

### **12.1 INTRODUCTION**

Cyclosporine A (CsA) is a potent immunosuppressive drug that selectively inhibits transcription of interleukin-2 and several other cytokines, mainly in T-helper lymphocytes (Borel et al., 1977). Its introduction has dramatically improved the outcome of solid organ transplantation and CsA is also used with increasing frequency for the treatment of autoimmune diseases. However, the long-term treatment with CsA in solid organ transplantation has been shown to be associated with the development of hypertension and nephrotoxicity. The pathophysiology of CsA-induced acute nephrotoxicity is not fully understood but glomerular hypoperfusion following CsA administration has been shown [Murray et al., 1985, Sullivan et al., 1985]. Several mechanisms, including endothelin-mediated systemic and renal vasoconstriction, sodium retension, impaired vasodilatation secondary to reduction in nitric oxide (NO), and altered cytosolic calcium translocation have been proposed to underline CsA-induced hypertension [Pichler et al., 1995, Van der et al., 1995]. Many drugs can injure the kidneys, but they cause renal injury via only a few common mechanisms. Many patients who develop renal injury after drug exposure have identifiable risk factors that could be modified or that should preclude the use of these drugs in the first place.

#### **12.2 FOUR DRUG-RELATED RENAL SYNDROMES**

Drugs can cause four major renal syndromes:

- Acute renal failure
- Nephrotic syndrome
- Renal tubular dysfunction with renal potassium wasting and acidosis.
- Chronic renal failure.

## **12.2.1 ACUTE RENAL FAILURE**

Acute renal failure is a rapid decrease in renal function associated with alterations in urine volume, azotemia, and derangement of biochemical homeostasis. An increase of creatinine by more than 0.5 mg/dL above a known baseline or a value higher than 1.5 mg/dL is generally considered

significant. In severity, it can range from asymptomatic azotemia to severe acute renal failure that requires dialysis.

Drugs can cause acute renal failure by three mechanisms:

- Prerenal
- Intrinsic
- Obstructive.

#### 12.2.1.1 Prerenal acute renal failure

Some drugs can cause acute renal failure by reducing the volume or pressure or both of blood delivered to the kidney; the resulting renal failure is therefore termed prerenal."

**Drugs implicated** include diuretics, highosmolar radiocontrast media,35 the immunosuppressive drugs cyclosporine and tacrolimus, nonsteroidal anti-inflammatory drugs (NSAIDs), interleukin-2, and angiotensinconverting enzyme (ACE) inhibitors.

#### 12.2.1.2 Three types of intrinsic acute renal failure

Drug-induced intrinsic acute renal failure falls

into three types:

- Acute tubular necrosis
- Acute interstitial nephritis
- Thrombotic microangiopathy.

#### A. Drug-induced acute tubular necrosis Drugs implicated.

Most of the drugs that can cause acute tubular necrosis are excreted by the kidney; these include aminoglycoside antibiotics, amphotericin B, cisplatin (causing renal failure in up to 25% of patients after a single dose), radiocontrast agents, pentamidine, cocaine, and intravenous immunoglobulins. Acute tubular necrosis can also be induced by statin drugs given in combination with immunosuppressive agents such as cyclosporine; clinical features of rhabdomyolysis such as myalgias, elevated creatine kinase levels, and myoglobinuria may be seen. Similarly, the combination of cisplatin and aminoglycosides may be more nephrotoxic than either agent alone.

For most drugs that cause acute tubular necrosis, the target is predominantly either the early or late segments of the proximal tubule, though other segments may suffer variable injury. Perhaps the most critical determinant of nephrotoxicity is the extent of drug or toxin uptake within cellular targets in the kidney.

#### B. Acute allergic interstitial nephritis

Acute interstitial nephritis presents with systemic manifestations of a hypersensitivity reaction such as fever, rash, and arthralgias. The onset after drug exposure ranges from 3 to 5 days with a second exposure, to as long as several weeks with a first exposure. However, the latency period may be as short as 1 day with rifampicin, or as long as 18 months with an NSAIDs.

