Original article

Corticosteroid actions on dengue immune pathology; A review article

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ABSTRACT

Introduction: Dengue infection causes significant morbidity and mortality in over 125 countries worldwide, and its incidence is on the rise. Currently, no therapy is available beyond supportive care. In fact, corticosteroids are used therapeutically for a broad spectrum of diseases including autoimmune, allergic, inflammatory diseases and organ transplant rejection. However, only a few studies were done to evaluate the effectiveness of corticosteroids in dengue infection, although the immune pathology of dengue is similar to other diseases treated effectively by corticosteroids for several decades.

Objectives: This review is aimed at identifying biological actions of steroids at molecular and receptor level in dengue immune pathology after reviewing pharmacological and immunological research findings of corticosteroids and dengue.

Methods: We searched medline/pubmed and Google scholar for publications with the search terms 'dengue' and 'steroid', 'corticosteroid', 'prednisolone', 'methylprednisolone' or 'dexamethasone' in the title and abstract. Then the publications were analyzed according to the action of steroids in dengue pathology under different subheadings.

Results: The results are presented under four categories and it shows that corticosteroids can suppress cells involved in innate immunity, T cells, B cells and antibodies, complements and hematological manifestations in dengue pathology.

Conclusion: This article explains strong supportive evidence for actions of corticosteroids in dengue pathology at receptors and molecular levels. Therefore it is suggested that a gold standard steroid protocol for each phase of dengue pathology that can be tested further with a double blind control trial study.

1. Introduction

Dengue fever (DF) often presents with fever, rash, headache and myalgia, which are caused by a flavi virus with four distinct stereotypes; DENV-1, DENV-2, DENV-3, and DENV-4.¹ An infection with one stereotype does not protect against the others and sequential infections put patients at a greater risk for dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)² that are mainly caused by immunological dysfunction.³ The World Health Organization (WHO) estimates that 50 to 100 million dengue infections occur annually and it affects more than 125 countries that are known to be dengue endemic. Moreover, it includes 500,000 cases of DHF and 22,000 deaths annually. Most of them are children. The incidence has increased by 30 folds during the past 50 years.⁴ In Sri Lanka, 110,372 cases and 301 deaths were reported within the first seven months of the 2017 epidemic.⁵ Currently, no specific therapy is available beyond supportive care while untreated complicated dengue fever can have a 50%

mortality rate.⁶ Therefore, unless exact or partial treatment is introduced to suppress this life threatening immune dysfunction pathology of dengue, 5 million cases of DHF and 0.22 million deaths will occur within the next decade (2018–2028).

Corticosteroids (CSs) are used therapeutically for a broad spectrum of diseases including autoimmune diseases, allergic and inflammatory diseases and in organ transplant. However, only a limited number of studies were done to evaluate the effectiveness of CSs in dengue infection,⁷ although the immune pathology of dengue has been within the range of immune pathology of other diseases, which have been treated effectively by CSs for several decades.

In fact, fear of administration of corticosteroid to dengue patients still exists because it is an infective illness. Very recently a review article stated favorable effect of steroid in dengue.⁸ In clinical trials there were no evidence of viremia with use of steroids and there were no significant side effects after the administration of low and high oral doses of corticosteroids and high doses of IV corticosteroids.^{9,10}

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Moreover, no evidence of harmful effects of corticosteroids was found¹¹ and beneficial effects were found.¹² In this recent review of dengue and steroids, it was concluded that the effectiveness of corticosteroid for immune suppression in dengue is depended on sustained maintained therapeutic blood levels of corticosteroids using a higher receptor affinity steroids for required time duration.

With the conclusion of clinical trial studies data, a hypothesis was formulated. It is suggested that different doses, routes of administration, and particular groups of steroids contribute to suppress immune pathology of dengue at different stages of dengue infection due to different pharmacological actions of CSs at molecular and receptor levels. This could open a new direction to assess molecular and receptor level evidence of CSs to prove this hypothesis.

2. Objectives

This review is aimed at identifying biological actions of steroids at molecular and receptor level in dengue immune pathology after reviewing pharmacological and immunological research findings of CSs and dengue under different subheadings.

3. Methods

We searched medline/pubmed and Google scholar for publications with the search terms 'dengue' and 'steroid', 'corticosteroid', 'prednisolone', 'methylprednisolone' or 'dexamethasone' in the title and abstract. Then the publications were analyzed according to the action of steroids in dengue pathology under different subheadings.

4. Results

The results are presented under four categories depending on steroids action on innate immunity, T cells, B cells and antibodies, complement system and hematological manifestations.

