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REVIEW

Inhaled corticosteroids: effects on the airway vasculature in bronchial asthma

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ABSTRACT: Inhaled corticosteroids suppress airway inflammation and components of airway remodelling in bronchial asthma. In the tracheobronchial (airway) vasculature, these include the inhibition of inflammatory hyperperfusion, microvascular hyperpermeability, mucosal oedema formation, and the formation of new blood vessels (angiogenesis).

Corticosteroids are now known to exert their effects on the airway vasculature through genomic and nongenomic mechanisms. Genomic actions involve the regulation of target genes, and suppress most of the vascular elements of inflammation and angiogenesis in the airway. In contrast, nongenomic actions are mediated by rapid cellular mechanisms, and induce transient vasoconstriction in the airway, thereby reversing inflammatory hyperperfusion.

The vascular actions of corticosteroids contribute to controlling clinical symptoms of asthma primarily by influencing airway calibre in the lung periphery and airway hyperreactivity.

In this review article, recent advances into the understanding of cellular mechanisms and the clinical implications of the interaction of inhaled corticosteroids and the airway vasculature in asthma are reviewed.

KEYWORDS: Airway vascularity, angiogenesis, asthma, inhaled corticosteroids, nongenomic action, vasoconstriction

Airway inflammation is a central feature of bronchial asthma. In addition to inflammatory cell infiltration in the bronchial wall [1], histological analysis of endobronchial biopsy specimens and new methods of blood flow measurements have revealed prominent alterations of the tracheobronchial (airway) vasculature in patients with asthma. The major structural and functional changes related to the airway circulation include the proliferation of blood vessels (angiogenesis) [2–4], increased blood flow [5, 6], increased microvascular permeability [7, 8], and oedema formation in the airway wall [9]. These vascular components seem to have significant clinical implications because of their correlations to asthma severity, including airflow limitation [10–13] and bronchial hyperresponsiveness [7, 11, 14–18]. Recent advances in the understanding of the cellular mechanisms responsible for these vascular abnormalities may ultimately lead to new therapeutic approaches for the treatment of asthma.

Since 1949, when HENCH *et al.* [19] published his findings on cortisone causing dramatic improvements in patients with rheumatoid arthritis, corticosteroids have become established as the most potent anti-inflammatory agents in the pharmacotherapy of various chronic inflammatory diseases, including asthma [20]. The inflammatory process in asthma involves the increased expression of various pro-inflammatory chemokines, cytokines, growth factors, lipid mediators, adhesion molecules, enzymes, and receptors for the same inflammatory mediators [21]. Corticosteroids are the most effective drugs to suppress airway inflammation, mainly by down-regulation of pro-inflammatory proteins [22, 23]. In addition, corticosteroids seem to reverse components of the asthma-induced structural changes (airway remodelling), including the increased vascularity of the bronchial wall [24].

The anti-inflammatory actions of corticosteroids occur with a considerable delay (within hours or days) because of the multiple steps of cellular actions required to change protein expression. However, evidence is now accumulating for rapid corticosteroid actions [25, 26], and the existence of membrane-bound steroid receptors that may mediate these rapid actions [27, 28]. The rapid effects of corticosteroids have also been

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demonstrated in the airway vasculature in recent years [29, 30]. These ''nonclassical'' actions might contribute to the anti-inflammatory effects of corticosteroids; however, the underlying cellular mechanisms are clearly incompatible with the transcriptionally mediated (genomic) actions.

Here, the current authors provide an overview of the rapidly expanding information on: 1) the molecular basis of the genomic and nongenomic actions of corticosteroids; 2) the vascular manifestations of asthma; and 3) the interactions of corticosteroids and airway blood vessels by which inflammatory changes of the airway vasculature can be reversed in patients with asthma.

GENOMIC MECHANISMS OF CORTICOSTEROID ACTIONS

Inhaled corticosteroids suppress airway inflammation, which is responsible for asthma-associated changes of the airway vasculature. The anti-inflammatory effects of corticosteroids are due to activation or repression of target genes involved in the inflammatory process (fig. 1). These genomic actions are mediated by intracellular receptors (glucocorticoid receptors; GRs), which ultimately alter transcription through direct DNA binding [31] or transcription factor inactivation [32]. Because genomic mechanisms require additional steps, such as protein synthesis, sorting, modification, and intracellular transport, anti-inflammatory effects of corticosteroids take at least hours to occur.

