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Role of Reactive Oxygen Species in Adriamycin Induced Nephrosis

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Summary. **To investigate the role of reactive oxygen species (ROS) generation in the pathogenesis of adriamycin (ADR) nephropathy, chemiluminescence was measured using isolated glomeruli from ADR rats. The results showed a marked increase of chemiluminescence which paralleled the increase of albuminuria. A superoxide dismutase (SOD) mimic, which enters easily into the cells was administred and a significant decrease of proteinuria was noted.**

Addition of SOD *in vitro* inhibited the enhanced chemiluminescence in rats administered with ADR. A slight glomerular cell increase, but no recruitment of leucocytes and/or monocytes/marcrophages, was observed. We therefore conclude that ADR-induced nephropathy is mainly due to ROS generation of intrinsic glomerular cells.

INTRODUCTION

Evidence has accumulated that shows the importance of reactive oxygen species in the development of tissue injury. The significance of ROS generation in immunologically mediated glomerular disease has particularly attracted the attention of many investigators. In glomerulonephritis, ROS generated by inflammatory cells such as polymorphonuclear cells (PMN) and monocytes/macrophages are known to contribute to the glomerular injury. Meanwhile, puromycin aminonucleoside (PAN) nephrosis has been recognized as an experimental model of minimal change glomerular disease. Its pathogenesis had not been clearly elucidated until recently when the role of ROS was put forward as an etiology of this glomerular lesion.[1] However, macrophages were concurrently noted in the mesangial region in PAN nephrosis, which makes it possible that inflammatory cells other than intrinsic glomerular cells may be contributing to the PAN induced glomerular lesion.[1,2]

To study whether the ROS generation by intrinsic glomeruli cells evokes glomerular lesions, we have herein chosen ADR nephrosis as an experimental model of nephrosis.

MATERIALS AND METHODS

Wistar male rats weighing 100-120 g were used. The rats were injected intravenously with 7.5 mg/kg of adriamycin and were divided into two groups. One group received subcutaneous injection of a total of 30 mg/100 g (in 2 divided doses) of a superoxide dismutase (SOD) mimic, Fe-tripirinidyl-methyl aminomethyl amine (FeTAPP) as a scavenger of ROS,[3] while the other group (control) received ADR alone. Twenty-four hour urine was collected at day 0, 4, 7, 10, 14, 19, 21, 24, 26 and 28 after the injection of ADR. Additionally, at day 0, 4, 10, and 28, some animals from each group were sacrificed, and glomeruli were isolated using the sieving method. The isolated glomeruli were checked for purity and only preparations with more than 90% purity were subjected to chemiluminescence measurement. The chemiluminescence was measured according to the method of Shah et al.[4] with a little modification. Briefly, the standard reaction mixture consisted of luminal (concentration adjusted to 3×10^{-5} M), and 2 ml of Hanks balanced salt solution. Glomeruli, preincubated at 37℃ for 15 min., were added to each vial and the count was taken. No stimulant was added to the vial and measurement was performed at 37℃ using Biolumat 9505. The background was counted in the vial containing only luminal and buffer.

After measurement, samples were stored at −30℃. They were subsequently thawed and the protein content was assayed by the method of Lowry et al.[5] after solublization with NaOH.

Background (count of the vial containing only luminal and buffer) was subtracted and the results were expressed as counts per 60 min. per milligram protein.

Twenty-four hour urine was used for the measurement of albumin by the immunodiffusion method. At day 28, the sacrificed animals were subjected to histological examination. The kidney was fixed in buffered 10% formalin, serially dehydrated in alcohol, and embedded in paraffin. The thin sections were subjected to the esterase staining for the detection of PMN and macrophages restrictively. Then, the number of glomerular cells, macrophages and PMN were counted in glomeruli and compared with controls.

RESULTS

After ADR injection, urinary albumin began to increase at day 14 and kept increasing until the day of sacrifice (day 28). Comparison of albuminuria between rats with ADR injection alone and those with ADR injection and TPAA treatment showed that in the treated rats albuminuria became mild after the day 14. The difference became significant after day 24 (Fig. 1). Glomerular chemiluminescence also increased after injection of ADR, peaking at day 14 and then decreasing gradually thereafter. Chemiluminescence, however, remained significantly increased compared to normal glomeruli (Fig. 2). To determine whether the increased chemiluminescence in glomeruli of ADR nephrotic rats was really due to the release of ROS, chemiluminescence of the glomeruli at day 14 was measured, adding varying amounts of SOD to the vial. The results showed a decrease of chemiluminescence in a dose-dependent manner (Fig. 3). Histological examination revealed no increase in PMN or macrophages, suggesting that increased chemiluminescence can be attributed to the increased ROS generation by intrinsic glomerular cells. The number of glomerular cells was slightly increased in ADR rats.

