Mitochondrial DNA and its respiratory chain products are defective in doxorubicin nephrosis

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Abstract

Background. Doxorubicin induces a self-perpetuating nephropathy characterized by early glomerular and late-onset tubular lesions in rats. We investigated the potential role of mitochondrial injury in the onset of these lesions.

Methods. Rats were treated with intravenous doxorubicin (1 mg kg⁻¹ week⁻¹) for 7 weeks and were sacrificed either 1 week (‘short-term’) or 30 weeks (‘long-term’) following the last dose. Additional rats received a single dose either 6 days or 2 h prior to euthanasia. All rats were killed at 48 weeks of age. Glomerular and tubular injury was monitored and correlated to the activity or expression of respiratory chain components. Finally, we quantified both nuclear and mitochondrial DNA (mtDNA) as well as superoxide production and the 4834 base pair ‘common’ mtDNA deletion.

Results. The ‘long-term’ group had significant glomerular and tubular lesions, depressed activities of mtDNA-encoded NADH dehydrogenase and cytochrome-c oxidase (COX) and increased citrate synthase activity. In addition, expression of the mtDNA-encoded COX subunit I was reduced and mtDNA levels were decreased. In ‘short-term’ rats, there were fewer tubular lesions, but similar numbers of glomerular lesions activity. Among all animals, glomerular and tubular injury were inversely correlated with mtDNA levels, mtDNA-encoded respiratory chain activities and with the expression of the mtDNA-encoded respiratory chain subunit COX-I. Injury was positively correlated with superoxide production and the activities of nucleus-encoded mitochondrial or cytoplasmic enzymes. Kidneys from the ‘long-term’ group showed more mtDNA deletions than in ‘short-term’ animals and these were not observed in the other groups.

Conclusions. These results suggest an important role for quantitative and qualitative mtDNA alterations through the reduction of mtDNA-encoded respiratory chain function and induction of superoxide in doxorubicin-induced renal lesions.

Keywords: cytochrome-c oxidase; doxorubicin; kidney; mitochondrial DNA; rat; reactive oxygen species

Introduction

The anti-tumour anthracycline antibiotic doxorubicin (Adriamycin⁶) is the drug of choice for the treatment of many solid malignancies and lymphomas. Rats that are given doxorubicin develop heart failure as well as a self-perpetuating glomerular nephropathy, which has a relatively early onset. Even in the absence of continued doxorubicin exposure, the glomerular damage progresses and late-onset tubular lesions are observed [1,2]. Within a few weeks after doxorubicin administration, the glomerular filtration rate declines gradually and the animals develop a nephrotic and a tubular syndrome. The exact pathophysiological mechanisms underlying the loss of the glomeruli and tubules and the causes of delayed progression remain to be determined.

We hypothesize that acute doxorubicin exposure causes an initial mitochondrial injury that accumulates with time and ultimately compromises the bioenergetic capacity of the organelles, which accounts for the delayed progression of renal damage. Our hypothesis stems from the finding that doxorubicin enters mitochondria, where it generates reactive oxygen species (ROS) that cause damage to mitochondrial DNA (mtDNA) [3]. Disruption of mtDNA may produce respiratory chain dysfunction, which in turn may generate additional ROS that attack the respiratory chain itself or again cause damage to mtDNA (Figure 1) [4]. ROS may, therefore, represent part of a vicious
cycle composed of interconnected insults to mtDNA and to the respiratory chain. In support of this, radical scavengers have been demonstrated to exert some form of nephroprotection against doxorubicin-induced renal injury [5,6].

The present study was the first to examine long-term effects of repeated doxorubicin doses in rats and it determined whether the drug exerts qualitative and quantitative alterations in mtDNA resulting in impaired mtDNA-encoded respiratory chain activity. Unlike previous models, which mostly investigated effects of repeated drug administration, we aimed to analyse toxicities that persist or even accumulate during the long-term following drug administration. To investigate the effect of time, we allowed both short and long survival of the animals from exposure to doxorubicin to the time of kidney analysis.