Gene activation by direct DNA binding (transactivation)

According to the classical model of steroid hormone action, the lipophilic corticosteroid molecules easily cross the lipid bilayer of the cell membrane to enter into the cell and bind to specific receptors [33]. After corticosteroid binding and dissociation of the two subunits of the heat-shock protein 90 (hsp90), which act as a molecular chaperone, activated GRs translocate to the nucleus and bind to palindromic DNA sequences (response elements) in the promoter regions of target genes. Depending on positive or negative activity of the regulatory elements, binding of the ligand-activated receptor complex may up- or downregulate gene transcription, and thus protein synthesis. Corticosteroids increase the transcription of some anti-inflammatory genes through direct DNA binding; however, this mechanism seems to have a minor role in the suppression of inflammation. For instance, corticosteroids have been reported to repress inflammation efficiently in mice with a defective GR, which cannot bind DNA [34]. Recently, it has been proposed that transactivation is responsible for some side effects (*e.g.* diabetes induction, skin atrophy) caused by corticosteroids [35].

Gene repression by protein–protein cross-talk (transrepression)

Transcription is upregulated for a variety of inflammatory genes that play a central role in asthma-associated airway inflammation [21]. Increased transcription is induced by pro-inflammatory transcription factors, such as nuclear factor-κB

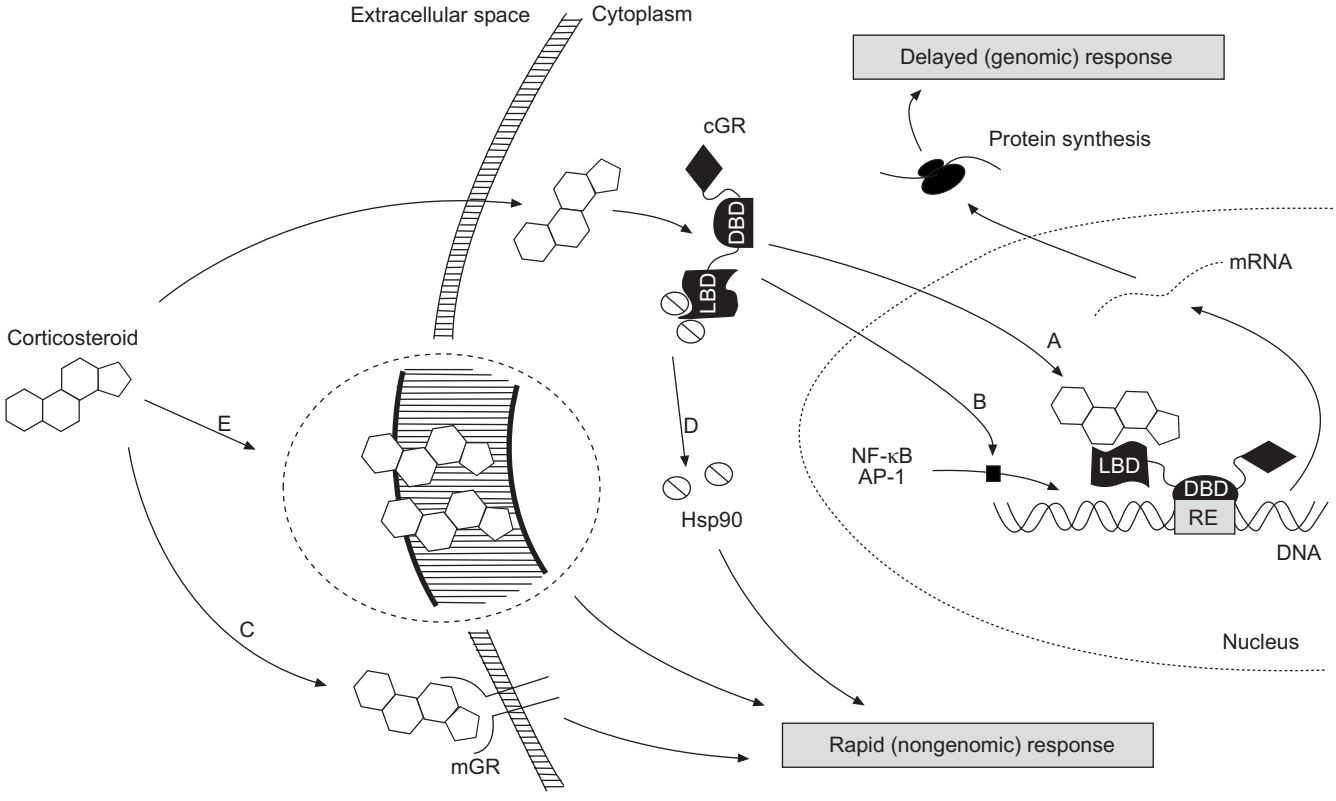

FIGURE 1. Schematic diagram of the complex cellular actions of corticosteroids. Genomic actions are mediated by cytoplasmic receptors, which ultimately alter transcription through A) direct DNA binding or B) transcription factor inactivation. In contrast, nongenomic actions are mediated by C) membrane-bound or D) cytoplasmic receptors, or E) nonspecific interactions with the cell membrane. cGR: cytoplasmic glucocorticoid receptor; mGR: membrane glucocorticoid receptor; LBD: ligand-binding domain; DBD: DNA-binding domain; Hsp90: heat-shock protein 90; RE: response element; NF-κB: nuclear factor-κB; AP-1: activating protein-1.

TABLE 1 Genomic and nongenomic actions of corticosteroids

	Genomic actions	Nongenomic actions		
Receptor location	Cytoplasm	Cytoplasm	Membrane	Nonspecific
Action's onset and reversibility	Hours–days		Seconds–minutes	
Sensitivity to inhibitors of transcription (actinomycin D) and protein synthesis (cycloheximide)	Inhibition		No effect	
Activity of membrane impermeant carrier (bovine serum albumin)-coupled drug	Inactive	Inactive	Active	Active

