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REVIEW

The experimental model of nephrotic syndrome induced by Doxorubicin in rodents: an update

Wagner de Fátima Pereira[1] · Gustavo Eustáquio A. Brito-Melo[1] · Cayo Antônio Soares de Almeida[1] · Lázaro Lopes Moreira[1] · Cleiton Willian Cordeiro[1] · Thiago Guimarães Rosa Carvalho[2] · Elvis Cueva Mateo[2] · Ana Cristina Simões e Silva[2]

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Abstract Nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminemia, generalized edema, and hyperlipidemia. It begins by changes in the glomerular filtration barrier, with increased permeability to plasma proteins. It affects all age groups and can progress to end-stage renal disease. NS pathophysiology is still unknown. However, the critical role of the immune system is well recognized. Animal models are useful tools for the investigation of NS. Among different experimental models proposed in the literature, disease induced by Doxorubicin has been considered helpful to the purpose of many studies. The aim of this review article is to describe the animal model of NS induced by the injection of Doxorubicin in rodents, with emphasis on action of the drug, potential mechanisms of renal injury, as well biochemical, histological, and corporal changes obtained with this model.

Keywords Nephrotic syndrome · Idiopathic nephrotic syndrome · Animal model · Doxorubicin · Proteinuria

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✉ Ana Cristina Simões e Silva
acssilva@hotmail.com

[1] Laboratório de Imunologia do Centro Integrado de Pesquisa em Saúde, Universidade Federal do Vale do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil

[2] Unidade de Nefrologia Pediátrica, Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Departamento de Pediatria, Universidade Federal de Minas Gerais, Avenida Alfredo Balena, 190, 2o andar, sala 281, Bairro Santa Efigênia, Belo Horizonte, Minas Gerais Cep: 30130-100, Brazil

Introduction

Nephrotic syndrome (NS) is a common renal disorder characterized by intense proteinuria, hypoalbuminemia, generalized edema, and hyperlipidemia. NS may occur in any age group as a primary renal disease, also known as Idiopathic Nephrotic Syndrome (INS), or secondary to diverse clinical entities such as diabetes, lupus nephritis, HIV nephritis, hepatitis B, and others [1, 2]. In many cases, NS leads to end-stage renal disease (ESRD), requiring renal replacement therapy [1]. Despite advances in INS studies in recent decades, the pathophysiology of this disease remains unknown [2].

Some experimental models of NS have been proposed in the literature [3]. These models have contributed to the understanding of the pathophysiological mechanisms and to the evaluation of new therapeutic approaches to this disease [3]. NS induced by intravenous injection of the chemotherapeutic agent Doxorubicin has served very well for the purpose of several studies [4, 5]. This review article will discuss animal models available to study the NS in rodents, with emphasis on NS induced by Doxorubicin. The description of this model will include action of the drug, methodology of the studies, potential mechanisms of renal injury, and histological, biochemical, and corporal changes following Doxorubicin injection.

Experimental models of NS

Animal models represent a good strategy to overcome the ethical and methodological difficulties of obtaining human material for various scientific investigations [6, 7]. Rats and mice have been used in several studies of NS [3, 4, 8]. Preference for these rodents is justified by lower

maintenance costs, rapid reproduction cycle, good human disease reproducibility, and possibility of genetic manipulation [6]. Furthermore, it is possible to obtain rapid induction of renal disease in rodents and there is availability of reagents for different assays [9].

Nephrotic syndrome can be induced in animal by protamine sulfate injection [10, 11], protein overload [12–14], bacterial antigens administration [8, 15], $CD4^+$ stem cell injections [16], dibasic sodium phosphate injection [17], anti-podocyte immunoglobulins infusion [18], and interleukin 13 overexpression [19]. NS may also be chemically induced by the administration of puromycin aminonucleoside (PAN) [20–22] or by the chemotherapeutic agent Doxorubicin, also known as *Adryamicin*™ [23, 24], a glycoside antibiotics belonging to anthracycline family, obtained from *Streptomyces peucetius var. caesius* [25, 26].

Genetic changes are responsible for many cases of human NS [27–29]. Therefore, some genetically modified animals have been developed for the study of this disease [30–33]. Besides all models obtained by experimental interventions, there is still a rat strain *Buffalo/Mna*, which develops spontaneously NS at 3 months of age [27, 34]. Even though there are several animal models for the study of NS, disease induced by Doxorubicin (*Adryamicin*) administration has been very frequently used in many studies [4–6, 35–38].

Animal model of NS induced by anthracyclines

Anthracyclines are glycosides antibiotics obtained from *Streptomyces peucetius var. caesius* [25, 26]. Doxorubicin is an anthracyclines red–orange crystalline powder, soluble in water and slightly soluble in alcohol [26], used in the treatment of solid tumors. Daunorubicin, another anthracycline, is used in acute myeloid leukemia [39, 40]. Anthracyclines were developed in 1960. The first two anthracyclines agents were Doxorubicin and Daunorubicin. Doxorubicin differs from Daunorubicin only by the binding of a hydroxyl group [41].

The mechanisms of action proposed for the anthracyclines are the interposition between base pairs of nucleic acids with inhibition of DNA and RNA synthesis [25, 40]; DNA alkylation; interference with separation of DNA strands; direct effects on membranes; topoisomerase II inhibition [40]; cellular apoptosis induction [40, 42]; and free radicals synthesis [43–45].

In rats and mice, Doxorubicin is rapidly removed from the plasma after injection and deposited in tissues. The drug is mainly excreted in bile and moderately in urine [46, 47]. Doxorubicin accumulates in kidney, liver, heart, and intestine with greater intensity than Daunorubicin. Plasma levels remain constant and lower after 20 min [47]. At intravenous doses from 5 to 20 mg/kg, about 34 % is excreted in bile and 6–8 % in urine over a period of 10 h [46]. Renal accumulation may be responsible for most of the Doxorubicin nephrotoxicity in comparison to Daunorubicin [5]. Doxorubicin distribution in rats after intravenous injection is shown in Fig. 1.

Similar to human disease, in animal models of NS, initial renal injury occurs during disease induction. Injury may be caused by immune complexes formation with renal antigens or by direct action of toxins [29] and drugs such as Doxorubicin [6]. Many substances produced acute nephrotoxicity leading to acute tubular necrosis. On the other hand, Doxorubicin shows not only mild acute effect, but also significant chronic effects that induce a nephropathy with NS features [35].