**Subjects and methods**

**Animals**

After approval by the animal ethics board, male Wistar rats were obtained from Charles River (Sulzfeld, Germany). The rats received an intravenous port (Rat-O-Port™; Uno Roestvaststaal, Zevenaar, Netherlands) following anaesthesia with Isoflurane™ (Abbott, USA) at 10 weeks of age. They were then divided into five groups (Figure 2). Group A animals (n = 7) served as controls and received seven intravenous injections of saline (300–700 μl) through the port, beginning at 11 weeks of age. Groups B, C, D and E received doxorubicin (1 mg/kg) in equivalent volumes (Pharmacia, Freiburg, Germany). Group B (the ‘long-term group’, n = 10) was administered seven weekly injections beginning at 11 weeks of age. Group C (the ‘short-term group’, n = 9) received the same seven injections, but the course was begun at 41 weeks of age. Groups D (n = 8) and E (n = 6) received one single injection either 6 days or 2 h prior to euthanasia, respectively. All animals were killed by cervical dislocation at 48 weeks of age, followed by immediate organ collection and postmortem examination.

The kidneys and the left gastrocnemius skeletal muscles were snap frozen and cryopreserved in liquid nitrogen until subsequent analysis. Aliquots were fixed in glutaraldehyde (3%) for subsequent electron microscopy.

**Histopathology**

The severity and extent of glomerulopathy was evaluated by semi-quantitatively scoring mesangial matrix and glomerular sclerotic lesions from 4 μm kidney sections stained with periodic acid–Schiff (PAS) [7].

Tubular involvement was evaluated with semi-quantitative scores from 4μm cryostat sections that were stained with oil red (30 min) and counterstained with Meyer’s haematoxylin (8 min). We randomly selected 25–60 tubuli from each section. The severity of lipid accumulation, which paralleled...
the loss of tubuli and the degree of interstitial fibrosis, was graded for each tubulus as ‘0’ (no obvious lesion), ‘1’ (borderline), ‘2’ (moderate), ‘3’ (severe) and ‘4’ (extensive). These values were then multiplied by the percentage of tubuli with the same degree of injury and then divided by the total number of tubuli examined.

Kidney sections of two randomly selected rats from groups A, B and C were examined by electron microscopy [8]. The evaluator was blinded to the treatment status of the animals. We also performed succinate dehydrogenase (SDH) and cytochrome-c oxidase (COX) histochemistry on 4 µm cryostat kidney transverse sections [9].

**Enzyme activity measurement**

We measured the activities of nicotinamide adenine dinucleotide hydrogen dehydrogenase (NADH), SDH, COX and citrate synthase in freshly prepared extracts from whole kidney cross sections using spectrophotometric assays [10]. NADH (complex I), SDH (complex II) and COX (complex IV) are all enzymes of the mitochondrial respiratory chain. COX and NADH are multi-subunit components, which are partly encoded by nuclear DNA (nDNA) and partly by mtDNA, whereas SDH is encoded entirely by nDNA. Citrate synthase is a nDNA-encoded component of the Krebs cycle and located in the mitochondrial matrix.

**Quantification of the mtDNA-encoded COX-I respiratory chain subunit**

Subunit I of COX (COX-I), which is encoded by mtDNA, was quantified by immunoblot and normalized to the signal of a simultaneously used antibody against subunit IV of COX (COX-IV), which is itself encoded by nDNA [9]. The intensities of the signals were quantified by scanning densitometry using the Scion ImageTM program (Scion Corporation, Frederick, USA). The ratio between the COX-I and COX-IV signals was calculated and the results were expressed as a percentage of the control group mean.

**Quantification of wild-type mtDNA**

Both mtDNA and nDNA were digested with the restriction enzyme *XhoI* and quantified by Southern blotting [8]. The mtDNA was probed with a 13.1 kilobase pair, random-prime digoxigenin-labelled polymerase chain reaction (PCR) fragment, spanning nucleotide positions 3192 and 16290 of rat mtDNA. The nDNA probe was directed against the multi-exon reverse transcriptase-polymerase chain reaction (RT-PCR) fragment spanning nucleotide positions 3192 and 16290 of rat mtDNA. The nDNA probe was directed against the multi-exon reverse transcriptase-polymerase chain reaction (RT-PCR) fragment spanning nucleotide positions 3192 and 16290 of rat mtDNA.

