CHAPTER II

THEORIES AND RELATED WORKS

Pathological Studies in Humans

A. Light Microscopic Studies

The characteristic of tubular necrosis found by most investigators includes tubular dilatation, tubular epithelial flattening, and intratubular casts. In some cases, absence of tubular nuclei and fragmentation of tubular basement membrane were seen. (Chugh et. al., 1984; Visith Sitprija and Vijitr Boonpucknavig, 1979) The picture of interstitial infiltration with mononuclear cells, usually seen around degenerating tubules, was commonly found in tubular necrosis. These interstitial changes could either be due to immunological reaction to tubular antigens released from necrotic tubules or cellular reaction to the venom. Vascular changes including necrotizing arteritis of interlobular arteries and thrombophlebitis of arcuate veins have been reported as well. (Visith Sitprija et. al., 1974)

In most cases, glomerular changes were unremarkable. Nevertheless, Visith Sitprija and Vijitr Boonpucknavig (1983) have reported the glomerular pathology in 38 patients including 27 cases of Russell's viper bite and 11 cases of green pit viper bite. The common findings were thickening of mesangial area and mesangial proliferation in varying degrees. In some cases, segmental fibrosis of glomeruli and tubularization of Bowman's capsules were also noted. Interestingly,

extensive proliferation of glomerular parietal epithelial cells, consistent with extracapillary proliferative glomerulonephritis, was found in 2 patients who developed prolong oliguria.

In cases of bilateral cortical necrosis reported from India, there was extensive glomerular and tubular cell necrosis with fibrin thrombi presented in glomerular capillaries. (Date and Shastry, 1981)

B. Electron Microscopic Studies

Date and Shastry (1982) have reported the electron microscopic study of renal pathology in nine patients who developed acute renal failure following Russell's viper bite. The glomerular capillaries showed irregular basement membrane thickening and focal endothelial cell swelling. Subendothelial electron-dense deposit was seen in one case. Mesangium expansion was found in all cases with swollen glomerular epithelium and patchy foot process fusion.

The swollen vascular endothelium was also noted, especially in medullary area. The tubular epithelium showed intracytoplasmic bodies, necrosis and sloughing. Long segments of acellular tubular basement membranes resulting from extensive shedding of tubular epithelial cells were also demonstrated. The interstitium was edema with inflammatory cells infiltration.

C. Immunofluoresence Studies

Previous immunofluoresence studies were scant; Visith Sitprija and Vijitr Boonpucknavig (1983) have demonstrated granular deposition of IgM and C_3 in mesangial area and along capillary loops in 20 cases of Russell's viper bite; nevertheless, in 18 of 20 patients, antivenom had been given.

Fibrin deposition in the glomeruli was found in majority of cases; this deposition was more intense in those two cases of extracapillary proliferative glomerulonephritis.

Pathological Studies in Experimental Animals

Surprisingly, only two animal experimental works on the renal histopathology have been reported. In 1978, Aung-Khin investigated the effects of Russell's viper bite on human pathology as well as experimental animals. Forty experimental animals (twenty albino mice, ten albino rats and ten albino rabbits) were injected with lethal dosages of Russell's viper venom. Necropsies were performed and the renal tissues were examined by both light and electron microscope. Their study also included 7 human cases of Russell's viper bite, 4 of whom died and 3 recovered after treatment. In animals which died before 16 hour, their renal histopathological changes were mild; only tubular vacuolization and dilatation was noted. After 24 hour, definite histopathological pictures of tubular necrosis were seen.

Electron microscopic examination showed that many glomerular capillary loops were occluded with fibrin thrombi, platelets and dense granular materials. The investigators suggested that intravascular coagulation and subsequent renal ischemia should be the primary pathogenetic mechanism of acute renal failure in cases of Russell's viper bite.

Chugh et. al. (1984) have reported the light microscopic study of renal histopathology in rhesus monkeys after Russell's viper envenomation. Their findings revealed mild tubular necrosis in only one of five animals received lethal dosages of Russell's viper venom. However, when the sublethal dosages were used, more than half of animals showed moderate to

severe degree of acute tubular necrosis and 50% to 75% of their glomeruli contained fibrin. They concluded that their results argued against the role of direct nephrotoxicity as the sole pathogenetic factor of acute renal failure following Russell's viper bite.