4.1. CSs suppress cells involved in innate immunity in dengue pathology (Fig. 1)

Dengue virus (DENV) is presumably injected into the bloodstream during the feeding of mosquitoes on humans and attacks the immune system cells. Firstly, it results in the infection of immature epidermal dendritic cells (DCs) in the epidermis and dermis.¹³ Infected DCs then migrate from the site of infection to lymph nodes where monocytes and macrophages are recruited. Thereafter the infection is amplified and the virus is disseminated through the lymphatic system.¹⁴ As a result of this primary viremia, several cells of the mononuclear lineage, including blood-derived monocytes, myeloid DCs and splenic and liver macrophages are infected.¹⁵ The time gap between the bite of an infected mosquito and the onset of clinical symptoms is believed to be 3–15 days.¹⁶

Immature dendritic cells (imDCs) express high levels of DC-SIGN (Dendritic Cell Specific Intercellular adhesion molecule-3 (ICAM-3) Grabbing Non-integrin)/CD209 which facilitates initial viral binding and entry. CD209 is a known target of dengue virus, having a high affinity for ICAM3 molecules expressed on T-cells.^{17,18} Thus DCs are the most effective antigen presenting cells (APC) able to acquire and display viral antigens and finally activate T-cells. In dengue pathology, infected imDCs also contribute to vascular leak by producing matrix metalloproteinase (MMPs).¹⁹ The maturation and sensitivity of DCs are promoted by dengue virus induced cytokines e.g. TNF α , IFN γ , IL-6, and activated T cells.^{3,20} Mature Dendritic Cells (mDCs) lose their ability to capture and process antigens, up-regulate their production of cytokines, increase their expression of MHC class which has been suggested as a mechanism by which the immune system may enhance viral pathogenesis.^{19,21} The mDCs do not possess high levels of DC-SIGN but they do facilitate Antibody-Dependent Enhancement (ADE) via Fc γ Ila and

Fc γ Ilb receptors.^{18,22} ADE in mDCs can increase viral RNA production over 100-folds making dendritic cells potent components in dengue pathogenesis.^{3,22} When DCs fail to mature properly, they will fail to stimulate T-cells and may induce tolerance.²² Moreover, mDCs can also activate B-cells through co-stimulation of CD40, IL-6 and IL-14. In addition to DCs, macrophage, monocyte and natural killer (NK) cells also mediate for viral replication, cytokines production and killing of infected cells.²² In fact, IL-15 produced by DCs has comparable activity with IL-2 for the induction of B and NK cell proliferation and differentiation.^{21,23}

Among CSs, methylprednisolone (MP) usually enhance antigen uptake and prevent DC differentiation and maturation and production of TNF- α , IL-6, and IL-12. But MP does not affect the viability of DC.^{24–26} By these ways, the maturity function of DCs is retarded. This may in turn indirectly suggests, reduce dengue viral replication that was 100 times in mDCs. In addition, MP treated DCs were deficient in their ability to elicit proliferative responses and production of MMPs.²⁴ This may contribute to prevent vascular leak in dengue. Furthermore, CSs exert potent suppressive effects on human DCs and thereby inhibit the induction of primary T and B cell responses preventing immune dysfunction induced by dengue virus. MP also cause up-regulation of the anti-inflammatory cytokine IL-10, which gets its anti-inflammatory effect by suppressing the production of macrophage inflammatory proteins such as IL-1, IL-6, IL-8, IL-12, TNF, the granulocyte-macrophage colony stimulating factor (GM-CSF), MHC class II molecules, B7 and intercellular adhesion molecule-1 (ICAM-1).^{27,28} More interestingly, IL-10 inhibits TNF-induced NF- κ B activity and acts to diminish Th1 cell activity by suppressing IL-2 and interferon- γ .²⁹ The anti-inflammatory effects of CSs on leucocytes is partly by inhibition of signaling of NF- κ B. Moreover, glucocorticoids (GCs) alter the trafficking and functioning of leucocytes such as neutrophils, eosinophils, mast cells and endothelial cells.³⁰

Elevated cyclooxygenase-2 (COX-2) and Prostaglandin E2 (PGE2) expression caused by viral infection have been reported to be associated with viral replication and viral pathogenesis.^{31,32} Administration of a single oral dose (4, 8, or 16 mg) of MP was found to cause a dose and time-dependent inhibition of whole-blood COX-2 activity and significantly lower the levels of PGE2.³² Thus it could be suggested that CSs contribute indirectly to reduce both replication and viral pathogenesis either through DCs or COX-2/PGE2.