**Drugs implicated** include penicillins, cephalosporins, cocaine, sulfonamides, NSAIDs (especially fenoprofen, but so far not cyclo-oxygenase [COX-2] inhibitors), diuretics, lithium, ranitidine, omeprazole, captopril, lithium, phenytoin, valproic acid, amphotericin B, streptokinase, 5-aminosalicylates, allopurinol, rifampin, and some Chinese herbs.

#### C. Thrombotic microangiopathy

Thrombotic microangiopathy can cause severe acute renal failure. In general, the pathologic hallmark of thrombotic microangiopathy is hyaline thrombi in the microvasculature of many organs. Changes in the kidney include afferent arteriolar and glomerular thrombosis and thickening of the glomerular capillary wall on electron microscopy due to the deposition of fibrin-like materials.

**Drugs implicated** include cyclosporine (Remuzzi and Ruggenenti, 1995 Pham et al., 2000), tacrolimus, chemotherapeutic agents (eg, mitomycin C, bleomycin, cisplatin), ticlopidine, clopidogrel, estrogen-containing oral contraceptives, quinine, and cocaine. The incidence of thrombotic microangiopathy is higher with the combination of cisplatin and bleomycin than with cisplatin alone.

#### **12.2.2 NEPHROTIC SYNDROME**

The nephrotic syndrome is due to glomerular dysfunction and marked by heavy proteinuria.

**Drugs implicated** include gold, NSAIDs, penicillamine, interferon, and captopril.

## **12.2.3 CHRONIC RENAL INSUFFICIENCY**

Chronic renal insufficiency caused by drugs generally presents as tubulointerstitial disease. This form of injury may be caused by chronic analgesic abuse, lithium, cisplatin, cyclosporine, nitrosourea, and Chinese herbs.

Amphotericin B is still the gold standard therapy for life-threatening systemic fungal sepsis, but many patients develop acute renal failure associated with urinary magnesium and potassium wasting, hypokalemia, renal tubular acidosis, and polyuria due to nephrogenic diabetes insipidus. The nephrotoxicity is related to direct tubular damage by deoxycholate used as a solubilizing agent for amphotericin B. The renal toxicity is reversible on cessation of therapy.

Liposomal amphotericin B is as effective as conventional amphotericin B in empirical therapy of fungal infections in febrile neutropenic patients, and it is associated with less infusion-related toxicity and less nephrotoxicity.

#### **12.3 EXPERIMENTAL PROCEDURE**

#### Animals

Albino male rats of Wistar strain weighing 150-200gms were used for the study. The animals were fed *ad libitum* with standard pellet diet and had free access to water.

# 12.3.1 NEPHROTOXICITY STUDY OF CYCLOSPORINE AND ITS LIPOSOMAL FORMULATIONS

The rats were divided into five groups of four animals each. Group I served as control. Group II received cyclosporine (20mg/kg, i.v.) for 14 days. Groups III, IV, V received liposomal formulations (CPL, CL and CNL) containing cyclosporine (20mg/kg, i.v.) respectively for 14 days. After 24 hours of the last dose of cyclosporine, blood was collected and serum was

separated for estimations of creatinine, urea, uric acid and blood urea nitrogen (BUN). These values were determined with kits of Span Diagnostics (India) Pvt. Ltd. The animals were then sacrificed and the kidney was examined macroscopically, and cross sections of kidney were collected in 5% formalin in saline for histopathological evaluation. The tissues collected in formalin at necropsy were processed, embedded in paraffin, sectioned at 5 microns, and stained with hematoxylin and eosin. Histopathologic examinations of the tissue sections were conducted by a pathologist and peer reviewed.

