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Original Article

Modulatory and regenerative potential of transplanted bone marrow-derived mesenchymal stem cells on rifampicin-induced kidney toxicity

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ABSTRACT

Introduction: Anti-tuberculosis agent rifampicin is extensively used for its effectiveness. Possible complications of tuberculosis and prolonged rifampicin treatment include kidney damage; these conditions can lead to reduced efficiency of the affected kidney and consequently to other diseases. Bone marrow-derived mesenchymal stem cells (BMMSCs) can be used in conjunction with rifampicin to avert kidney damage; because of its regenerative and differentiating potentials into kidney cells. This research was designed to assess the modulatory and regenerative potentials of MSCs in averting kidney damage due to rifampicin-induced kidney toxicity in Wistar rats and their progenies. BMMSCs used in this research were characterized according to the guidelines of International Society for Cellular Therapy.
Methods: The rats (male and female) were divided into three experimental groups, as follows: Group 1: control rats (4 males & 4 females); Group 2: rats treated with rifampicin only (4 males & 4 females); and Group 3: rats treated with rifampicin plus MSCs (4 males & 4 females). Therapeutic doses of rifampicin (9 mg/kg/day for 3-months) and MSCs infusions (twice/month for 3-months) were administered orally and intravenously respectively. At the end of the three months, the animals were bred together to determine if the effects would carry over to the next generation. Following breeding, the rats were sacrificed to harvest serum for biochemical analysis and the kidneys were also harvested for histological analysis and quantification of the glomeruli size, for the adult rats and their progenies.
Results: The results showed some level of alterations in the biochemical indicators and histopathological damage in the rats that received rifampicin treatment alone, while the control and stem cells treated

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group showed apparently normal to nearly normal levels of both bio-indicators and normal histological architecture.

Conclusions: Intravenous administration of MSCs yielded sensible development, as seen from biochemical indicators, histology and the quantitative cell analysis, hence implying the modulatory and regenerative properties of MSCs.

1. Introduction

The most frequently used and efficacious medication for treating tuberculosis (TB) is rifampicin. Although rifampicin-induced acute kidney malfunction might occur, this is a common complication during therapy, as it occurs in 0.1% of those suffering from TB [45]. Acute interstitial nephritis as an immune-mediated cause of acute kidney malfunction is also related to rifampicin, which can lead to 1.6% mortality [2]. It is still a severe impediment that can proceed to Fanconi syndrome, a proximal renal tubule defect leading to mal-absorption of major elements, such as phosphorus, bicarbonate, sodium, potassium, glucose and amino acid that can lead to numerous signs such as bone pain and fracture, fatigue, and muscular weakness [67]. The epidemiological statistic associated with medication provoked renal damage was placed at 18–27% of all occurrences of acute renal malfunction in the USA hospitals [59]. All the kidney physiological tasks can be disturbed in one way or another due to drug effect on the kidneys. In an event of persistent poor glomerular filtration rate, dialysis and transplantation remains the only cure option. Several reports revealed that rifampicin-induced acute kidney malfunction is a complication when the medication is re-administered or used irregularly [15,17,43]. The aftermath of rifampicin-induced acute kidney malfunction is mostly promising upon discontinuation of medication, in which 96% of those suffering from TB can attain complete revitalization within 90 days as at the start of the renal injury [15]; however, to eradicate *Mycobacterium tuberculosis* from the patient, the medication must be taken for 6 months or more. Yet, the eradication of this disease remains elusive. This has been largely attributed to the ability of *Mtb* to maintain a latent or dormant infection in a host despite the evidence for a vigorous host immune response [26,38,71]. Moreover, many research papers have shown that prolonged use of rifampicin can cause kidney injury [4,52]. A remarkable elevation in the serum sample of the AST level due to anti-TB medication was reported by Ref. [51]; in patients after receiving treatment by Ref. [6] and in mice treated with rifampicin [65]. A remarkable rise in the serum urea and creatinine levels was reported in patients treated with isoniazid, rifampicin, pyrazinamide, and ethambutol for a period of eight months [70]. In another experiment, a reduction in serum urea levels were recorded in rats treated with isoniazid and rifampicin for a period of one month. Furthermore, an extraordinary elevation in serum urea was reported, but with no meaningful changes in the creatinine level of rats that received rifampicin for a period of 4 weeks. Moreover, an elevation in AST, bilirubin and urea was reported [55], with no remarkable changes in the creatinine level. Histopathological kidney damage such as glomerular injury increased, and mesangial matrix expansion and renal tubule regeneration were observed [55] in albino rats that were treated with rifampicin for a period of 4 weeks via oral gastric tubes [54]. Prolonged rifampicin therapy can also cause hemolysis and subsequently acute kidney failure and can lead to interstitial nephritis (which is due to its direct toxic effect) and is seen as part of pan-nephropathy [40]. Renal lesions were observed and were due to the formation of immune complexes that were detected on capillary glomerular basement membranes using immunofluorescent and electron microscopy. Deterioration in kidney activity appears to be acute when rifampicin is reintroduced [18]. MSCs can be used together with rifampicin to avert kidney damage because they have the ability to home to damaged tissue when injected intravenously. Clinical complications like ischemic acute renal failure (ARF), described by severe regression in the glomerular filtration rate, is usually seen in hospitalized patients and predominantly in multi-organ failure patients. Intravenous administration of Bone marrow mesenchymal stem cells after ARF, was able to histologically locates the injured kidney and considerably boost the restoration of kidney function due to their ability of trans-differentiation into kidney tubular or vascular endothelial cells [37,47]. A single intra-renal administration of BMMSCs 7 days after ischemia-reperfusion significantly improved renal function and modified renal remodeling. The improvement of renal function was associated with a reduction in extracellular matrix accumulation. In a renal ischemia rat model, administration of MSC was able to decreased tubular dilation that is known to be a typical characteristic of progressive kidney failure [1]. Since physicians are not currently required to monitor renal function during the course of TB treatment unless the patient is at risk for hepatic or renal abnormalities [12]. Here, we want to investigate the therapeutic potentials of transplanted bone marrow-derived mesenchymal stem cells for the rat kidney and their subsequent generation (F1 generation) in managing the toxicity of rifampicin.

2. Methods

2.1. Isolation and culture expansion of mesenchymal stem cells (MSCs)

The isolation of mesenchymal stem cells from 8-weeks old wistar male rats was done according to the methods described by Refs. [20,50].

2.1.1. Procedure

Anesthesia cocktail of 50 mg/kg ketamine and 10 mg/kg xylazine were injected to the rats. At the back limbs, the femurs and tibias were cut off, the skin and muscles were removed. The dissected femurs and tibias were put in 70% alcohol for a few seconds and later transferred to phosphate buffered saline (Cat. No. P5368, Merck, USA). Within a biosafety hood, the dissected femurs and tibias were then relocated to a 10 cm bowl containing PBS; the individual bone was then held with tweezers, and the two terminals of the bone were cut open using a scissor. Using a 3 ml syringe filled with PBS and 22G needle, the marrow were flushed (2–3 times) to 50 ml collection tube by putting the needle to the open end of the bone. The collected cells were then resuspended by spinning at 200 g (1294 rpm) 40 °C for 5 min. The supernatant was aspirated and the cells were then resuspended in 120 ml MSCs medium, Dulbecco's Modified Eagle's Medium (Cat. No. D5546, Merck, USA), comprising of 10% Fetal Bovine Serum (Cat. No. 12303C, Merck, USA) and 1% Pen-Strep (Cat no. P4333, Merck, USA).