4.2. CSs suppress T cells, B cells and antibody in dengue pathology (Fig. 2)

Another important molecule in dengue pathology is the antibody. The normal interaction of dengue virus with anti-dengue antibody generally leads to neutralization. However, in heterotypic dengue viral infections, the antibodies are non-neutralizing and lead to enhancement. Thus pre-existing antibodies (Ig G and Ig M) are associated with antibody-dependent enhancement and complement activation.³³ In infants also dengue hemorrhagic fever can occur in primary dengue viral infection that could be due to maternally derived nonneutralizing IgG facilitated antibody-dependent enhancement.³⁴ The non-neutralizing levels of dengue virus-reactive IgG were postulated to be a critical risk factor for severe dengue during infancy. Therefore live attenuated dengue vaccine is a cause for risk of severe dengue in such infants, even in their primary infection. In addition the disease severity due to a robust immune response in infants with primary infections would be associated with a consequence of higher viral burdens in vivo and an activation phenotype of peripheral-blood NK cells and CD8⁺ and CD4⁺ T cells.³⁵

Type1 T helper (Th1) cells produce IFN γ , IL-2 and the tumour necrosis factor (TNF)-beta, which activate macrophages.^{36,37} By contrast, type 2 T helper (Th2) cells produce IL-4, IL-5, IL-10 and IL-13, which are mainly responsible for antibody production, eosinophil activation and inhibition of several macrophage functions.³⁷ IFN γ and TNF α were also strongly associated with dengue disease severity and correlate well with T-cell activation.³ IFN γ secretion by dengue specific T-cells has

03 - CS s suppress DENVs induced complement activation

(DENV = Dengue virus
DC = Dengue virus
MBL = Mannose-binding lectin
MP = Mannose-binding lectin
CSs = Corticosteroids).

when their concentrations are high enough to invoke a general systemic response.⁵⁶ Thus higher complement levels correlate to increasing disease severity of dengue illness.⁵⁵ Cross reactive antibodies activate complements further. Finally increased alternative complement proteins, complement receptors and C proteins facilitate a positive feedback loop that can lead to dangerous consequences in a dengue-infected patient.

In this life threatening steps of dengue pathology, it is observed that MP directly inhibits the alternative and amplification pathway of complements.⁵⁷ Moreover, steroids in higher doses may regulate multiple steps in the immunological apparatus, including stabilization of membranes, modulation of in vivo component levels and inhibition of complements, which help to stop or prevent histamine release, vascular permeability and circulatory collapse.⁵⁸

4.4. Corticosteroids suppress DENV induced hematological manifestation (Fig. 4)

Hematological abnormalities that are generally observed in severe dengue, such as thrombocytopenia, coagulopathy, bleeding and vasculopathy are related to platelet and endothelial dysfunction.⁵⁹ Disseminated intravascular coagulation and major hemorrhages in a minority of patients with severe or prolonged shock may be due to severe thrombocytopenia and the secondary effects of hypoxia and acidosis.⁵⁹ Thrombocytopenia may be due to bone marrow suppression, immune mediated platelet destruction, augmented platelet adhesiveness to vascular endothelial cells and high levels of platelet activating factor (PAF).⁶⁰ Moreover marked thrombocytopenia is evident in patients with elevated IL-1 β , IL-8, TNF- α and Monocyte chemo-attractant protein-1 (MIP-1).⁶¹ Spontaneous bleeding is strongly associated with

Fig. 3. Steps from complement activation to dengue pathogenesis and action of corticosteroid

DENVs(1) infect both DCs and T-cells(2)and up-regulate C3a and C5a receptors (3) and produce C3 peptide (4).Viral infected macrophage also involve in C3 peptide production (5). The complements are activated through antigen antibody complexes(6) named the classical pathway(7); recognition of carbohydrate structures on pathogens(8) by mannose binding lectin/MBL pathway (9) and hydrolysis of C3(H₂O) (10) named as alternative pathway(11). DENVs can be directly neutralized via the MBL pathway (12). The increase in alternative complement proteins, complement receptors(3) and C protein (4), all facilitate (13) a positive feedback loop (amplification pathway)(14). They mediate basophiles, neutrophils, eosinophils and mast cells to release histamine (15). Dengue pathology is increased by complement molecules and enzymes (C3a and C5a) that can induce oxidative bursts, generation of cytotoxic oxygen radicals, chemotaxis, inflammation, circulatory collapse as well (e.g.anaphylaxis)(16). MP(17) directly inhibits the alternative(18) and amplification pathway (19) of the complement but not the MBL path way. CSs in higher doses (20) may function by regulating multiple events in the immunological apparatus including inhibition of compliments, stabilization of membranes and modulation of vivo components levels (21).

