Beneficial effect of retinoic acid on the outcome of experimental acute renal failure

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Abstract

Background. Retinoic acid (RA) exerts beneficial effects on vascular remodelling and experimental nephritis, and plays a role in kidney development. Pathological changes caused by acute renal failure (ARF) result in high mortality. We determined whether RA ameliorates ARF-induced pathology caused by potassium dichromate (PD).

Methods. Adult Wistar female rats (210–250 g) were randomly allocated to four groups: (i) an ARF group that received PD [15 mg/kg body weight (bw), single dose subcutaneously]; (ii) a group that received PD plus RA (1 mg/kg bw) beginning at 5 days before PD and that continued for 14 additional days; (iii) a group that received PD plus thyroxine (T4; 8 μg/100 g bw) with RA; and (iv) a group that received only the vehicle for PD (saline solution). We evaluated functional, biochemical and morphological characteristics of the kidneys.

Results. PD-induced alterations in serum creatinine, creatinine clearance (Ccr) and fractional excretion of sodium (FeNa) were less severe when rats received RA. PD increased lipoperoxidation and this alteration was partially blocked by RA. Animals undergoing ARF showed severe histological injury (brush border loss, acidophilia, oedema, pyknosis, karyorhexis, cell detachment and disruption of the basement membrane). These alterations were less severe in RA-treated rats, indicating a protective effect on functional and morphological alterations. Alterations in urinary sediment were reduced by RA. The simultaneous administration of T4 with RA did not produce additional protection.

Conclusion. RA exerted beneficial effects on the duration and severity of renal damage induced by PD in a model of renal failure resembling ARF in humans. The protective effect of RA may be mediated by diminished lipoperoxidative damage.

Keywords: acute renal failure; creatinine clearance; FeNa; potassium dichromate; retinoic acid; T4

Introduction

Acute renal failure (ARF) is associated with a high mortality that reaches >50% even during adequate medical care. In intensive care units, mortality rates reach up to 70% [1]. ARF is associated with several medical, surgical and obstetric conditions as well as with overexposure to heavy metals, such as lead, mercury or chromium, that accumulate in the proximal tubule, their main site of toxicity. Parenteral administration of potassium dichromate (PD) provokes acute renal damage in rats, resulting in polyuria, a decline in glomerular filtration rate (GFR), tubular obstruction and back-leakage of filtrate [2]. Deleterious alterations in proximal cells are dependent upon the type and dosage of toxic insult as well as the duration and intensity of ischaemia. Also observed are fragmentation and detachment of the brush border and changes in phospholipids and cholesterol leading to modifications of membrane fluidity, electrical resistance and epithelial permeability [3]. Recovery depends on the restoration of haemodynamic alterations and on recovery of tubular epithelium. In recent work, the importance of collagen IV–integrin interactions in epithelium recovery has been emphasized [4].

During ARF, there is severe damage to the epithelial transporting mechanisms. In proximal tubules, the loss of brush border is an early feature associated with
alterations in transcellular transport of water, ions and organic compounds. Chemical or ischaemic insults also modify the functions of the paracellular transporting route by breaking intercellular tight junctions, desmosomes and gap junctions, which leads to increased permeability with abnormal backflow and loss of intercellular communications. Chemical- or ischaemia-induced loss of extracellular matrix causes apoptosis accompanied by detachment of tubular cells. Alterations of intercellular unions may also lead to loss of viable cells [5].

Conservative treatment or dialytic procedures are given according to the severity of ARF. Although other therapeutic approaches such as the use of thyroid hormones have shown benefits in experimental animal models, the results in humans are still unresolved.

In recent years, retinoic acid (RA) has been shown to play multiple roles in kidney function. It also participates in tubular cell differentiation, and in the synthesis of collagen, fibronectin and laminin-1 [6]. During compensatory hypertrophy in remnant kidneys after uninephrectomy, retinol-binding protein was increased, suggesting its participation in the hypertrophy process [7]. Dietary deficiency of retinol during pregnancy led to inborn nephron deficits in rats [8]. In addition, lower than normal concentrations of blood retinol were found in human ARF [9].