DISCUSSION

ADR is one of the clinically active drugs used for the treatment of a range of neoplasms. The cardiotoxicity of this drug has been a focus of clinical concerns.[6] Several factors have been proposed as being responsible for this side effect: Enzyme inhibiting mitochondrial effects, alteration of calcium transport, release of vasoactive substances and free radical dependent lipid damage.[6] Of these, free radical formation has been strongly advocated and two mechanisms have been postulated to show how this quinone drug-generated ROS. One is electron reduction of the drug to a semiquinone free radical intermediate followed by a cascade of oxygen-reactive species. The second is related to the interaction of ADR with metal ions, particularly with iron.

Despite the paucity of renal lesions in humans,

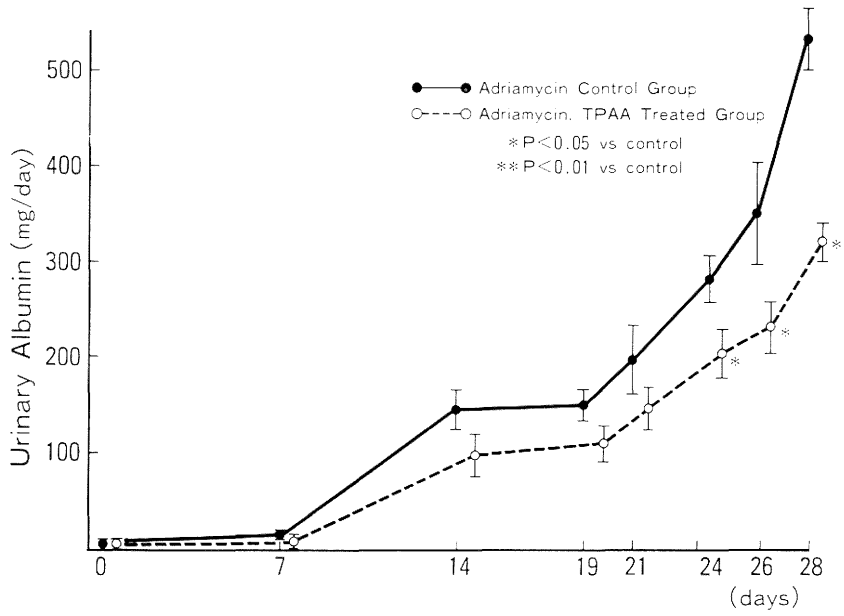

Fig. 1. Albuminuria in ADR rats.

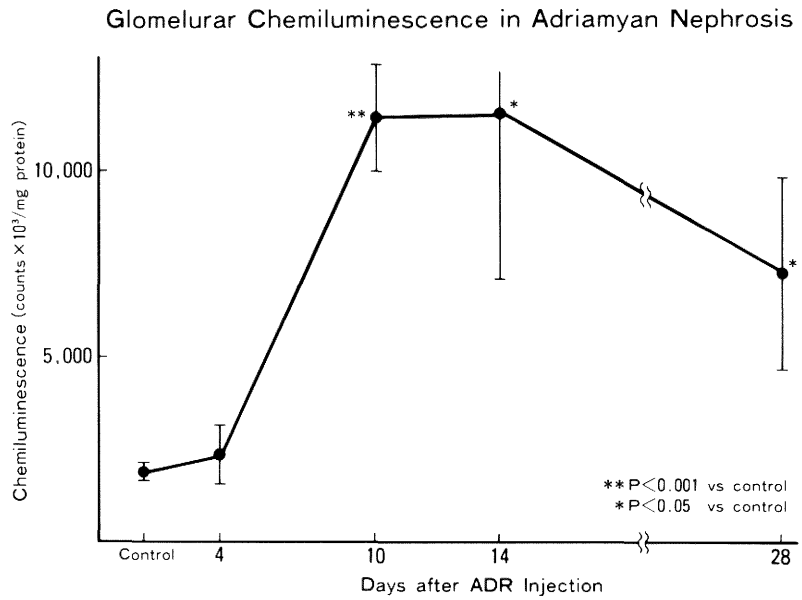

Fig. 2. Chemiluminescence in isolated glomeruli.