platelet count below 100,000 cells/c.mm or a rapid drop in the platelet count.⁶¹ In addition, the cause for increased spontaneous bleeding may be brought about by low plasma fibrinogen levels, release of Heparansulphate and secretion of Von Willebrand factor (VWF) in DHF. Low plasma fibrinogen may be due to permeation of fibrinogen through the endothelial cells, development of antibodies potentially cross-reactive to plasminogen and impaired synthesis of fibrinogen.^{62,59} Heparansulphate in blood vessels acts like an anti-coagulant and is damaged by the initial cytokine response in DHF and liberated to the circulation. This may be the reason for the prolonged Activated partial thromboplastin time (APTT) in DHF.⁶³ Severe dengue is associated with increased circulating levels of VWF, which is associated with thrombocytopenia, clinical bleeding and plasma leakage.⁶⁴ However, the tendency to bleed after a high dose of CSs could not be observed because non-genomic effects of GSs enhance the rapid activation of endothelial nitric oxide synthase (eNOS), which is a possible inhibitor of VWF secretion.⁶⁵

Activation of both platelets and complements and release of inflammatory mediators such as vascular endothelial growth factor (VEGF), TNF α , PAF and Monocyte chemoattractant protein-1 (MCP-1) is proposed as the mechanism that causes vasculopathy leading to the plasma leakage.⁶⁶ VEGF may induce permeability via prostanooids and NO or via PAF.⁶⁷ TNF α may lead to permeability via cyclooxygenase induction and the release of prostanooids.⁶⁸ In fact, it was observed that CSs inhibit the pro angiogenic gene VEGF⁶⁹ resulting in reduced plasma leakage. PAF is produced and secreted by several types of cells, including mast cells, monocytes, tissue macrophages, platelets, eosinophils, endothelial cells and neutrophil which has also been shown to be involved in the production of many inflammatory cytokines such as TNF α and IL-18.⁷⁰ PAF levels were significantly higher in more severe

Fig. 4. Steps from hematological manifestation to dengue pathogenesis and corticosteroid action in the steps.

DENVs(1) infect and suppress megakaryocyte cells (MCs) (2) in bone marrow leading to thrombocytopenia(3) that is aggravated by endothelial cells adhesion molecule (4), antiplatelet antibodies(5) and platelet adhesion molecule (15). Infected immune cells(7) secrete platelet activating factor (PAF) (8) and cytokines(9) and chemokines. These effects are suppressed or reduced by CSs (6) PAF is implicated in platelet aggregation and activation (10) resulting thrombocytopenia (3), increased release of VWF (11) and Heparan sulphate(12), low levels of fibrinogen (13) contribute to aggravated bleeding (14) tendency in dengue. The dengue induced pathological effect such as production of cytokines (9), platelet adhesive molecules (15) NS-1 antibody (16) formation are suppressed by CSs/MP. The ability of increased endothelial NO (e NO)(17) production by MP leads to reduced secretion of (18). All contribute to reduce the tendency to bleed(13) and its cause in dengue. Activation of platelets(10), complements(19) and release of inflammatory mediators such as PAF (8), MCP-1(20), VEGF(21), TNF(22) leading to plasma leakage and contribute to the development of vasculopathy (23). It is brought about by PAF through altered expressions of the pattern of the tight junction protein ZO-1 (24). Dexamethasone (DEX) (25) increases the ex-

pression of ZO-1 and decreases levels of PAF via suppression of immune molecules. CSs suppress immune cells(26) and reduce production of prostanoids (PTs), NO (27) and COX-2 (28) which are the permeability facilitators of VEGF and TNF respectively. (29) CSs also suppress the action of NS-1 directly and indirectly.

(DENV = Dengue virus

MCs = megakaryocyte cells

PAF = platelet activating factor

VWF = Von Willebrand factor

CSs = Corticosteroids

MP = Methylprednisolone

MCP = Monocyte chemoattractant protein

VEGF = Vascular endothelial growth factor

TNF = Tumor necrosis factor alpha

DEX = Dexamethasone

PTs = prostanooids

NO = Nitric oxide

COX-2 = Cyclooxygenase-2).