In 1970, Sternberg showed structural changes in rats' glomeruli after Daunorubicin injection [48]. Subsequently, studies have pioneered Doxorubicin use to induce renal injury in rats [37, 49–51] and in mice [4, 9, 52]. Even in the 70s, it was described a clinical case of renal damage in humans after chemotherapy with Doxorubicin [53].

Proteinuria is an early feature of NS, both in humans and in animal models, particularly albuminuria. Doxorubicin induces renal injury in rodents similar to those described in patients with focal segmental glomerulonephritis [4, 9, 35, 54]. The absence of immunoglobulins and complement system components in renal tissue of animals at initial stages of lesion indicates a direct toxic effect of the drug on renal tissue [9, 50, 55]. Moreover, mechanical obstruction of blood flow in a kidney immediately before and some minutes after injection of the drug protects this kidney from the lesion, thus confirming acute and local effects of Doxorubicin [55, 56].

Advantages and disadvantages of Doxorubicin-induced NS model

Although there are several animal models for the study of NS, disease induced by Doxorubicin administration has been very frequently used [24, 35, 36]. Among the advantages of using this experimental model, low cost of the drug, lower complexity of management, good reproducibility of the model [6], and ability of the drug to induce renal injury after a single dose [23, 24, 36, 37] can be mentioned. It is also possible to use lower or fractionated doses, for long-term studies [35, 45, 58], as shown in Table 2. The disadvantages of using this animal model are mainly related to administration techniques and tissue toxicity of Doxorubicin, since vascular extravasation of the drug during injection may cause serious tissue damage [26]. The total bioavailability of injected Doxorubicin is an important factor for the induction of NS, since differences

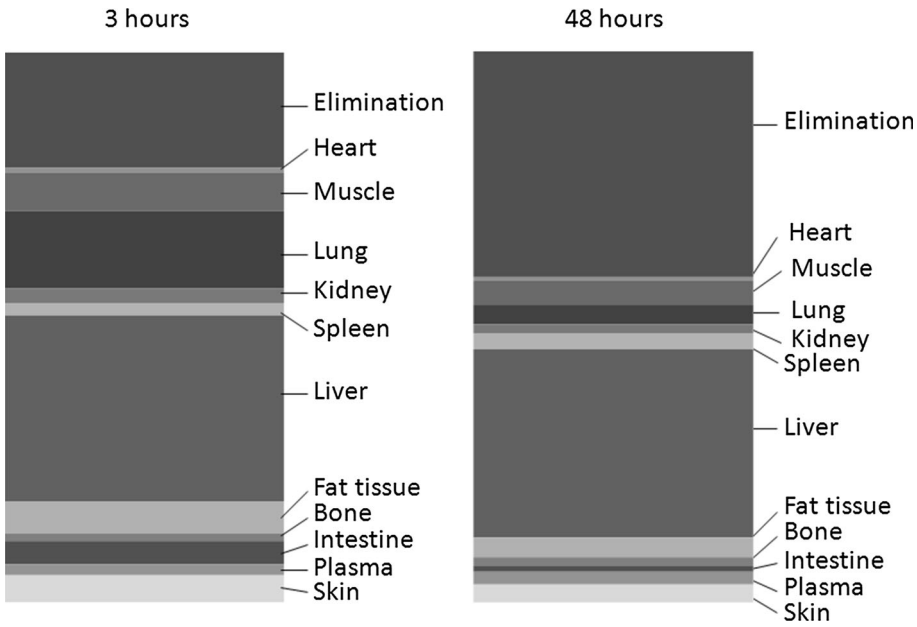

Fig. 1 Body distribution of Doxorubicin in rats at 3 and 48 h after intravenous injection (modified from Wang et al. [4])

of only 0.5 mg/kg in the injected dose can cause breakdown in disease generation, especially in mice [5].

Induction of progressive renal injury with only one injection of Doxorubicin is also an advantage when considering technical difficulties of intravenous injection in rodents and potential tissue injury by extravasation of the drug. Moreover, it has been shown that multiple doses of Doxorubicin were associated with cardiomyopathy and heart failure in rats [59].

Besides Doxorubicin injection, systemic administration of the aminonucleoside antibiotic, Puromycin, can also induce NS, resembling human focal segmental glomerulosclerosis (FSGS). Puromycin is an antibiotic that inhibits protein synthesis. Puromycin can be given by multiple intraperitoneal injections with initial administration of 10 mg/kg followed by 40 mg/kg every 4 weeks or as a single intravenous dose of 50 mg/kg to cause puromycin aminonucleoside-induced nephrosis (PAN). After injection, rats show an early nephrotic phase peaking at 10 days with complete foot process effacement followed by apparent resolution. Between 10 and 13 weeks, progressive lower-level proteinuria develops with early segmental sclerotic lesions leading to well-defined segmental sclerosis at 18 weeks [60].

Both Doxorubicin (or adriamycin) and puromycin are frequently used to induce FSGS because of their strong dose–response effects [61]. These models have been used to study serial micropuncture analysis of a single nephron, while glomerulosclerosis is developing [61]. FSGS treatment studies for which Doxorubicin and Puromycin animal models are used show that the combination of Angiotensin-converting enzyme inhibitors (ACE-I) and Ang II blockers does not have a better effect than ACE-I alone [62]. In addition, they show that MAPK is essential for podocyte injury making p38 MAPK a potential therapeutic target [63] and that vaccination with CCL2 DNA protects against kidney injury after adriamycin injections [64]. Both drugs cause direct toxic damage to the podocytes, increase the permeability of glomerular endothelial cells for larger molecules, and reduce glomerular charge selectivity, which leads to tubulointerstitial injury [5].

Which animal should be used for Doxorubicin-induced NS: rat or mouse?

Rats and mice are generally used to study glomerulopathies. There are advantages and disadvantages of both animal strains, as shown in Table 1. Rat strains used in most studies are *Sprague–Dawley* [48, 58, 65], *Wistar* [23, 55, 66], and *Lewis* [56, 66]. In experiments with mice, BALB/c strain is almost exclusively used [52, 67–72]. Mice of *129/SvJ* strain also develop NS after Doxorubicin administration [6, 68, 73], but this strain has been less commonly used.