Pathogenesis

Many factors have been suggested for their roles in the pathogenesis of renal lesions including hemodynamic alteration, disseminated intravascular coagulation, direct nephrotoxicity, intravascular hemolysis and hemoglobinuria and hypersensitivity reaction. (Rastegar et al.,1988)

A. Hemodynamic Alteration

Hypotension and circulatory collapse are not uncommon in cases of Russell's viper bite; this condition may be the result of bleeding. However, Russell's viper venom can directly cause vasodilation by its effects on prostaglandin, histamine and kinin production. (Fearn et. al., 1964; Huang and Lee, 1984; Oshima et. al., 1969)

Prapaporn Tungthanathanich et. al. (1985) studied the effect of Russell's viper venom on renal hemodynamic in dogs. Their results showed that initially, blood pressure, heart rate and pulse pressure decreased, while total peripheral vascular resistance and renal vascular resistance increased. After 2 hour following envenomation, blood pressure and heart rate returned to the baseline level ;but renal vascular resistance remained high and the total peripheral vascular resistance decreased for 24 to 48 hours. In this study, the changes in cardiac output and blood volume and the evidence of disseminated intravascular coagulation were not found. There were also the decrement of renal blood

flow and inulin clearance. The investigators concluded that the initial effect of Russell's viper venom causing hypotension and bradycardia would be consistent with vagal stimulation, since these effects could be prevented by vagotomy (Lee and Lee,1979). In the later phase, the reduction of total peripheral vascular resistance could be explained by the vasodilating effect of Russell's viper venom. Consequently, there was the increment of renal vascular resistance which was possibly mediated through compensatory renin-angiotensin activation.

The study in rabbits by Lee (1948) showed that the hypotensive action of Russell's viper venom was not effected by heat treatment (80°C, 30 minutes) and was neither restored by adrenaline injection nor by normal saline infusion. He concluded that the hypotensive effect of the venom was due to peripheral vasodilatation induced by some thermostable vasculotoxin. Russell's viper venom consists of kinin-forming enzymes (kininogenase), histamine and phospholipase A_2 (PLA_2), (Fearn et. al., 1964; Huang and Lee, 1984; Oshima et. al.,1969) all of which are able to decrease blood pressure. The effects of PLA_2 in the venom were extensively studied by Huang (1984); he found that PLA_2 were thermostable and had hypotensive action mediated through prostaglandin and leukotriene production.

B. Disseminated Intravascular Coagulation

Since the putatative action of Russell's viper venom causing disseminated intravascular coagulation (DIC) is generally accepted, the role of intravascular coagulation in causing ischemic renal failure has been mentioned by many authors. (Aung-Khin,1978;Chugh et al., 1984) Most supporting data came from the renal histopathological studies which demonstrated fibrin thrombi in glomerular capillaries and renal microvasculature in patients who developed cortical necrosis after

Russell's viper bite. This evidence suggested that intravascular coagulation would play the major pathogenetic role. (Date and Shastry, 1981) Nevertheless, the role of intravascular coagulation in patients who developed acute tubular necrosis is uncertained.

The possible role of fibrin thrombi causing glomerular lesions should be considered as well. As previously mentioned, Visith Sitprija et.al. have reported 2 cases of extracapillary proliferative glomerulonephritis from Russell's viper bite. In both cases, the immunofluorescent studies showed intense fibrin deposition in crescentic area. To determine whether intravascular clotting could by itself result in glomerular changes, Vasselli et al. (1966) carried out an electron microscopic study of glomerular changes in rabbits receiving the injections of Liquoid, thromboplastin or thrombin, all of which are capable of producing intravascular clotting. Their results revealed that not only glomerular thrombosis but also subsequent glomerular structural alterations were found. These alterations included swelling and proliferation of endothelial cells, accumulation of hyaline materials, abnormalities of the basement membrane, and formation of crescents. These changes sometime led to complete glomerular obliteration.

C. Direct Nephrotoxicity

Many investigators have suggested the possible role of direct cytotoxic effect of Russell's viper venom on the kidney. One of the supportive evidence came from an experimental study in isolated perfused rat kidneys. When Russell's viper venom was added to the perfusate, inulin clearance decreased and fractional excretion of sodium increased. These effects could be lessened by preincubation of Russell's viper venom with horse anti-serum. (Ratcliffe and Pukrittayakamee, 1985) Narongsak

Chaiyabutr et al (1985) used the renal micropuncture technique to measure the transmembrane potential of proximal tubule of Triturus kidney. Their result showed that there was progressive depolarization of cellular membrane when Russell's viper venom was added to the perfusate. This effect was concentration dependent and similar to the effect of 2-4 dinitrophenol, the agent which inhibited intracellular aerobic phosphorylation.

D. Intravascular Hemolysis

Russell's viper venom contains many proteolytic enzymes which can cause hemolysis. The role of intravascular hemolysis in causing acute tubular necrosis is known, for example in cases of G6PD deficiency. However, sincemost patients who developed acute renal failure following Russell's viper bite did not shown evidence of significant intravascular hemolysis, this factor would play only a minor role in the pathogenesis.

E. Other Factors

The immunologic mechanism has been suggested to cause interstitial infiltration but the role in renal failure following Russell's viper bite has not been firmly established. Although low serum complement C_3 was consistently found in Russell's viper victims, it would be only a non-specific concomitant finding. Since the Cobra venom, which does not cause acute renal failure, also has the capability in reduction of serum C_3 level. Moreover, the absence of renal failure in patients who exhibited IgM and/or C_3 deposits in their renal specimens and the occurence of renal failure in patients who had negative immunofluorescent studies were against the role of immunologic mechanism in pathogenesis of renal failure.