding acquisition:** D.S.; **Investigation:** D.S., R.F., A.P.; **Methodology:** D.S., J.I., F.H.; **Project administration:** R.F.; **Supervision:** D.S.; **Writing – original draft:** R.F., A.P.; **Writing – review & editing:** D.S., R.F., J.I., F.H., Z.Z. All authors have read and agreed to the published version of the manuscript.

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