Regardless of experimental model and animal strain used, most studies are conducted in males and young adult animals (Table 2). Some studies have shown that ovarian hormones interfere with the development of renal injury in NS model induced by Doxorubicin [58, 74]. Male mice after castration were less susceptible to drug-induced renal injury [58]. Still, some studies with female animals have obtained success in NS induction [52, 55, 67, 71].

Regarding animal age, mice at 6–8 weeks (20–25 g) are usually used [64, 75, 76] or rats at an average age of 8 weeks

Table 1 Advantages and disadvantages in the use of rats and mice as models of renal disease (modified from Pippin et al. [6])

Advantages		Disadvantages	
Rats	Mice	Rats	Mice
Large amount of renal tissue enables various analyzes	Greater availability of genetically modified animals	Limited availability of genetically modified animals	Existence of strains resistant to Doxorubicin
Models of podocyte injury well defined	Short pregnancy time	Greater expenditure of reagents to induce or treat renal disease	Limited glomerular complement activation
Easy isolation of glomeruli free of tubular fragments which provide a lot mRNA and protein	Low cost of acquisition and maintenance	Existence of strains resistant to Doxorubicin	Difficult isolation of glomeruli free of tubular fragments
Highest urine volume available for several analyzes	Increased availability of reagents and markers for immunological studies	Reduced availability of reagents and markers for immunological studies	Few models of podocyte injury
			Minor blood volume available for various analyzes
Bigger animals facilitate surgical procedures			Mice monoclonal antibodies use increase the depth markings
Larger blood volume enables different analyzes			Require greater surgical skills
			Minor venous diameter requires more skill to injection
			Minor urinary volume and higher evaporation during collection

[35, 58] and weighing 150–350 g [60, 77]. Some experiments were performed in younger [55] or older animals [67] according to the aim of the study. In this regard, Hahn et al. [67] investigated age effect on the induction of renal damage by Doxorubicin in mice at 5 and 12 weeks of age and found that injury was more severe in older animals. This difference in toxicity may be related to higher plasma and tissue peaks of the drug and the lowest rate of urinary excretion in older animals [78]. Table 2 summarized several studies of NS induced by Doxorubicin in rodents.

Advantages and disadvantages of each animal strain must be considered in choosing the experimental model. However, the fact that several mice strains are resistant to NS induced by Doxorubicin should also be considered [5, 6, 73]. Therefore, almost all studies with mice have used BALB/c [9, 54, 67–72] or 129/SvJ [6, 68, 73]. This resistance is an autosomal recessive Mendelian inheritance and may be related to increased activity of arginine methyl transferase-7 (Prmt7) protein that inactivates Doxorubicin [68]. Despite resistant to Doxorubicin action, mice of C57BL/6 strain can develop NS after receiving higher doses of the drug [32, 71].

Doxorubicin: administration routes and doses

Doxorubicin hydrochloride should be used intravenously with caution due to the risk of extravasation during injection [26]. In rodents, the tail vein is preferred for injection [67, 68, 79]. Other routes have been used less often, such as the femoral vein [80], intraperitoneal [65], intracardiac [57–81], and penile vein [56] (Table 2). Intraperitoneal route is easier to administrate, whereas intravenous injection provides direct availability of the drug and eliminates the absorption dependence on peritoneal membrane [6], since complete absorption of the drug is important for the induction of kidney damage.

In one of the first studies using Doxorubicin to induce NS in rats, it employs single intravenous injection of 7.5 mg/kg of body weight [37]. In subsequent studies, the doses generally used in rats ranged between 5.0 and 7.5 mg/kg. However, lower and higher doses have also been used ranging from 1.5 mg/kg [56] to 20.0 mg/kg [48] (Table 2). In mice, the doses used to induce NS varied on average between 10.0 and 11.0 mg/kg of body weight. As occurred for rats, lower and higher doses of the drug have also been used according to mice strain, ranging from 5.3 mg/kg in BALBc strain [54] to 25 mg/kg in C57BL/6 mice, a strain partially resistant to the Doxorubicin action [71].

There is general preference for single injection of Doxorubicin. However, lower or fractionated doses appear to be better for long-term studies [35, 45, 58] (Table 2). According to Bertani and co-workers, to induce NS in rats,

Table 2 Summary of studies using rodent model of nephrotic syndrome induced by Doxorubicin

References	Animal strains	Dose (mg/kg)	Administration route	*N*	Interval	Sex	Age or weight	Duration (days)
Studies in mice								
Lee et al. [54]	BALB/c (SCID)	5.3	i.v (tail)	01	–	M	6–8 weeks	42
Wu et al. [75]	BALB/c	9.5	i.v (?)	01	–	M	8 weeks	42
Chen et al. [9]; Zheng et al. [67]; Shui et al. [68]	BALB/c	10.0	i.v (tail)	01	–	F/M	6–8 weeks	18/15/20
Wang et al. [4]; Lenderink et al. [69]	BALB/c	11.0	i.v (tail/tail/?)	01	–	M	20–25 g	42/35
Vielhauer et al. [74]; Heikkila et al. [32]	BALB/c/C57BL/6	13.0	i.v (tail)	02/01	14 days/–	M	22–25 g	42/6
Takiue et al. [71]	(C3H/AnLCsCs)	15.0	i.v (?)	01	–	M	8–10 weeks	56
Chen et al, [52]; Hahn et al. [66]	BALB/c	20.0	i.v (tail)	02/01	?/–	F	5–12 weeks	4/12
Jeansson et al. [70]	C57BL/6	25.0	i.v (tail)	01	–	F	?	6
Silveira et al. [38]	BALB/c/FVBN	10.0	i.v. (tail)	01	7 days	M	20–25 g	28
Studies in Rats								
Lebrecht et al. [45]	Wistar	1.0	i.v (?)	07	Weekly	M	11–48 weeks	210
De Boer et al. [56]	Wistar/Lewis	1.5	i.v (penis)	01	–	M	312 g	84
Kim et al. [65]; Fogo et al. [61]; Okuda et al. [35]; Sakemi et al. [58]	Wistar/Lewis/SD	2.0	i.p/i.v (?/?/?)	06/02	Weekly/20 days	M/F	150–300 g	35/217/196/140
Zoja et al. [95]	Lewis	5.0	i.v (tail)	01	–	M	200–250 g	30
Rangan et al. [76]; Mandelbaum et al. [79]	Wistar	3.5	i.v (tail/femoral)*	01	–	M/F	186–320 g	35/35
Ma et al. [85]	SD	4.0/3.5	i.v (tail)	02	7 days	M	180–220 g	28
Sternberg [48]	SD	5.0 to 20.0	i.v/(?)	01	–	M	200–400 g	28
Abassi et al., [23]; Zima et al. 1998; Wang et al. [78]	Wistar/SD	5.0	i.v (tail/tail/femoral/tail)	01	–	M/F	80–350 g	21/21/42
Muñoz et al. [82]; Wang et al. [105]; Wu et al. [86]	SD	6.0	i.v (tail)	01	–	M	150–250 g	28/49/49
Rangan et al. [94]	Wistar	7.0	?	01	–	M	222–296 g	28
Buranakarl et al. [24]; Weening and Rennke [55]	SD/Wistar	7.5	i.v (?/?)	01	–	M/F	150–350 g	14/55
Bricio et al. [36]; Bertani et al. [37]; Zheng et al. [104]; Guo et al. [103]	SD	7.5	i.v (tail)	01	–	M	180–350 g	21/30/35/56
Bertani et al. [50]	SD	7.5	i.v (?)**	01	–	M	280–300 g	60
Van Goor et al. [83]	Wistar	8.0	i.v (?)	01	–	M	12 weeks	8