[36] and activating protein-1 [37]. To suppress inflammation, the ligand-activated GR directly binds to the pro-inflammatory transcription factors and interferes with their functions [32]. Transcription factor inactivation results in decreased transcription and synthesis of inflammatory proteins, thereby disrupting the inflammatory process. Transrepression is now considered the principal mechanism by which corticosteroids suppress airway inflammation in asthma. In a search for novel anti-inflammatory drugs with reduced risk of side effects caused by transactivation, several selectively transrepressing compounds are under clinical investigation [38].

Post-transcriptional regulation of gene expression

Post-transcriptional mechanisms regulate mRNA stability and translation of many pro-inflammatory genes involved in the pathophysiology of asthma [39]. In contrast to major pathways activated in response to inflammatory cellular stimuli to stabilise mRNAs (*e.g.* p38 mitogen-activated protein kinase [40]), corticosteroids have been shown to increase mRNA degradation, and thus block the production of several of pro-inflammatory cytokines and other proteins. In fact, steroid hormones were one of the earliest molecules shown to downregulate gene expression by increasing mRNA decay rates. Since then, an increasing number of genes have been shown to be inhibited by corticosteroids through a post-transcriptional mechanism [41]. However, the clinical implications of corticosteroid actions on mRNA stability have not been clarified in asthma [42].

RAPID, NONGENOMIC MECHANISMS OF CORTICOSTEROID ACTIONS

Although the major anti-inflammatory effects of corticosteroids are due to transcriptional mechanisms, evidence is growing for actions manifested within seconds or minutes. These effects are mediated by cellular mechanisms that are too rapid to involve gene expression and have been termed nongenomic actions [43]. Nongenomic actions are initiated by specific interactions with membrane-bound or cytoplasmic GRs, or nonspecific interactions with the cell membrane (fig. 1). Despite a high mechanistic diversity among different cell types, nongenomic actions have some common features (table 1).

In contrast to genomic actions, much less is known about the clinical significance of rapid nongenomic actions of corticosteroids. In bronchial asthma, inhaled fluticasone propionate has been shown to acutely decrease airway mucosal blood flow [29, 44], inhibit inhaled AMP-induced bronchoconstriction [45], and protect against exercise-induced bronchial obstruction in children [46]. Corticosteroids have also been shown to acutely decrease nasal itching in allergic rhinitis [47]. In keeping with early reports of acute potentiation of bronchodilator actions by corticosteroids [48, 49], budesonide acutely enhanced the fast onset of bronchodilator action of formoterol in patients with chronic obstructive pulmonary disease in a recent study [50]. Although rapid effects on airflow limitation have also been suggested in patients with acute asthma [51], there is insufficient evidence that inhaled corticosteroid therapy results in clinically important rapid changes in pulmonary function [52], whereas systemic corticosteroids probably require >6 h to 24 h to improve pulmonary function [53].

