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Mini-review

Inflammation and oxidative stress in viper bite: An insight within and beyond

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ABSTRACT

Though systemic and local manifestations of snakebite are considered serious, the relevance of oxidative stress in viper bite pathology is largely denied. However, over the past decade, studies have provided substantial evidence for the presence of persistent oxidative stress in viper bite victims. This review aims at highlighting the disturbances in redox homeostasis soon after viper envenomation and its implications in the pathomechanism of secondary/long term complications including thrombocytopenia, hypopituitarism, infertility, renal abnormalities and persistent local tissue degradation. Both enzymatic and non-enzymatic components of viper venom play a pivotal role in bringing redox turbulence in victims. Venom-induced hemorrhage and necrosis with subsequent release of damage associated molecular pattern (DAMPs) molecules also contribute to sustained oxidative stress and inflammation. Studies have demonstrated that along with anti-venom therapy an antioxidant treatment during the early stages of viper bite and also long term treatment could help to reduce the occurrence of secondary/long term complications. Further, proper knowledge regarding the pathophysiology will allow for exploration of new avenues in the treatment of viper bite.

1. Introduction

It is a well-known fact that envenomation by vipers accounts for thousands of death and much more cases of morbidity worldwide when compared to elapid bites. Viper bite has fascinated the venom researchers and medical practitioners because of its distinctive way of killing the prey. Viper envenomation particularly disturbs the circulatory system by altering the hemostasis of prey/victim, which in turn causes death by unrestrained bleeding. Further, it also induces myotoxicity, nephrotoxicity and serious local manifestations, which includes myonecrosis, dermonecrosis, extracellular matrix (ECM) degradation, blistering, hemorrhage and edema. This would eventually end up with tissue loss, deficient functional recovery and amputation of the affected part (Jorge et al., 1994; Warrell, 1996; Gutiérrez et al., 2007). In addition, viper bite may also lead to several debilitating secondary/long-term complications like thrombocytopenia, renal abnormalities, hypopituitarism, persistent local tissue degradation and infertility, which has consistently puzzled the health authorities (Jeevagan et al., 2013; Herath et al., 2012; Torrez et al., 2014). Some of these manifestations may appear after few months or years of envenomation. Substantial evidences report the failure of classical anti-venom treatment in reversing these complications. Thus, viper bite management is often a challenging task for medical practitioners till today (Girish and Kemparaju, 2011).

Even though, in the past decade, advancement in mass spectroscopic techniques and proteomic approaches has revealed the proteome of viper venoms. An array of studies have emphasized the pronounced and subtle pathological events involved in both local and systemic manifestations of viper bite, however, the pathomechanism behind the secondary/long-term complications is not clear (Rucavado et al., 2012; Lomonte et al., 2014). Further, a lot more events can happen soon after envenomation that could contribute to these pathologies, which however have not received much attention in venom research. One such neglected protagonist in the scenario of viper bite pathology is 'oxidative stress' and the

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present review focuses on the involvement of oxidative stress in eliciting secondary/long-term complications in envenomed patients.

2. Viper bite induced oxidative stress

Though oxidative stress is one of the unrecognized scenarios in the snake bite pathology, in the recent past quite a number of studies have witnessed the prevalence of oxidative stress in viper envenomation. A study by Al Asmari et al. (2006) for the first time underscored the importance of oxidative stress in *Echis pyramidum* venom-induced cytotoxicity. Administration of venom to mice followed by the measurement of MDA levels in vital organs at different time intervals revealed significant lipid peroxidation, which persisted for several hours suggesting distortion of redox homeostasis. Further, Da Silva et al. (2011) evidenced the occurrence of oxidative stress and alteration in hepatic metabolism in rats injected with *Crotalus durissus terrificus* snake venom. Venom injection significantly increased the lipid peroxidation, catalase and glutathione-S-transferase activity in liver as well as plasma levels of ALT and AST signifying the hepatotoxicity.

In a recent study, Sebastin Santhosh et al. (2013) reported oxidative stress induced by *Vipera russelli* venom and its amelioration by a phytochemical crocin (isolated from *Crocus sativus*) using experimental mice. *V. russelli* venom injection significantly produced oxidative turbulence by elevating the levels of stress markers (MDA, H_2O_2 and ROS), antioxidant enzymes [superoxide dismutase (SOD), Catalase (Cat), Glutathione-S-transferase (GST)] in both liver homogenate and RBC fractions. It also remarkably disturbed the endogenous glutathione (GSH) cycle and caused severe hematological alterations along with increased pro-inflammatory cytokine levels. On the other hand, they also demonstrated that administration of the phytochemical crocin effectively ameliorates the venom-induced oxidative stress by restoring the altered stress markers and antioxidant enzymes (Sebastin Santhosh et al., 2013). The study signifies two aspects: venom induces oxidative stress in association with systemic inflammation; consequently, long term antioxidant therapy along with anti-venom treatment might help treating oxidative stress-associated secondary complications in viper-envenomed patients. Yet another study reported the viper venom-induced depletion in platelet count and its restoration by crocin phytochemical treatment. It revealed that the observed platelet loss was due to oxidative stress-driven apoptotic events in platelets, which was efficiently neutralized by crocin treatment (Santhosh et al., 2013a). Besides platelets, the effect of venom on blood components has been evaluated by the same group. The study claims that viper venom induces perturbances in blood components by eliciting oxidative stress and by activating inflammatory cytokines which could enhance systemic inflammatory events in the victims (Santhosh et al., 2013b).