**Detection of the ‘common’ deletion**

The sequence of normal rat mtDNA contains two 16 base pair (bp) direct repeats between which 4834 bp of the mtDNA molecule may be deleted by slipped mispairing during replication [11]. We probed for the ‘common’ 4834 bp deletion in rats by amplifying 100 ng of genomic DNA with two extradeletional primers in a PCR. The 459 bp product, corresponding to the deleted mtDNA molecule, was preferentially amplified by choosing a short extension cycle (30s). The PCR products were sequenced by MWG Biotech (Ebersberg, Germany).

**Assessment of superoxide production**

Kidney transverse sections were prepared for in situ imaging of superoxide generation with the oxidative fluorescent dye dihydroethidium (Sigma, Taufkirchen, Germany) [12]. The intensity of the fluorescence was quantified using Scion ImageTM.

**Statistical analysis**

Group means were compared using unpaired *t*-tests or Wilcoxon analysis, as appropriate. Regressions were computed by non-linear exponential regression analysis. All calculations were performed using the Sigma Plot 2000™, version 6.0, statistical package (SPSS Inc., Chicago, USA).

**Results**

**Findings at autopsy**

Three of the 10 rats from the long-term group B died at weeks 38, 44 and 46, respectively. Postmortem examination revealed pleural effusions and a dilated myocardium in the animals that died at weeks 38 and 44. One of the nine animals in group C died at week 48. All of these rats were excluded from the analysis because their survival times no longer matched that of their respective groups.

The mean body weight gain for group B was lower than that in the other doxorubicin-exposed groups and was continuously lower than that of the controls. Of the seven group B animals that survived until euthanasia, five had pleural effusions. The kidneys in groups B and C were enlarged, had an irregular surface and were yellowish in colour. Groups D and E had kidneys that were macroscopically normal.

PAS staining revealed that glomeruli of the cumulative long-term group B and of the cumulative short-term group C rats had enlarged mesangial, segmental or global sclerosis and an obliterated capillary lumen (Figure 3.1). There was also an increase of triglycerides in the glomeruli (Figure 3.2). All these changes were absent in groups D and E. The degree of histological damage (glomerulopathy score) was elevated in groups B and C compared with controls (both *P* < 0.001), whereas the other groups had normal glomeruli (Table 1). Groups B and C were not different from each other (*P* = 0.08). The tubulo-interstitial area in group B was characterized by marked luminal dilatation and tubular epithelial atrophy. In the oil-red stains, a prominent accumulation of intracellular lipids was noted in the preserved epithelial cells of the tubuli (Figure 3.3). This lipid accumulation was more prominent in the basal rather than in the apical area. Only group B showed a markedly elevated tubulopathy score (*P* < 0.001).
score for group C was 10-fold lower than that of group B and did not significantly differ from controls. Groups D and E (Table 1) were also not different from controls. The ultrastructure of group B kidneys was characterized by end-stage nephropathy whereas only an increased number of mitochondria was noted in group C (Figure 4).

Enzyme activities and COX-I expression

The enzymatic activity of citrate synthase was increased by 170% in group B (513 ± 182 μmol min⁻¹ g protein⁻¹; \( P = 0.01 \)) and by 153% in group C (462 ± 160 μmol min⁻¹ g protein⁻¹; \( P = 0.02 \)) compared with controls (100%; 302 ± 44 μmol min⁻¹ g protein⁻¹). Citrate synthase activity was not different between controls and all of the other groups (Table 1). The enzymatic activity of SDH showed a similar, albeit less pronounced, up-regulation in kidneys from groups B and C. Complex I (normalized for SDH activity), expressed as the NADH/SDH ratio, was depressed by 41% (\( P = 0.02 \)) in group B and 34% (\( P = 0.05 \)) in group C compared with controls. In groups D and E, the NADH/SDH ratio was essentially normal.

Similarly, the mean enzymatic activity of complex IV (COX) in group B was reduced to 55% (9 ± 4 μmol min⁻¹ g protein⁻¹) of controls (17 ± 4 μmol min⁻¹ g protein⁻¹; \( P = 0.003 \)). Although there was some decline

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Table 1. Effects of doxorubicin on kidney morphology, respiratory chain function and mtDNA and superoxide production