The aim of this study was to evaluate the potential beneficial effects of RA on the evolution and severity of ARF induced by PD. Both thyroid hormones and RA show protective effects for kidneys against the actions of several deleterious factors. Therefore, we predicted that co-administration would exert a summated effect on kidney protection. We evaluated the combined effects of thyroxine (T$_4$) with RA. We found a protective effect of RA, without additional benefit of simultaneously administered T$_4$.

**Materials and methods**

**Animals**

Adult Wistar female rats (210–250 g) were reared in our animal house and fed standard rat chow (PMI, 5008) with tap water *ad libitum*. They were maintained at 22–24°C, with an average humidity of 55%. The handling and experimental procedures were performed according to recommended international standards.

The experimental design included four groups of 36 animals: (i) the ARF group had renal damage induced by a single dose of PD [15 mg/kg body weight (bw), subcutaneously]; (ii) the second group was given with PD with concomitant RA (1 mg/kg bw); (iii) the third group received a combination of PD, T$_4$ (8 μg/100 g bw) and RA, at the previously described doses; and (iv) the fourth group received the vehicle for PD (saline solution).

In the ARF group, renal damage induced by PD was evaluated functionally and histologically. For the functional evaluation, rats were lodged individually in metabolic cages for 24 h urine collections taken during scheduled days (see below). Body weight was recorded and blood samples were obtained from the tail vein. Serum and urine sodium and creatinine were measured. Triiodothyronine (T$_3$) and T$_4$ were measured in blood by radioimmunoassay [10] (Diagnostic Products Corporation, Los Angeles, CA). Serum and urine sodium concentrations were measured by flame photometry using an automated analyser (Instrumentation Laboratory, Hollyton, MA). Creatinine was measured according to the Jaffe method. Creatinine clearance (C$_{cr}$) and fractional excretion of sodium (FeNa) were calculated as follows:

$$C_{cr} = \left(\frac{Cr_u \times J_v}{Cr_s}\right)/100 \, \text{g bw}$$

where $Cr_u$ is urinary creatinine, $Cr_s$ is serum creatinine and $J_v$ is urine volume/min.

$$\text{FeNa} = \frac{(Na_u \times Cr_u)/(Na_u \times Cr_p) \times 100}{Cr_s}$$

where $Na_u$ and $Na_s$ are serum and urinary sodium, and $Cr_s$ and $Cr_u$ are serum and urinary creatinine, respectively. These parameters were estimated before PD and on days 1, 3, 7, 10 and 14 after administration of PD. For each of these periods, six rats were selected randomly to study renal functional parameters. At the end of the study, they were sacrificed.

**Histological evaluation**

For the ARF group, histological damage was studied in a separate group of rats ($n = 4$ for each period for a total of 24 rats), treated with PD and sacrificed at days 1, 3, 7, 10 and 14 after induction of ARF. Animals were anaesthetized with sodium pentobarbitial (30 mg/kg bw, intraperitoneally) and kidneys were exposed, perfused with saline solution through a PE90 catheter, and rapidly removed. They were then decapsulated and sagittal sections including cortex, medulla and papilla were obtained. Slices 2–3 μm thick were fixed in Bouin solution and stained with haematoxylin and eosin. Histological damage was evaluated blind by two independent observers. Loss of brush border, cell oedema, piknosis, karyorhexis, acidophila, cell detachment and loss of basal membrane were scored for histological sections obtained from animals on days 0, 1, 3, 7, 10 and 14.

In the ARF plus RA group, the animals received PD as described for the previous groups ($n = 4$, for each period for a total of 24 rats). The animals were pre-treated with RA (p.o. daily; all-trans-retinoic acid, Sigma Co., MA) for 5 days before and for 14 days after induction of ARF. Functional and histological evaluations were carried out as previously described.

In the group receiving ARF plus simultaneous administration of RA and T$_4$, ARF was induced with PD as in previous groups ($n = 4$ for each period for a total of 24 rats). In addition, this group received simultaneous RA and T$_4$ at the previously described doses. Functional alterations and histology were evaluated as previously described.

In the vehicle-treated group ($n = 24$), animals received subcutaneous saline (the vehicle for PD). Functional and histological evaluations were performed as previously described.