i.v intravenous, *i.p* intraperitoneal, *fem* femoral, ? unknown, *N* number of injections, *interval* time between injections, *M* male, *F* female, age (in weeks) or weight (in grams), *duration* total duration of the experiment in days, *SD* sprague–dawley

* i.v injection associated to unilateral nephrectomy

** i.v injection associated to unilateral renal clipping

doses of 3.0, 5.0, or 7.5 mg/kg were able to induce proteinuria, which persists for several months, but lower doses resulted in less pronounced renal damage [50]. In the study of Vielhauer and co-workers, NS was induced in mice by two injections of 13.0 mg/kg with an interval of 14 days between injections [75]. According to these authors, injections of 11.0 mg/kg of Doxorubicin in mice induced only transient proteinuria during 4–6 weeks [75].

Injection techniques

Peripheral veins represent the main route for Doxorubicin injection (Table 2). Animal restraint facilitates the injection procedure. For best vein location in rats' tail and, particularly, in mice, the use of heating boxes can promote vasodilatation before venous injection [5]. In general, intravenous injection is performed in awake animals [5, 37, 54], but some authors prefer to prior anesthetize the animal [24, 71, 80].

One option to reduce Doxorubicin injected dose and consequently side effects is the use of techniques that potentiate drug effect. This way, De Boer and co-workers obstructed blood flow in one kidney (renal artery clamping) during drug injection [56]. This maneuver allowed the use of only 1.5 mg/kg in rats [56]. Blood flow obstruction technique of the contralateral kidney was also used, along with injections of 3.5 mg/kg [77] and 7.5 mg/kg [50] of Doxorubicin in rats.

In order to overcome technical difficulties of intravenous injection, Rangan and co-workers proposed the use of intracardiac injection in pre-anesthetized animals [57].

Since Doxorubicin is a chemotherapeutic agent only for hospital use, biosafety precautions are necessary for the manipulation of this drug such as use of disposables gloves, masks, goggles, and appropriate clothing. The solution should be carefully handled. In case of contact with skin or mucosa, the area should be washed thoroughly with soap and water [26].

Reproducibility of NS induced by Doxorubicin

The animal model of NS induced by Doxorubicin has a good reproducibility [4–6, 35, 37]. For this reason, the model has been frequently used [23, 36, 38, 54–56]. In addition, studies using rodents model of NS induced by Doxorubicin have similarities in histological findings of renal injury. However, there is a chronological variability between studies, probably due to the differences in animal strain and Doxorubicin dose [45, 82].

Most studies are conducted in males and young adult animals (Table 2). A protective role of ovarian hormones on renal injury induced by Doxorubicin [58, 74] may probably interfere with the reproducibility of this model in female animals. Concerning age, renal injury by Doxorubicin is more severe in older animals [67] probably due to higher plasma and tissue peaks of the drug and lowest rate of urinary excretion in these animals [78].

The reproducibility of the NS model induced by Doxorubicin is mainly related to the dose of drug used, since small variations in bioavailability of injected Doxorubicin may cause failure in disease generation [5]. It is possible to induce NS after single injection of Doxorubicin [23, 24, 36, 37] although some researchers prefer intermittent doses [45, 65], as shown in Table 2.

As a consequence of the narrow therapeutic index of Doxorubicin, small differences in injected doses can cause large variations in intensity of renal damage [5]. Therefore, Pippin and co-workers recommend a pilot study to determine the dose of Doxorubicin and to confirm induction of NS in rodents [6].

Thus, the reproducibility of animal model of NS induced by Doxorubicin appears to be related to dose of the drug and duration of the experiment. Since lower doses of Doxorubicin cause milder renal lesions, it is necessary to consider that injury may take longer to reach the typical histological pattern of the disease. In this regard, each study should follow specific methodology based on proposed goals, but always considering duration of the experiment. Additionally, a pilot study should be performed to determine optimal experimental design.

Biochemical and corporal changes in rodents with NS induced by Doxorubicin

Biochemical changes

Proteinuria is the major characteristic of NS and serves to confirm effectiveness of the animal model. Albumin was not only detected in urine between 5 and 7 days after Doxorubicin injection [4, 6, 37] or a little before, but may also occur urinary loss of immunoglobulins, especially IgG [9, 52]. Besides proteinuria, there are hypoalbuminemia, high levels of serum creatinine [4, 54], hematuria, reduction in creatinine clearance [9], and increase in albumin/creatinine ratio in spot urine [52, 71]. On the other hand, some authors did not find significant changes in serum levels of albumin [9, 35] or urine and plasma creatinine [35, 37, 52].