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>B (n = 7)</th>
<th>C (n = 8)</th>
<th>D (n = 8)</th>
<th>E (n = 8)</th>
<th>P-value (control vs B)</th>
<th>P-value (control vs C)</th>
<th>P-value (B vs C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulopathy score</td>
<td>4 ± 3</td>
<td>173 ± 98</td>
<td>104 ± 35</td>
<td>12 ± 7</td>
<td>3 ± 1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Tubulopathy score</td>
<td>2 ± 3</td>
<td>128 ± 59</td>
<td>12 ± 13</td>
<td>4 ± 6</td>
<td>2 ± 2</td>
<td>&lt;0.001</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Citrate synthasea</td>
<td>302 ± 44</td>
<td>513 ± 182</td>
<td>462 ± 160</td>
<td>358 ± 64</td>
<td>331 ± 80</td>
<td>0.01</td>
<td>0.02</td>
<td>0.6</td>
</tr>
<tr>
<td>SDHa</td>
<td>54 ± 11</td>
<td>71 ± 9</td>
<td>68 ± 12</td>
<td>55 ± 11</td>
<td>57 ± 9</td>
<td>0.008</td>
<td>0.04</td>
<td>0.55</td>
</tr>
<tr>
<td>NADH/SDHb</td>
<td>100 ± 39</td>
<td>59 ± 16</td>
<td>66 ± 24</td>
<td>97 ± 28</td>
<td>92 ± 22</td>
<td>0.02</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>COX/SDHb</td>
<td>100 ± 26</td>
<td>42 ± 17</td>
<td>52 ± 25</td>
<td>104 ± 34</td>
<td>98 ± 16</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.4</td>
</tr>
<tr>
<td>COX-I/COX-IVb</td>
<td>100 ± 11</td>
<td>68 ± 22</td>
<td>93 ± 11</td>
<td>88 ± 9</td>
<td>105 ± 20</td>
<td>0.004</td>
<td>0.26</td>
<td>0.01</td>
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<tr>
<td>mtDNA/nDNAb</td>
<td>100 ± 18</td>
<td>67 ± 27</td>
<td>79 ± 36</td>
<td>95 ± 19</td>
<td>113 ± 27</td>
<td>0.02</td>
<td>0.19</td>
<td>0.5</td>
</tr>
<tr>
<td>Superoxide productionb</td>
<td>100 ± 47</td>
<td>345 ± 210</td>
<td>200 ± 41</td>
<td>90 ± 41</td>
<td>108 ± 43</td>
<td>0.006</td>
<td>&lt;0.001</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\( ^a \mu \text{mol min}^{-1} \text{ g protein}^{-1}. \)

\( ^b \text{Percentage of control mean. The values are means ± SD. The 'common' mtDNA deletion was present at low levels (+) in group C, at higher levels (++) in group B, but was absent (−) in groups A, D and E. n/a, not applicable.} \)
and with COX/SDH ratios. COX-I expression and with mtDNA levels

<table>
<thead>
<tr>
<th></th>
<th>Glomerulopathy score</th>
<th>Tubulopathy score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase</td>
<td>$r = +0.79$, $P &lt; 0.001$</td>
<td>$r = +0.67$, $P &lt; 0.001$</td>
</tr>
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<td>SDH</td>
<td>$r = +0.68$, $P &lt; 0.001$</td>
<td>$r = +0.58$, $P = 0.002$</td>
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<tr>
<td>NADH/SDH</td>
<td>$r = -0.69$, $P &lt; 0.001$</td>
<td>$r = -0.64$, $P = 0.002$</td>
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<tr>
<td>COX/SDH</td>
<td>$r = -0.81$, $P &lt; 0.001$</td>
<td>$r = -0.68$, $P = 0.001$</td>
</tr>
<tr>
<td>COX-I/COX-IV</td>
<td>$r = -0.69$, $P &lt; 0.001$</td>
<td>See Figure 5</td>
</tr>
<tr>
<td>mtDNA/nDNA</td>
<td>$r = -0.82$, $P &lt; 0.001$</td>
<td>$r = -0.70$, $P = 0.002$</td>
</tr>
<tr>
<td>Superoxide production</td>
<td>$r = +0.53$, $P &lt; 0.001$</td>
<td>$r = +0.68$, $P &lt; 0.001$</td>
</tr>
</tbody>
</table>

Table 2. Glomerular and tubular injury correlated positively with the activity of nDNA-encoded mitochondrial enzymes (citrate synthase and SDH) and with superoxide production, but negatively with the NADH/SDH and COX/SDH ratios, COX-I expression and with mtDNA levels.