For each of these groups, 2–3 slides from each period were examined. For each period, 10–14 fields were observed and 136–167 tubules were studied. We examined brush border integrity, cell oedema, cell detachment, piknosis, karyorhexis, acidophila and loss of basal membrane.
Urinary sediment

Detachment of damaged cells and their elimination into the tubular fluid in ARF contributes to backflow due to partial occlusion of the tubular lumen and increased intratubular pressure during decrements in GFR. We therefore studied the presence of dead and live cells in urine from the animals with ARF. We formed two additional groups \( n = 24 \) for each group, i.e. a group having ARF induced by PD, and an ARF plus RA group, that were both created using previously described methods. Urine was collected on an individual basis in metabolic cages for 2 h under mineral oil to avoid evaporation. Samples were centrifuged at 1300 r.p.m. for 5 min. Extrusion of trypan blue (0.4%) was used to discriminate live from dead cells and both populations were counted in a Neubauer chamber. The presence of cells of renal origin and from lower urinary epithelia was recorded on days 0, 1, 3, 7, 10 and 14 after induction of ARF with PD. Identification was performed on a morphological basis during microscopic examination at 100x.

Lipoperoxidation measurements

The degree of peroxidation was estimated from the concentrations of malondialdehyde in renal tissues from control and ARF animals that were or were not given RA. We followed the methodology of Buege and Aust [11].

Reagents

All-trans-retinoic acid, T4 and trypan blue were purchased from Sigma (St Louis, MO). PD was obtained from Merck-Mexico. Other high purity reagents were obtained from local dealers.

Statistical analysis

Values are means ± SEM. ANOVA was used for multiple comparisons followed by Tukey–Kramer tests. Differences at the \( P < 0.05 \) level were considered significant.

Results

RA partially prevented the decrement in creatinine clearance induced by PD

As expected, PD induced severe decreases in GFR, as estimated from \( C_{cr} \). The maximal effect was observed on day 3 after PD administration. Spontaneous recovery was observed by day 7, and on days 10 and 14 clearances were similar to initial values. The administration of RA partially prevented the decrease in GFR (Figure 1).

Increments in serum creatinine induced by PD were partially prevented by RA

In agreement with the decrement in \( C_{cr} \), serum creatinine increased from 0.6 to a maximum of 3.75 mg/dl on day 3 after PD injection. RA significantly reduced the augmentation of serum creatinine observed on days 1 and 3 (Figure 2), which agreed with \( C_{cr} \), to indicate a protective effect. A recovery by day 14 was complete in both groups.

RA blunted the increment in fractional sodium excretion elicited by PD

As in experimental or human ARF, FeNa sharply increased after administration of PD. Maximal increments appeared on day 3 \( (P < 0.001) \) after PD, and FeNa returned to control values on day 10. FeNa values in RA-treated rats were comparable with those of the ARF group on day 14. Importantly, RA prevented the augmentation of FeNa, and functional damage was less severe in this group than in the group that received PD alone (Figure 3). The vehicle for RA
(ethanol/distilled water, 70/30%, by gavage) did not alter the time course of FeNa for RA or vehicle groups during any period of the study.

RA partially blocked the increment in lipoperoxidation induced by PD

We found that (i) lipoperoxidation under resting conditions was lower in RA-pre-treated rats than in control rats (Figure 4); and (ii) increments in lipoperoxidation induced by PD were partially blocked by RA treatment. These findings suggest that RA was effective in reducing oxidative damage.

Changes in urinary volume during ARF

Urinary volume increased after administration of PD (day 0 = 1.2 vs day 3 = 2.8 μl/min per 100 g bw; P < 0.01). In contrast to the beneficial effects of RA on serum creatinine and FeNa, increments in urinary volume were not prevented by simultaneous RA (day 3: ARF = 2.8 ± 0.4, ARF + RA = 3.4 ± 0.6 μl/min per 100 g bw; NS), suggesting some selectivity of the drug on alterations in renal function.

Loss in body weight during ARF was prevented by RA

Administration of PD induced severe weight loss with a maximal effect by day 7 (−51.8 ± 7.0 g) which was partially recovered by day 14 (−36.6 ± 7.0 g). In contrast, animals that received RA did not lose body weight (Figure 4). Together with the serum creatinine and FeNa findings, these suggest a protective effect of RA against PD-induced toxicity.