#### **12.4 RESULTS AND DISCUSSION**

#### **12.4.1 NEPHROTOXICITY STUDIES**

Administration of cyclosporine (group II) resulted in a significant (p<0.001) elevation in serum creatinine, urea, uric acid and BUN levels, the markers of renal injury, as compared to control group (group I). The administration of liposomal formulations (CPL, CL, CNL) showed significant decrease in these levels (table 12.1). The nephrotoxicity of cyclosporine, characterized by the elevation of serum creatinine, urea, uric acid and BUN, was also reversed to a significant extent by the liposomal formulations containing cyclosporine (CPL, CL, and CNL).

Rats dosed iv with cyclosporine (20mg/kg/day) or its liposomal formulations containing cyclosporine (20mg/kg/day) exhibited no drugrelated histopathology in kidney at 24 h. However, in the 14<sup>th</sup> day cyclosporine-treated kidneys, necrosis occurred in the tubular epithelial cells located in the outer stripe of the outer medulla (figure 12.2), necrosis was also accompanied by scattered apoptosis, as evidenced by diminished cells when compared to the control rat kidney (figure 12.1). Liposomal treatment for 15 days had shown comparatively less necrosis on kidney than free drug as shown in figures 12.3 and 12.4.

Groups	Creatinine	Urea	Uric acid	BUN
	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
Group I	0.56 ± 0.02	29.19±0.05	0.67±0.04	13.46±0.025
(Control)	0.00 ± 0.02	29.19-0.03	0.07±0.04	10.40±0.020
Group II	1.2±0.04***	52.23±3.33***	1.93±0.09***	24.39±1.56***
(CsA)	1.20.04	02.20±0.00	1.95±0.09	24.09±1.00
Group III	0.45± 0.03	24.26±2.52	0.66±0.04	11.33±1.18
(CPL)				
Group IV	0.45 ± 0.02	33.47±0.917	0.63±0.08	15.63±0.43
(CNL)				
Group V	0.55± 0.01	31.03±0.64	0.68±0.01	14.31±0.29
(CL)	0.00-0.01			

# Table 12.1 Nephrotoxicity study of Cyclosporine and its liposomes onthe serum levels of creatinine, urea, uric acid and BUN

\*\*\*P < 0.001- extremely significant

# Comparison of groups: Group I and Group II, Group II and Group III, Group II and Group IV, Group II and Group V.

Light micrographs (10X) comparing kidney from control male rats with kidney from male rats treated with cyclosporine and liposomal cyclosporine.

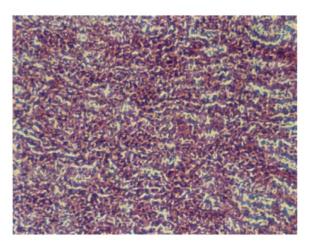


Figure 12.1 Kidney from control male rat showing normal morphology of epithelial cells located in the outer stripe of the outer medulla.

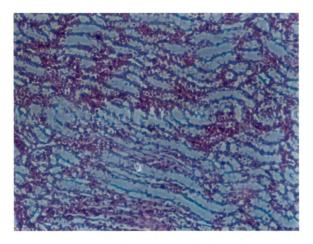


Figure 12.2 Kidney from male rat treated with 20mg/kg/day cyclosporine for 15 days.

Nephrotoxicity Study of Cyclosporine Liposomes

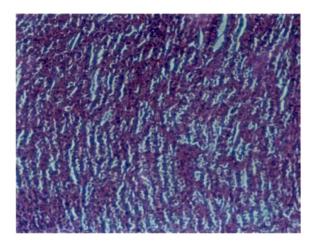


Figure 12.3 Kidney from male rat treated with 20mg/kg/day CPL for 15 days

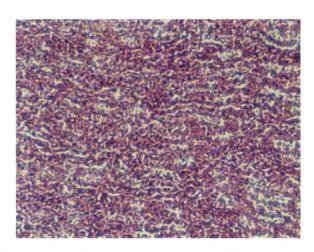


Figure 12.4 Kidney from male rat treated with 20mg/kg/day CNL for 15 days

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