forms of dengue and were associated with a reduced expression of tight junction proteins and reduced cell layer integrity resulting in increased para cellular leak.⁷¹ Moreover, PAF acts as a potent activator and a mediator of both immune-mediated and non-immune mediated anaphylaxis. It is implicated in platelet aggregation and activation through oxidative bursts, chemotaxis of leukocytes, augmentation of arachidonic acid, metabolism and release of vasoactive amines in the inflammatory response, resulting in increased vascular permeability, circulatory collapse, decreased cardiac output and various other biological effects.^{70,71} In dengue patients, PAF was found to alter the expression pattern of the tight junction protein ZO-1 and had decreased the integrity of human endothelial cell monolayer, as measured by trans-endothelial resistance.⁷¹ Among the CSs, dexamethasone was investigated earlier and proved that it can increase transendothelial electrical resistance and ZO-1 expression.⁷² In addition, dexamethasone has also been found to influence tight junction expression leading to a reduction of permeability.⁷² Furthermore, it was observed that the amount of plasma extravasations produced by PAF was decreased by dexamethasone in adrenalectomized rats.⁷³ On the other hand, TNF and

IL-1 were found to stimulate the synthesis and release of the platelet-activating factor (PAF) by neutrophils and vascular endothelial cells,⁷⁴ which could be effectively suppressed by CSs as mentioned above.⁷²

5. Conclusion

Lack of knowledge and clinical practice on the use of corticosteroids in relation to immune pathology exists and causes clinical manifestations of DSS/DHF and their complications. Therefore all clinicians must be provided with adequate scientific evidence in these regards. Apart from the existing fluid management for dengue disease, this immune pharmacological approach is suggested as a treatment option to suppress the immune dysfunction that leads to DHF and other complication of dengue. Therefore the value of steroid on dengue immune pathology cannot be underestimated and this could open a completely new scientific approach and will be suggested for management of more than 250,000–500,000 patients in the world who suffer from severe dengue disease annually. This can be tested further with a double blind control trial study using a standard steroid protocol considering the stages of

immunopathology of dengue. This review article (a) provides an immunological link between dengue and steroid treatment (b) offers scope for corticosteroid based treatment as an approach for DF and DHF, DSS (c) provides a better understanding of actions of corticosteroid in dengue immune pathology (d) provides and opens well known arguments, completely new to dengue, but with strong scientific evidence to reconsider use of steroid to manage dengue and its complications (e) offers scope to consider steroids for primary and secondary prevention of dengue and complication and treatment. (f) provides information on the main cause for aggravation of existing dengue pathology under non-steroid therapy (g) provides a plausible explanation as to why not all patients with dengue develop DHF/DSS and differently associated complications. (h) can be used to explain other treatment methods that are used to explain such as herbal and other approaches to control dengue disorders and its complications (i) offers a novel coherent picture of the immune pathology of dengue related to the pharmacological approach of corticosteroid treatment at different severity of dengue immune pathology.

Declaration of Competing interest

None to declare.

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Abbreviations

DF	Dengue fever
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
WHO	World Health Organization
CSs	Corticosteroids
DENV	Dengue virus
DCs	Dendritic cells
imDCs	Immature dendritic cells
DC-SIGN	Dendritic Cell Specific Intercellular adhesion molecule-3 (ICAM-3) Grabbing Non-integrin
MMPs	Matrix metalloproteinase
TNF α	Tumor necrosis factor alpha
IFN γ	Interferon gamma
IL	Interleukin
mDCs	Mature Dendritic Cells
MHC	Major histocompatibility complex
ADE	Antibody-Dependent Enhancement
NK	Natural killer
MP	Methylprednisolone
GM-CSF	Granulocyte-macrophage colony stimulating factor
ICAM-1	Intercellular adhesion molecule-1
GCs	Glucocorticoids

COX-2	Cyclooxygenase-2
PGE2	Prostaglandin E2
Th1	Type1 T helper
Th2	Type 2 Th
NS1	Nonstructural protein 1
iNOS	Inducible nitric oxide synthetase
NO	Nitric oxide
MBL	Mannose-binding lectin
PAF	Platelet activating factor
VWF	Von Willebrand factor
APTT	Activated partial thromboplastin time
eNOS	Endothelial nitric oxide synthase
VEGF	Vascular endothelial growth factor
MCP-1	Monocyte chemoattractant protein-1

Ethical approval

Not required.

Availability of data and material

The data set for this publication is available upon request from the authors.

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Authors contribution

SMRB Formulated hypotheses on following topics such Corticosteroid actions on dengue immune pathology effectiveness of corticosteroid in dengue treatment and management of dengue and post dengue Syndrome hypothesis for dengue, devised the project and review articles, the main conceptual ideas and proof outline, wrote the manuscript. HMMTBH corrected, edited the manuscript and supervised the review.

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