RA decreases the severity of histological alterations in ARF

Kidneys from rats treated with PD showed extensive damage, including necrotic proximal tubules containing cellular debris and tubular dilation. Epithelial cells were detached and the brush border could not be identified (Figure 5A). Glomeruli and distal tubules were preserved. In sections from RA-treated rats (Figure 5B), tubular damage was less severe than in animals receiving only PD. The proximal epithelium was preserved even though some tubules showed necrotic cells and tubular debris in the lumen. The glomeruli and distal tubules had a normal appearance (not shown).

The brush border was lost in all PD-treated animals from the first day after induction of ARF until day 10, with partial recovery (60%) on day 14. In the animals treated with RA and PD, this damage was delayed and was less severe, and they showed complete recovery
Acidophilia and cell oedema were present during all ARF time periods. In the animals treated with RA, acidophilia was completely prevented and cell oedema gradually diminished and disappeared by day 14 (Figure 8A and B). In rats undergoing ARF, piknosis and karyorhexis were observed on day 3 but recovered afterwards. In animals that received RA, neither piknosis nor karyorhexis were observed (Figure 8C and D), further supporting a protective role for this compound.

We observed detached cells in the lumen of tubules from PD-treated rats. The cells were present on day 1 and reached a maximum of 89.4% by day 3. They then decreased on subsequent days and on day 14 they were still present in 25% of the tubules. In PD animals treated with RA, cell detachment was less frequent and recovery was complete on day 14 (Figure 8A). In PD rats, the basement membrane was disrupted with a maximal alteration on days 7 and 10. Recovery was observed by day 14. In PD + RA rats, treatment with RA prevented these alterations in the basement membrane (Figure 8B).

### RA reduced the presence of dead and live cells in the urinary sediment

In animals with ARF, elimination of renal and lower urinary epithelial cells increased, beginning on day 1, with a peak on day 3, followed by a return to basal elimination after day 10. In contrast, animals that received RA showed a reduction in the number of cells. The time course of increased cell elimination mimicked that of functional and histological alterations. It is noteworthy that RA treatment showed a double effect. First, there was a reduction in the number of dead cells, indicating a protective effect against the mechanisms producing cell necrosis. Secondly, RA diminished the number of live cells in the urine, suggesting an additional protective effect against alterations in proteins responsible for intercellular junctions and in proteins related to cell attachment to the basal membrane (Figure 9).

**Combined T4 and RA treatment did not produce additive protective effects**

We examined whether combined RA and T4 would show additive protective beneficial effects. We administered T4 during 14 days following induction of ARF. To assess the behaviour of the hormone, we measured serum T4 and T3 in rats treated with either PD alone, PD in combination with T4 or PD in combination with RA and T4. T4 was elevated in the group that received the hormone (PD alone, 2.63 ± 0.16; PD plus T4, 8.9 ± 1.28). T3 did not increase after receiving the hormone. Protective effects with RA treatment were not enhanced when we gave simultaneous T4. Increments in serum creatinine were blunted similarly by both treatments, and FeNa was equally and less altered, respectively, by those treatments compared with PD alone rats.
Heavy metals induce severe nephrotoxicity and their main site of action is the proximal tubule [2]. We found that PD caused severe decreases in Ccr with maximal damage on day 3. Importantly, pre-treatment with RA significantly accelerated the recovery of Ccr. In parallel with the alterations in Ccr, serum creatinine increased in the animals undergoing ARF, and RA attenuated this increase. Moreno-Manzano et al. [12] reported that all-trans-retinoic acid (tretinoin) caused increases in Ccr in 18-month-old rats when it was fed for 3 months to the rats at the same dose used in this study. They suggested that tretinoin may slow the rate of progression of glomerulosclerosis by acting on the balance of synthesis/degradation of extracellular matrix and/or on the cellular hypertrophic changes that occur as ageing progresses [12].

The fractional excretion of sodium has long been recognized as a useful tool for the diagnosis of ARF [13]. As expected, FeNa increased maximally on day 3 in PD-treated rats and it partially returned to control values, remaining 53% above baseline on day 14. In contrast, animals treated with RA showed a lower increment in FeNa. This finding suggests that RA exerted similar protective effects on tubular damage as it did on Ccr. These effects on FeNa and Ccr are the first that have been reported for ARF.