Membrane receptor-initiated actions

Corticosteroids induce a number of rapid cellular effects without entering into the cell. In some cases, these cell membrane-initiated actions are also sensitive to blockers of the cytoplasmic GR. These observations ultimately resulted in the discovery of corticosteroid binding sites and receptors associated with the cell membrane. After the early demonstration of high-affinity cell membrane binding sites for corticosteroids in rat liver [54], membrane-bound receptors have been shown in amphibian brain [55] and mouse lymphoma cells [56]. Membrane-bound GRs are also present in various human cells, including leukaemic [57] and peripheral blood mononuclear cells [28]. Recently, membrane binding sites for corticosteroids have been demonstrated in smooth muscle cells isolated from human airway blood vessels [58, 59]. Ligand binding to this membrane receptor might be responsible for the hormone's inhibitory effect on norepinephrine uptake into these cells [60].

The molecular structure of the membrane GR is still unknown. Receptor distribution analysis suggests that the same receptor may function in both nuclear and membrane locations [27]. The membrane receptor turned out to be a modified form of the classical GR in some cases of neuronal cells [61]. In contrast, overexpression of His-tagged cytoplasmic receptors did not increase expression of membrane receptors in a recent study [28], which suggests that these are not interchangeable receptor populations. Supporting the existence of distinct receptor proteins, the amphibian brain neuronal membrane receptor has been identified as an acidic glycoprotein with an apparent molecular weight of 63 kD [62], which is inconsistent with the characteristics and predicted weight (*i.e.* 96 kD) of the cytoplasmic GR protein. Membrane receptor activation has been shown to induce rapid effects on a variety of second

messenger systems (Ca^{2+}, adenosine 3′,5′-monophosphate, inositol trisphosphate, protein kinase C) to alter cellular processes [63, 64]. In addition to membrane-associated receptors, corticosteroids could bind other receptors, ion channels, enzymes, transporters, and previously undescribed proteins in the cell membrane [27].

Intracellular receptor-initiated actions

Activation of the cytoplasmic GRs has been shown to initiate rapid nongenomic actions. Following ligand binding, heat-shock proteins (*e.g.* hsp90) and kinases of the mitogen-activated protein kinase system (*e.g.* Src) are rapidly released from the GR–chaperone protein complex, and the released proteins are thought to induce rapid cellular responses [65]. For instance, heat-shock proteins appear to mediate dexamethasone-induced increase in calcineurin activity of renal ducts [66], whereas Src proteins have been reported to be responsible for dexamethasone-induced inhibition of phospholipase A_2 in lung adenocarcinoma cells [67]. Ligand binding of the cytoplasmic GR also leads to rapid activation of the nitric oxide (NO) synthase in endothelial cells; a mechanism by which acute administration of pharmacological doses (40 $mg \cdot kg^{-1}$) of dexamethasone seem to decrease vascular inflammation and reduce myocardial infarct size following ischaemia and reperfusion injury in mice [68].

Nonspecific actions

Corticosteroids may cause rapid effects by changing the physicochemical properties of the cell membrane. The lipophilic corticosteroid molecules intercalate into the phospholipid bilayers of cellular membranes. At high concentrations, corticosteroids tend to accumulate in membranes, change membrane fluidity, and thus induce rapid changes in cellular functions [69]. Since membrane fluidity effects require corticosteroid concentrations $>10^{-4}$ M, the functional importance of this mechanism is highly questionable. Corticosteroid intercalation in the cell membrane, however, could influence the function of integral proteins (*i.e.* ion channels, transporters, and receptors) well below this concentration range [61]. For instance, membrane intercalation of corticosteroids alters membrane transport of cations and increases the proton leak of the mitochondria [70]. In smooth muscle cells isolated from human bronchial arteries, corticosteroids acutely inhibit the uptake of organic cations (or extraneuronal uptake) [60]; this is critical in norepinephrine inactivation in the airways [71]. This rapid nongenomic action is likely to increase the duration of the norepinephrine/vasoconstrictor signal, and consequently decreases airway blood flow as seen in healthy and asthmatic subjects after inhalation of corticosteroids [29]. A similar phenomenon, inhibition of norepinephrine uptake into glial cells in the brain, is proposed to enhance the accumulation of norepinephrine in the synapse, and may speed up the clinical effects of norepinephrine reuptake inhibitor antidepressant drugs [72].