Our results showed that rats with NS induced by doxorubicin also have severe dyslipidemia (Table 3). Accordingly, other studies reported high serum cholesterol levels [24, 83], apolipoproteins, and triglycerides [84, 85], highlighting the role of dyslipidemia in NS pathogenesis in this animal model [56, 84, 85]. A positive correlation was

Table 3 Biochemical and corporal alterations in rats with nephropathy induced by doxorubicin

	Control Group	Doxorubicin group			
	Mean (SEM)	T-07 Mean (SEM)	T-14 Mean (SEM)	T-21 Mean (SEM)	T-28 Mean (SEM)
Total cholesterol (mg/dL)	66.6 (7.6)	97.6 (12.4)	350.1 (33.1)*	544.3 (73.9)*	513.7 (96.6)*
Triglycerides (mg/dL)	72.9 (6.5)	41.1 (7.4)*	569.4 (164.5)*	686.9 (54.8)*	574.3 (122.9)*
Proteinuria (mg/L)	33.2 (3.1)	44.5 (5.3)	45.3 (5.5)	58.7 (6.8)*	71.5 (3.4)*
Food consumption (mg/day)	30.2 (0.7)	20.5 (3.2)*	22.2 (2.2)*	22.5 (1.6)*	26.9 (2.3)
Kidney weight (mg)**	9.3 (0.4)	9.45 (0.3)	10.7 (0.5)*	12.8 (0.4)*	13.8 (0.8)*

Kidney weight was corrected for body weight (personal archive)

* $p < 0.05$, *T* time in days

** *SEM* standard error of mean

detected between plasma levels of cholesterol and albumin loss in urine [85]. Furthermore, glomerular sclerosis rate presented higher correlation with plasma levels of cholesterol than with proteinuria [56]. In addition to these classical biochemical parameters, blood urea nitrogen (BUN) [35, 37, 86], plasma and urinary sodium [3, 23, 24] and potassium levels [24], alanine aminotransferase, uric acid [86], and cystatin C (CyC) [87] have also been measured in this animal model.

Hemostatic changes were also reported in Doxorubicin-induced NS, with an increased tendency to blood clotting [88, 89]. This characteristic is also common to patients with idiopathic NS, and is associated with high incidence of thromboembolic events [90–92].

Corporal changes

In general, there are weight loss reports in this NS animal model at first weeks after Doxorubicin injection [35, 71, 77, 82]. Later there is weight gain in animals with NS, but so much slower than in control animals [4, 54, 83]. According to Mihailovic-Stanojevic and co-workers, weight loss may be related to dose of the drug and animal strain used, not being, therefore, a constant finding [93]. The weight loss may be also related to side effects of the drug, as discussed later.

In relation to internal organs, although it was reported a progressive reduction in renal weight of animals injected with Doxorubicin [54], majority of the studies, including data from our group, found an increase in this organ weight [4, 35, 82]. Table 3 and Fig. 2 show our results of biochemical and physical changes in rats with NS induced by Doxorubicin. Increased kidney weight in Doxorubicin-injected rats is probably due to local edema and renal tissue fibrosis [4, 35]. Other internal organs such as heart, lung, and liver have been poorly investigated in this model. According to Zheng and co-workers, these organs are not affected by the doses of Doxorubicin usually used to induce NS in mice for a period of 15 days [68]. Other changes reported in this animal model of NS were ascites [37, 94], pulmonary congestion, pleural effusion [94], and hypertension [35, 80].

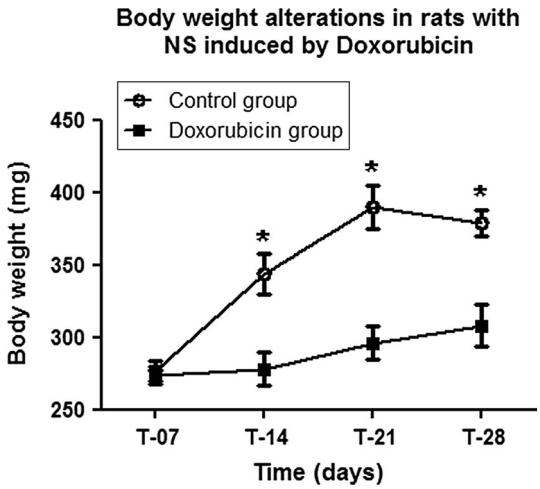

Fig. 2 Body weight alterations in rats with nephropathy induced by doxorubicin, *$p < 0.05$, *T* time in days, *NS* nephrotic syndrome (personal archive)

Renal histology in rodents with NS induced by Doxorubicin

In NS induced by Doxorubicin, tubule-interstitial lesions are minimal on day 7, moderate on day 14, and severe between 21 and 28 days after drug injection [95]. Renal histology has been similar to what is commonly seen in patients with NS [6, 54, 79].

By light microscopy, rats and mice with doxorubicin nephropathy showed renal tissue alterations like interstitial inflammatory infiltrate, tubular hypertrophy, increased collagen deposition (fibrosis), thickening of the basement membrane, Bowman's space enlargement, and reduced

number of glomerular cells (atrophy), as shown in Fig. 3. By electron microscopy, the main characteristic of rats and mice with experimental NS is the wide effacement of foot process [9, 35, 37, 54, 96]. Due to proteinuria, renal damage normally begins with intratubular crystals formation [50]. With disease progression, it detected reduction in cellularity, atrophy and glomeruli tuft collapse, in addition to mesangial expansion [4, 9], glomerular adhesions of capillary tuft to Bowman's capsule (synechiae) and crescent-like lesions [96]. At the late phase, it can be observed glomerular sclerosis and vacuolation, pronounced interstitial fibrosis [35], and tubular atrophy [4, 54], with reduction in tubular cells size, loss of brush border, and cellular vacuolation [4, 9].

Inflammatory response contributes to renal injury, since persistent proteinuria promotes continuous stimulus for tubular cells, which secrete chemokines and cytokines [2, 97]. Albumin excess in renal tubules increased the monocytes chemoattractant protein-1 (MCP-1/CCL2) expression, a cytokine responsible for macrophage chemotaxis. However, in the absence of inflammatory infiltrate, proteinuria *per se* was not sufficient to induce renal interstitial fibrosis [75]. Furthermore, changes in distribution of nephrin protein, CD2AP and ZO-1 were associated with increased podocyte expression of CD80, a co-stimulatory molecule generally present in cells related to immune response [15].