Among all animals there was a positive correlation between nDNA-encoded enzymatic activities (citrate synthase and SDH) and both glomerulo- and tubulopathy scores (Table 2), whereas there were negative correlations with the mtDNA-determined activity ratios (NADH/SDH and COX/SDH). The expression of the mtDNA-encoded respiratory chain protein (COX-I/COX-IV) correlated negatively with the glomerulopathy score. No such correlation was observed with the tubulopathy score. Instead, there appeared to be a sharp threshold, since the degree of tubular damage increased abruptly, when the COX-IV-adjusted COX-I expression decreased below ~75% of the control mean (Figure 5). Although all group B rats, except one, had a COX-I/COX-IV ratio of <75% of normal, none of the other rodents showed ratios that were as low.

**MtDNA content**

Whereas the mean wild-type mtDNA content (mtDNA/nDNA ratio; Table 1) in group B kidneys was reduced compared with controls (67±27% vs 100±18%; $P = 0.02$), this was only slightly (79±36%) and non-significantly ($P = 0.19$) depressed in group C kidneys. There was also no reduction in the mean mtDNA content of groups D and E (95±19% and 113±27%, respectively). In all animals, the mtDNA content (mtDNA/nDNA ratio) was inversely correlated with the degree of glomerular ($r = -0.82$, $P < 0.001$) and tubular ($r = -0.70$, $P = 0.002$) damage (Table 2).

**MtDNA deletions**

A 459 bp PCR product was amplified from all group B and C kidneys (Figure 6) and was confirmed by sequencing to represent the ‘common’ mtDNA deletion. The intensity of the PCR products from group C

![Representative electron micrographs of epithelial cells from proximal tubules, revealing increased numbers of mitochondria of apparently normal structure in group C (2), compared with the control (1). (3) End-stage nephropathy within group B. Mitochondria appear to be swollen and some contain electron-dense amorphous materials, but are lacking cristae. Magnification: ×2800.](image-url)
kidneys was always lower than the lowest intensity observed in group B kidneys. We failed to detect the ‘common’ deletion in control, group D or group E kidneys. Southern blot analysis failed to detect potential mtDNA deletions in any of the samples of any tissue, indicating that the level of heteroplasmy of the ‘common’ mtDNA deletion, as detected by PCR, is likely to be <5%.

Superoxide production

Superoxide levels (Figure 7) were highest in group B (345±210% of control values; \( P = 0.006 \)), significantly elevated in group C (200±41%; \( P < 0.001 \)) and essentially normal in groups D (90±41%) and E (108±43%).

Skeletal muscle

Skeletal muscle ultrastructure did not differ among the groups and showed no pathology (data not shown). Citrate synthase activity, NADH/SDH, COX/SDH activity ratios and COX-I (normalized to COX-IV) in gastrocnemius muscle were not different in control, group B and group C rats. The mtDNA levels did not differ between the groups and mtDNA deletions were not detected in any of the muscle samples (data not shown).

Discussion

In the present study, we treated five groups of rats with single or repeated intravenous doses of doxorubicin in order to analyse kidney mitochondria and to investigate the mechanism and time course of drug-induced renal injury. Although single doses of doxorubicin did not cause significant renal or mitochondrial damage, cumulative administration led to substantial glomerular and tubular lesions. Tubular damage was not prominent in rats sacrificed shortly after repeated doses (group C), but was observed in group B animals a few weeks after multiple doxorubicin doses (group B).

The onset of the nephrotic lesions was paralleled by a dose- and time-related decrease in the enzymatic activity of mtDNA-encoded enzymes and a simultaneous enhancement of the nDNA-encoded respiratory chain and matrix components. This up-regulation of mtDNA-independent enzymes can be interpreted as a compensatory response to defects in the mtDNA-encoded respiratory chain. The histochemical analysis revealed that tubuli accounted for the brunt of the enzymatic changes, whereas glomeruli displayed relatively little activity (Figure 3). Our hypothesis that the glomerular and, especially, the tubular changes may have resulted from mitochondrial lesions was supported by the marked reduction in the COX-I/COX-IV ratio in the long-term group. We also found a marked

Fig. 6. Agarose gel electrophoresis for detection of the ‘common’ mtDNA deletion. Lane 1: PCR without DNA template; Lanes 2 and 8: 100 bp DNA size ladder. Representative PCR-products amplified from 100 ng of genomic DNA from group B (lane 4) and group C (lane 5) kidneys. Amplified PCR products were not detected from control (lane 3), group D (lane 6) or group E kidneys (lane 7).