Apart from blood components, venom-induced oxidative stress has also been largely implicated in acute kidney injury following viper bite. Several studies have witnessed that an antioxidant strategy can reduce the complications associated with viper bite-induced acute kidney. Barone et al. (2011) demonstrated that altered renal function as well as redox status in *Bothrops jararaca* envenomed mice can be restored by treatment with lipoic acid and simvastatin. *B. jararaca* venom depleted the GSSG/GSH ratio in renal tissue, and induced hyperurecemia. Simvastatin was reported to be a better inhibitor compared to lipoic acid in preventing the above-mentioned renal toxic events. Nevertheless, lipoic acid was found to exert deleterious effect as evidenced by increased urinary urea, creatinine and protein in envenomed mice. Similar effects were observed when lipoic acid was administered to *C. durissus terrificus* envenomed mice (Alegre Vde et al., 2010). Recently, Frezzatti and Silveira (2011) showed the elevated hyperuricemia, oxidative stress, and lethality caused by direct nephrotoxic effect of *C. durissus terrificus* envenomation in animal model. *C. durissus terrificus* venom effectively induced uricemia, and altered renal GSSG/GSH ratio, which were effectively ameliorated by allopurinol and probenecid. However, compared to probenecid, allopurinol was found be efficient in reducing lethality induced by venom. *B. jararaca* or *C. durissus terrificus* venom significantly damaged redox homeostasis and renal function in mice, which was effectively abrogated by a well-known clinically approved antioxidant, N-acetylcysteine (NAC). NAC treatment effectively restored the GSSG/GSH ratio in both renal cortex and renal medulla of envenomed mice. Further, it mitigated the creatinuria, protein urea and amino peptidase activities efficiently, whereas, it failed to offer protection against the lethality induced by venoms (Barone et al., 2014).

A recent study by Katkar et al. (2014) have shown that besides viper venom, an antivenom treatment itself can alter the redox homeostasis to a certain extent in envenomed animals and was also unsuccessful in blocking venom-induced organ damage. Melatonin treatment ameliorated venom-induced local toxicity, inflammation, oxidative stress and organ damage. Thus, the study claims that combination of antivenom therapy and antioxidants like melatonin could become a coherent therapeutic approach in the management of viper bite.

A study by Mukhopadhyay et al. (2008) has reported a fortuitous finding that patients with snakebite-mediated acute renal failure (SARF) were shown to have increased oxidative stress compared to normoglycemic chronic renal failure (CRF) cases. The decreased serum GSH levels, total antioxidant status, intracellular erythrocyte GSH levels, and increased protein carbonyls, thiobarbituric acid reacting substances and methylgloxyl (MG), levels were observed in SARF patients with higher values compared to CRF patients. In addition, the r value of MG-creatinine correlation analysis in SARF patients was found to be greater than that of CRF. Thus, MG formed in the due course of oxidative stress is thought to mediate the pathophysiology of renal diseases associated with snake bite and the study claims that oxidative stress can aggravate the renal abnormalities in viper-envenomed patients. Zengin et al. (2012) have investigated the antioxidant and oxidant status and oxidative stress index in viper-envenomed patients soon after they are admitted to hospital and also after one month. A significant increase in oxidant status and oxidative stress index were observed on arrival, and according to the data the patients continue to be in a state of persistent oxidative stress even after a month of snake bite. Thus, from the above evidences it can be stated that oxidative stress is a highly relevant phenomenon in the pathophysiology of viper bite and could play an essential role in the perseverance of secondary/long-term complications of viper bite.

3. The possible role of venom components in inducing oxidative stress and inflammation

3.1. Phospholipase A_2

PLA_2s are abundant components in the proteome of viper venom and belongs to group II (Lomonte and Gutiérrez, 2011). These digestive enzymes are well known for their necrotic and inflammatory actions. The necrotic action of PLA_2s mainly involves the degradation of membrane lipids followed by its disruption and release of intracellular contents (Fernandes et al., 2014). All these events indeed contribute profoundly to elicit oxidative stress and inflammation. The catalytic hydrolysis of the sn-2 acyl bond of glycerophospholipids of plasma membrane generates lysophospholipids and free fatty acids including arachidonic acid. The

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