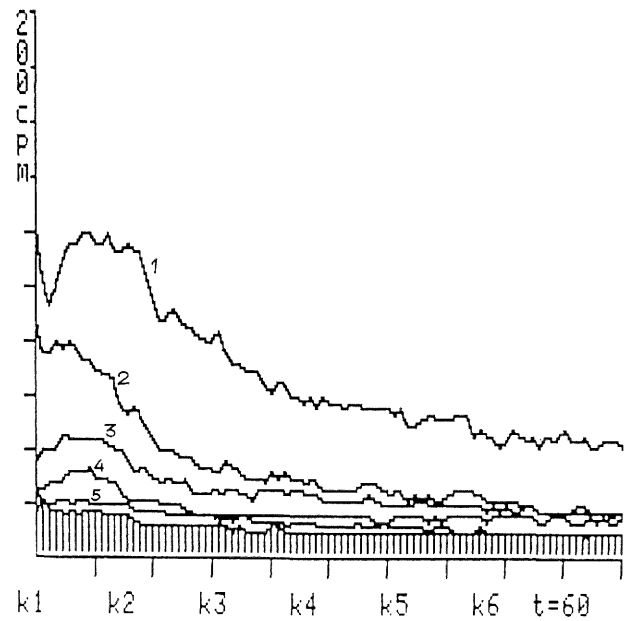

Fig. 3. Effect of SOD on the chemiluminescence generated by glomeruli of ADR rats.
1) Control (without SOD) 2) 200 μ/ml
3) 4,000 μ/ml 4) 6,000 μ/ml
5) 8,000 μ/ml 6) 1,000 μ/ml
Note chemiluminescence is inhigited in a dose-dependent pattern.

ADR nephrosis in rats has been recognized as an experimental model of nephrotic syndrome. The glomerular lesion induced by ADR is characterized by sustained prominent proteinuria.

Studies by some investigators have demonstrated that the heavy proteinuria is associated with eventual development of glomerular sclerosis,[7] while those by other investigators have indicated no glomerular sclerosis.[8] The difference may have arisen from the difference in the length of the observation period: the

former for 28 weeks and the latter for only 5 weeks. Nephrosis induced by administration of PAN is also well known as being an experimental model of human nephrosis and repeated injections of small doses of PAN will induce eventual glomerulosclerosis. In attempts to elucidate responsible mechanisms, physiological studies have been performed and indicate that glomerular hemodynamic abnormalities may play a role in progressive nature of glomerular sclerosis of PAN- and ADR-induced glomerular lesions. The results, however, are conflicting.[7] Although these lesions are principally chemically mediated, little has been studied about the role of ROS in inducing them. Recently, however, the glomerulosclerosis of PAN nephrosis has been attributed to ROS generation. Because macrophages were observed in the glomerulus, reservations exist as to whether these cells were involved in inducing the lesion.[2] This is in contrast to ADR nephrosis in which our study revealed no evidence of inflammatory cell involvement. Furthermore, our study showed increased glomerular chemiluminescence of ADR rat glomeruli compared to normal glomeruli. This increase coincided with an increase of albuminuria suggesting that the glomerular injury is induced by the generation of ROS by glomerular cells.

It is well documented that in the inflammatory process, ROS generated by PMN and/or macrophages causes glomerular injury and thereby proteinuria. These inflammatory cells also release other chemical mediators whhich contribute to tissue injury, and it is uncertain to what extent ROS generation plays in tissue damage.

Rehan et al.[10] recently provided evidence that intra-arterial injection of phorbol myristate acetate (PMA) induces glomerular injury. Catalase administration in this experiment prevented the glomerular injury significantly. They concluded that the injury was due to the PMN-derived ROS, but could not exclude the possibility that the injury was induced cooperatively with the release of proteolytic enzymes.

Shah and his group[4] reported that isolated glomeruli will give chemiluminescence not only in response to PMA but also at resting state. However, it was not shown which of the constituent cells of glomeruli generates ROS. ROS is known to be generated in cultured mesangial cells. We have shown that in ADR rat glomeruli, chemiluminescence is significantly increased without any stimulation. This was inhibited by adding SOD *in vitro*. SOD is not incorporated intracellularly so it can be assumed that ROS is released from the glomerular cells. If this is true, it is likely that released ROS reciprocally injures adjacent glomerular cells and glomerular basement membrane leading to proteinuria. We conclude that the inhibition of proteinuria by administration of TPAA to ADR rats may be due to its entry into the cell.

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