Fig. 7. Increased superoxide production within glomeruli (1) and tubuli (2) in representative sections from group B (B) rats compared with controls (A).
accumulation of intracellular triglycerides in glomeruli and tubuli, which is a common finding in many conditions characterized by impaired respiratory chain activity [13]. For example, a similar pattern of down-regulated COX with preserved SDH activity and accumulation of triglycerides is the diagnostic hallmark of mitochondrial myopathies caused by inherited mtDNA-alterations [14]. Taken together, the histological and enzymatic patterns observed in the current experiments suggest a marked dysfunction of mtDNA-encoded respiratory activity in doxorubicin nephropathy.

We detected reduced levels of wild-type mtDNA in group B kidneys. These reductions may have been due to inhibited mtDNA replication via DNA strand cross-linking or adduct formation, resulting from inhibition of a topoisomerase or from the intercalating properties of the anthracycline [15]. In addition to decreased amounts of wild-type mtDNA, there may also have been increased levels of mtDNA mutations. The ‘common’ deletion was present in group B and C kidneys, but with a greater frequency in group B kidneys. This deletion has been associated with oxidative stress and senescence in rats [4]. However, ageing alone does not explain the presence of the deletion, since the mutation was absent in the age-matched controls. Oxidative stress might be a more likely explanation, since oxidative lesions impede the replication of mtDNA by polymerase-gamma, which is thought to promote slip replication. Although we only screened for the ‘common’ mtDNA deletion, additional deletions might have been present.

The tubulopathy that we observed was of relatively late onset and, like the glomerular lesions, was self-perpetuating even in the absence of continued doxorubicin exposure. What might be the mechanism, given that most of the direct effects of doxorubicin on mtDNA are probably reversible? Acute doxorubicin exposure generates ROS through redox cycling [16]. Oxidative stress may damage the lipid architecture of the mitochondrial membrane, compromise its fluidity, attack mitochondrial proteins necessary for mitochondrial bioenergetics or alter mtDNA itself. Mutated or decreased mtDNA, in turn, leads to depressed respiratory chain function, which causes further increases in ROS [17] (Figure 1). Such vicious cycles, consisting of interconnected mtDNA and respiratory chain insults, might also explain the late onset of diseases associated with inherited mtDNA-mutations [14]. These cycles might be initiated during acute doxorubicin treatment and might be perpetuated even in the absence of drugs. The tubulopathy might become evident when the degree of combined respiratory chain and mtDNA insults exceeds a certain threshold. Indeed, we observed increased levels of superoxide in glomeruli and even greater levels in the tubuli of groups C and B, whereas ROS were undetectable immediately following doxorubicin administration. This suggests that the major source of superoxide is the respiratory chain rather than doxorubicin-induced redox cycling. Superoxide may also react with nitric oxide to induce apoptosis, providing a mechanism that has been implicated in the sclerotic processes of a variety of kidney diseases [18]. Although respiratory chain decline in doxorubicin nephropathy per se may limit the survival of renal structures, increased apoptosis by superoxide might represent a final pathway that is common to other models of glomerulosclerosis, such as puromycin-induced nephropathy [19].

We believe that the present findings have targeted mtDNA-damage and related respiratory chain dysfunction as important factors in the pathogenesis of doxorubicin nephrotoxicity in rats. The changes were tissue-specific, as they were not detectable in skeletal muscle. Humans do not exhibit doxorubicin nephrosis, indicating species specificity, and the causes of the tissue and species specificities are not known.

Why should these rat data be important for human medicine? Respiratory chain failure and limited oxygen supply might exert similar cellular consequences. Insights into the pathogenesis of doxorubicin nephropathy might therefore help in the understanding of renal ischaemia in transplantation research. Furthermore, damage to kidney mitochondria in humans may result from inherited mtDNA-mutations. Acquired forms may also arise from treatment with cisplatin [20] or nucleoside analogue inhibitors of mtDNA replication, such as adefovir and cidofovir [9]. For these conditions, doxorubicin nephrosis may provide an interesting animal model.

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