Renal immunohistochemistry of animals with lesions induced by Doxorubicin exhibits, at early stages, interstitial accumulation of macrophages [4, 54], with subsequent decline, and an increase in CD4+ and CD8+ T lymphocytes [4]. Infiltration of macrophages, an important component of innate immunity, is one of the most striking and constant features of chronic renal injury, and the degree of mononuclear cell infiltrate is predictive of subsequent disease progression [98]. Macrophages can contribute extensively to tissue damage and progressive renal failure via a number of mechanisms, including their production of proinflammatory cytokines and their T cell stimulatory capacity [98, 99]. Tissue factors determine the phenotype of monocytes/macrophages recruited into the renal tissue, whereas the profile of locally released cytokines regulates the differentiation of mononuclear cells. Th1-type cytokines induce differentiation into classical macrophages, denominated M-1, that produce cytotoxic and proinflammatory cytokines, while Th2-type cytokines induce alternative macrophages, denominated M-2, responsible for the synthesis of anti-inflammatory cytokines [100, 101]. More recently, it is shown that M-2 macrophages originating from the action of IL-10 and TGF-b also inhibited M-1 macrophages and TCD4 and TCD8 lymphocytes. In addition, this cell line also induced the differentiation of regulatory T cells at the renal interstitium of rats with NS induced by doxorubicin, with consequent improvement of the disease [100]. Accumulation of inflammatory cells occurs only in the interstitium. In general, B lymphocytes have not been detected [4]. Tissue changes are also characterized by increased expression of type IV collagen, fibronectin, and laminin in glomerular tuft and Bowmans's capsule [9].

By means of electron microscopy and immunohistochemistry, it has been possible to detect podocytes' changes [37, 52, 96]. After first hours of Doxorubicin injection, partial loss of podocytes architecture is already observed [37, 52]. Significant decrease in the number of podocytes, by apoptosis, is already observed 3 days after Doxorubicin injection [91]. At the late phase of NS induction (after 21 days), generalized fusion of podocytes and intracytoplasmic vesicles can be observed [9, 35, 37]. Renal injury progresses to complete loss of podal process [54] and formation of vacuoles containing fibrin [35]. Due to Doxorubicin-induced podocyte injury, glomerular adhesions of capillary tuft to Bowman's capsule (synechiae) were also observed, starting from 16 days, and followed by crescent-like lesions at 30 days [96].

Loss of selectivity of glomerular filtration barrier causes intraglomerular accumulation of macromolecules with subsequent mesangial matrix deposition and glomerular sclerosis [35]. It has been suggested that early urinary albumin excretion is due to sialoproteins loss during the first hours after Doxorubicin injection [37]. Further studies have demonstrated that electrical changes in glomerular filtration barrier were subsequent to structural lesions [9, 55]. More recently, proteinuria was associated with a reduction in thickness of glomerular endothelium glycocalyx layer, inducing changes in both electrical selectivity and size specificity of the glomerular filtration barrier [71].

Focal segmental glomerulosclerosis (FSGS) resulted from most rat models of nephron injury despite original etiology. Podocytes injury plays a major role in FSGS, since loss of podocytes leads to capillary tuft adhesions to Bowman's capsule, followed by altered filtration and ultimately nephron degeneration and fibrosis [98]. According to Kriz [97], major mechanisms contributing to the progression of segmental glomerular injury to global sclerosis, tubular degeneration, and local interstitial fibrosis probably are misdirected filtration and filtrate spreading [98].

Recently, in Doxorubicin-induced lesions, a sequence of events leading to glomerulosclerosis starting by early podocyte loss, abnormal podocyte migration, proliferation of glomerular parietal epithelial progenitor cells, and formation of hyperplastic lesions (synechiae and crescents) was described [96, 102]. Podocyte injury with consequent proteinuria depends on the beta-catenin activity [32], which is induced by endothelin-A receptor (ETAR) activation, and resulting in increased beta-arrestin-1, in podocytes