**Discussion**

Fig. 8. Pre-treatment with RA protected renal tissue from PD-induced damage. (A) Diffuse acidophilia was observed after day 1 of ARF (filled squares) and remained present until day 14. In contrast, this alteration was prevented in animals pre-treated with RA (open triangles). (B) Cell oedema was present from the beginning of ARF (filled squares), while in RA-treated rats (open triangles) it had a similar initial time course but rapidly subsided and on day 14 it was absent. (C) Piknosis was observed on day 3 in ARF (filled squares) but spontaneously diminished to control levels on day 10. It was prevented in RA-treated rats (open triangles). A similar action was observed for karyorhexis (D). Values are means ± SEM. ***P<0.001.

Fig. 9. Cell detachment and alterations in basement membrane were less severe in RA-treated rats. (A) Detached cells were observed from day 1 after induction of ARF (filled squares), and RA treatment diminished this alteration (open triangles). (B) Basement membrane was lost with maximal damage on day 10, followed by spontaneous recovery on day 14. RA pre-treatment prevented this alteration. Values are means ± SEM. **P<0.01; ***P<0.001.
RA attenuated the body weight loss caused by ARF. This beneficial effect may have been due to less severe renal failure resulting in a less pronounced catabolism. This finding may be of clinical relevance, since in humans intense catabolism is observed during ARF and RA may facilitate recovery. A more rapid recovery could also decrease the appearance of malnutrition and may reduce mortality. Wagner et al. [14] observed toxic effects of RA in Wistar rats that received 10-fold the dose we used. They observed weight loss, hair loss, cheilitis and keratitis. In the present study, we observed no adverse effects.

We found that severe histological damage ensued after PD administration and this was more marked on day 3. As expected, these alterations were more evident in proximal tubules. In agreement with a more rapid recovery of altered function in RA-pre-treated rats, these animals also showed less severe histological alterations than rats receiving only PD. Loss of brush border, acidophilia and cell oedema were the more persistent alterations, and RA pre-treatment accelerated their recovery. A conspicuous finding in our model was loss of brush border, which was also reported by Solez et al. [15] in biopsies from patients with ARF.

We found increases in dead and live cells in the urinary sediment, and pre-treatment with RA diminished these alterations. These findings suggest that RA may exert double protective effects against renal damage. First, it may act as an antioxidant to prevent damage due to oxidative stress, thereby decreasing the number of necrotic cells. Secondly, the decrease in the number of live cells suggests a protective effect of RA on the epithelial mechanisms involved in the attachment of cells to each other and to the basement membrane. Thus, the expected protective effects of RA were confirmed by the present results. In support of this, Kitamura et al. [16] reported that RA inhibits apoptosis induced by hydrogen peroxide in mesangial cells, and that it acted through suppression of c-fos/c-jun expression and c-Jun N-terminal kinase [16].

Importantly, animals pre-treated with RA showed a small number of live cells in the urine. In agreement with this, RA has been shown to induce the appearance of proteins, such as laminin, that are involved in the attachment of cells to basal membranes, suggesting that this vitamin plays a role in the expression of proteins that maintain epithelial integrity [17]. Furthermore, RA induces tight junction (TJ) structure and expression of TJ-associated molecules, such as ZO-1, occludin, claudin 6 and claudin 7 [18]. We previously reported that ZO-1 and claudin 7 are located in proximal isolated tubules [19], which are the site of action of chromium. In our current model, administration of RA may have helped to protect intercellular junctions.
from oxidative effects of PD, or it may have contributed to restoration of the damaged junctions. A combination of these two protective mechanisms is also possible. Meyer et al. [20] reported that oxidative stress induced by hydrogen peroxide in MDCK cells markedly reduced transepithelial resistance and disrupted the staining patterns of the TJ proteins ZO-1 and occludin [20].

In summary, PD-induced ARF in rats caused renal damage that was reduced by RA. RA protected against both functional and histological alterations. The beneficial effects of RA were not enhanced by the simultaneous administration of T4.

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