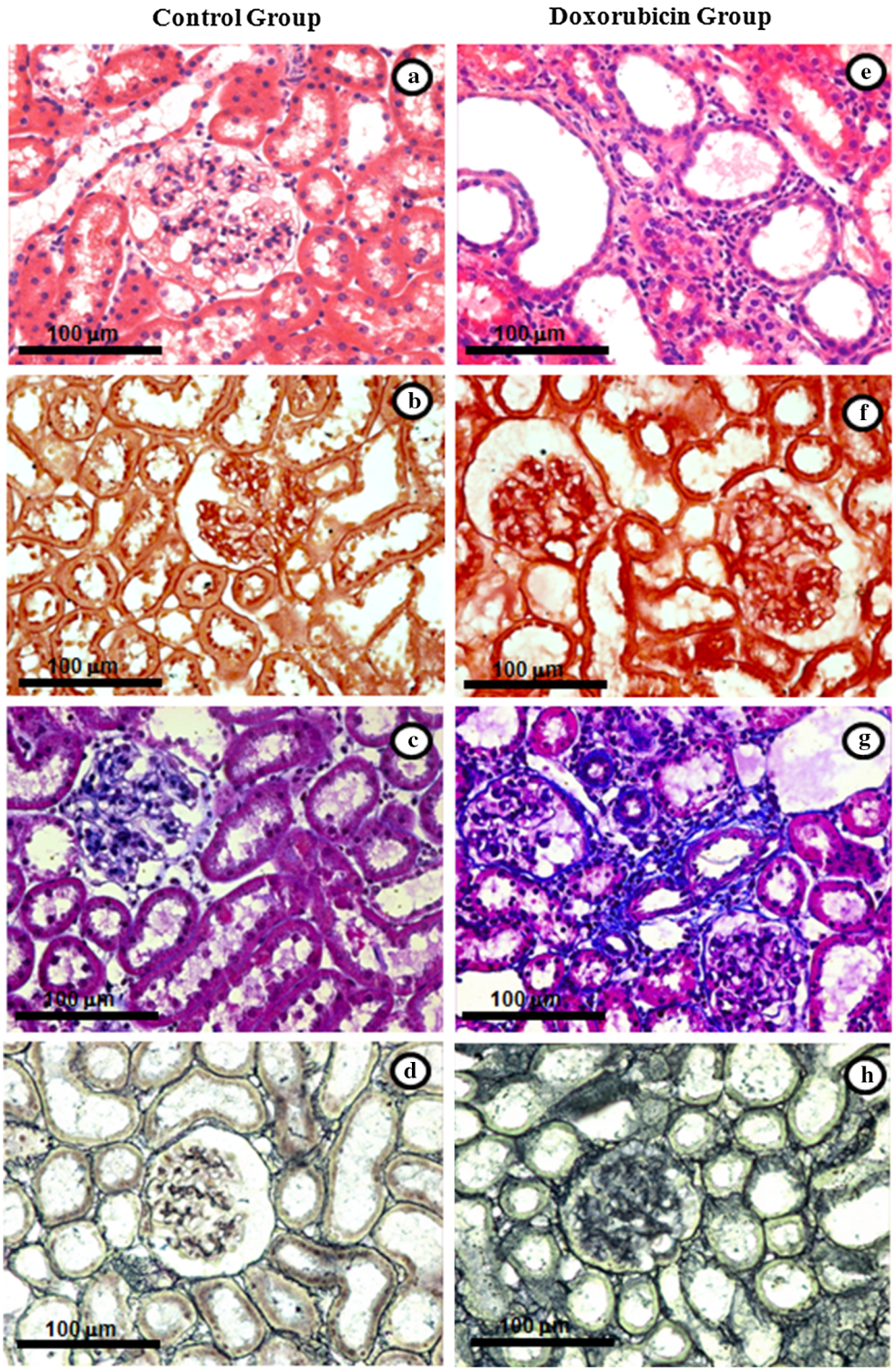

Fig. 3 Renal histology in rats with Doxorubicin-induced nephropathy (personal archive). Control group (**a**–**d**) showing normal renal tissue; Doxorubicin group (**e**–**h**) exhibiting interstitial inflammatory infiltrate, tubular hypertrophy, interstitial fibrosis, and thickening of the basement membrane. Representative microphotographs stained by hematoxylin eosin (*HE* **a** and **e**), periodic acid of Schiff (*PAS* **b** and **f**), Masson's trichrome (**c** and **g**) and Ammoniacal Silver (**d** and **h**)

[102]. Beta-catenin is a protein that sets P-cadherin to cytoskeleton in podocyte slit diaphragm [32]. Proteinuria is also related to reduced nephrin, podocin [19, 94], and synaptopodin expression [103]. Some therapies against proteinuria in animal model of NS induced by Doxorubicin were related to the maintenance of these structural proteins

[103–105]. Therefore, it is clear that podocytes are extremely important for glomerular architecture and function [6, 106, 107].

Figure 4 illustrates podocyte and inflammatory changes in Doxorubicin-induced nephropathy.

Inflammation profile in Doxorubicin-induced nephropathy

The susceptibility to renal injury by doxorubicin may be related to T-helper response, since C57BL/6 mice, which present a predominance of Th1 response, are resistant to nephropathy induced by doxorubicin, while BALB/c mice, in which Th2 response predominates, are susceptible to the disease [5]. Recently, high concentrations of IL-4 and of eoxtaxin/CCL11, both mediators related to Th2 response, were detected in renal tissue of animals with NS induced by doxorubicin [108].

Several studies also showed the predominance of Th2 response in human NS. As an example, Lama et al. [109] detected high levels of IL-2 and of IFN-gama in children with steroid-sensitive NS. However, according to Araya et al., there is no compelling evidence to define a predominance of Th2 response in human NS [110]. Some types of human glomerulonephritis, including crescentic and membranoproliferative glomerulonephritis, have a predominantly Th1 immune response pattern, while others, such as membranous nephropathy, IgA nephropathy and minimal change NS, show a predominantly Th2 response [2]. Some studies have considered that primary NS (including FSGS) presents an imbalance between Th1/Th2 responses [110], with a trend toward greater Th2 response [108, 111].

Other animal strains and experimental models of NS showed varied patterns of immune response. The Buffalo/Mna rat, an animal strain with spontaneous NS, exhibited early changes in the balance between Th1 and Th2 with predominance of Th2 (IL-10 and IL-13) and inhibition of Th1 (IL-2 and IFN-gama) before the onset of proteinuria [34, 112]. Mice transfected with IL-13 developed NS with overexpression of receptors for IL-4 and IL-13 in glomeruli [113]. Serum levels of IL-13 were correlated with the glomerular expression of B7-1 (CD80) in these animals [113]. CD80 is a co-stimulatory molecule generally present on the surface of B lymphocytes and of antigen-presenting cells that is associated with decreased apoptosis and induction of proliferation of TCD4 cells [114].

New therapeutic perspectives for Doxorubicin-induced nephropathy

Renin Angiotensin System (RAS) and Kallikrein-Kinin System (KKS) have been recently evaluated as potential targets for the treatment of Doxorubicin-induced nephropathy. Renoprotective and anti-proteinuric effects of ACE inhibitors and angiotensin type 1 receptor antagonists are well known [115]. However, recent studies also showed important renal effects for the counterregulatory RAS axis

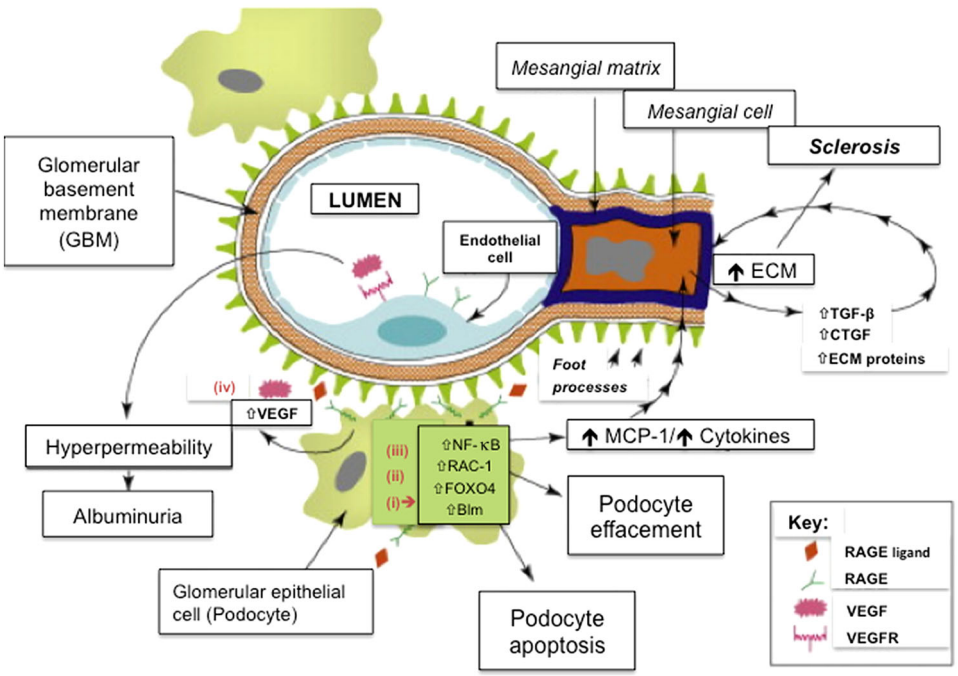

Fig. 4 Schematic view of podocyte and inflammatory changes in Doxorubicin-induced nephropathy. *ECM* extracellular matrix, *TGF-β* transforming growth factor beta, *CTGF* connective tissue growth factor, *NF-κB* nuclear factor kappa B, *RAC-1* Ras-related C3 botulinum toxin substrate 1, *FOXO4* forkhead box protein O4, *RAGE* receptor for advanced glycation end products, *VEGF* vascular endothelium growth factor, *VEGFR* vascular endothelium growth factor receptor

formed by the enzyme homologue to ACE (ACE2), the mediator Angiotensin-(1-7) and its receptor, Mas receptor (for review, see reference [116]). In this regard, Silveira et al. [38] showed that both antagonism of angiotensin type 1 receptor with losartan and stimuli of Mas receptor with AVE0991 exerted beneficial effects in Doxorubicin-induced nephropathy. Both compounds attenuated biochemical changes and reduced tissue inflammation and fibrosis [38]. In addition, beneficial effects of losartan were absent in mice with genetic deletion of Mas receptor, suggesting a critical role of Angiotensin-(1-7) in renoprotective actions of AT1 antagonists [38].

Concerning the role of KKS, Pereira and co-workers used knockout animals and kinin receptor antagonists to unveil the role of kinin receptor 2 (B2RBK) in Doxorubicin-induced nephropathy [117]. The disease was induced in wild-type and B2RBK-knockout mice, using a single intravenous injection of Doxorubicin. In wild-type mice, blockage of the receptor with antagonists prevented FSGS when administered soon after disease induction and reversed signs of disease—including proteinuria—when administered during the later stages. Treatment with the B2RBK antagonist also downregulated fibrotic and inflammatory proteins that are associated with renal lesions. The authors report that FSGS is exacerbated in B2RBK-knockout mice, and, consistent with previous studies, higher B1RBK receptor expression was observed in these animals. Interestingly, treatment of B2RBK-knockout mice with a B1RBK antagonist ameliorated disease. The results reported indicate that kinin receptors are potentially important targets in FSGS, because their blockage with antagonists can restore podocyte architecture and protect against clinical symptoms, such as proteinuria. Although this work focused primarily on B2BRK, the data suggest cross-talk between the two receptors, which should be explored further in future studies. The understanding of molecular mechanisms provided by experimental models could help in the development of new therapeutic approaches against FSGS [117].

Side effects of Doxorubicin use in rodents

The first problem is the risk of Doxorubicin extravasation during injection. Due to direct toxicity to tissues, Doxorubicin can cause severe necrosis [26] that makes the model impracticable [57]. Figure 3 shows tissue damage produced by Doxorubicin extravasation.

Doxorubicin toxicity is more pronounced in highly proliferative tissues, being bone marrow, digestive tract, and gonadal tissues the most affected sites [26]. Studies in humans showed cardiac abnormalities induced by anthracyclines [40, 118] and related to the Doxorubicinol metabolite [118]. Congestive heart failure in humans has been related to the total dose of 550 mg/m^2 [26], which is significantly higher than dose used in animal models of renal injury. For example, in young adult rats (200–300 grams), it has been used an average dose of 7.5 mg/kg of body weight, which corresponds to approximately 105 mg/m^2 [37].

In studies aiming to assess Doxorubicin cardiotoxicity in mice, the dose of 15.0 mg/kg was used [59, 119]. This dose induced early oxidative changes [59] and congestive heart failure [119]. There are also reports of cardiac changes in rats with the administration of only 6.0 mg/kg of the drug intravenously [120] or 10 mg/kg intraperitoneal [121], which were not related to Doxorubicinol metabolite [120]. However, cardiac alterations generally have not been the focus of evaluation in studies of NS induced by Doxorubicin in rodents.

Doxorubicin causes anorexia at the first 24 h after injection, which can last for several days. There is also the possibility of oral mucosa lesions [26]. In addition, as previously described [37, 84, 85] and shown by our results (Fig. 2), a side effect rather described in rodents is reduction of weight gain during the first weeks after drug injection. According to the literature [24, 72], this alteration has been associated with reduction in food intake or inhibition of protein synthesis [59]. The use of intraperitoneal injection with glucose and electrolytes solutions can prevent weight loss in animals treated with Doxorubicin [72].

Concluding remarks

Animal models have provided advances in research on renal diseases, including NS. NS induced by Doxorubicin in rodents is broadly used in studies with different approaches and aims. In order to choose the animal model of NS, one should consider the strain susceptibility, as well as commercial availability of reagents and biological markers for measured parameters.

Considering technical difficulties and potential complications during Doxorubicin application, there seems to be a general trend to use single dose injected into the tail vein. Despite the variety of methods employed, especially in relation to disease induction and Doxorubicin dose, renal lesions and biochemical alterations in this model are very similar to those of human FSGS. Therefore, NS induced by Doxorubicin is a quite feasible model for research.

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Conflict of interest None declared.

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