THE AIRWAY VASCULATURE IN PATIENTS WITH ASTHMA

In addition to serving as the source of nutrients to the bronchial wall [73], the airway circulation has important roles in heat and water exchange [74, 75], airway hyperreactivity [7, 14, 17, 76], and possibly the regulation of airway calibre in the lung periphery [10–13, 77–80]. Furthermore, the airway circulation functions as the conduit for inflammatory cell recruitment [81, 82], and participates in the clearance of locally released, biologically active substances [83, 84] and inhaled materials transported from the surface to the deeper tissue [85].

As an airway disease characterised with continuous inflammation and repair processes simultaneously, asthma is known to lead to both functional (vasodilation, hyperperfusion, increased microvascular permeability, oedema formation, and inflammatory cell recruitment) and structural changes (angiogenesis) in the airway vasculature. The vascular changes have significant pathophysiological consequences, and thus likely participate in the clinical manifestations of asthma [86, 87]. Despite the complex actions of inflammatory mediators, neurotransmitters, and neuropeptides on vascular endothelial and smooth muscle cells, the cellular mechanisms leading to the vascular manifestations of asthma have not been completely clarified.

Increased blood flow

Hyperaemia and hyperperfusion are consistent features of inflammation. Asthma, therefore, is expected to be associated with an increased airway blood flow. In the atopic sheep model of asthma, inhalation challenge with antigen has been shown to cause increases in airway blood flow, which were related to inflammatory mediators [88–90]. Using a recently invented noninvasive method for airway blood flow measurements, these findings subsequently have been confirmed in human subjects. By measuring the uptake of an inhaled, soluble inert gas (dimethyl ether) [91, 92], airway mucosal blood flow (comprising ~70% of total airway blood flow) has been shown to be increased in patients with stable bronchial asthma [5, 29, 93, 94]. Calculated as in the volume of the conducting airways from the trachea to the terminal bronchioles (disregarding the most proximal 50 mL), mean airway blood flow values were 24–77% higher in asthmatics than in healthy controls [5, 94].

In the asthmatic airway, increased blood flow is due to dilatation of resistance arteries and increased number of vessels. In animal models, most inflammatory mediators cause vasodilation [73]. Histamine has a triphasic effect with an initial vasodilation followed by vasoconstriction, and then a long-lasting vasodilation. Sensory neuropeptides released from afferent nerves are also strong vasodilators [95]. Despite the apparent vasodilation, some mediators released in the airways of asthmatics are vasoconstrictors, including endothelin-1 [96], which seems to be negatively regulated by pro-inflammatory tumour necrosis factor-α [97]. Further studies are required to reveal the contribution of airway hypervascularity to increased airway blood flow in asthmatics.

Abnormalities of blood flow regulation

The sympathetic nervous system provides local control of airway blood flow by releasing norepinephrine, which causes vasoconstriction through activation of α_1-adrenoreceptors on vascular smooth muscle [98–100]. The α_1-adrenergic responsiveness of airway blood flow has been confirmed *in vivo*, and additionally shown to be potentiated in asthmatics [94]. This airway vascular hyperresponsiveness, which has also been

reported in animal models of atopy [99], is similar to the bronchial hyperresponsiveness to α-adrenergic agonists seen in asthmatics [101]. The possible mechanisms of asthma-associated vascular hyperresponsiveness include increased expression and function of α-adrenoceptors, altered signal transduction, impaired inactivation of α-adrenergic agonists by cellular uptake processes, or a combination thereof [102, 103]. A putative endothelial contractile factor has also been proposed to be responsible for the increased vascular sensitivity to α_1-adrenergic agonists [99].

Although β_2-adrenergic agonists cause vasodilation in the bronchial circulation predominantly through increased synthesis of endothelial NO [104, 105], recent studies disclosed blunted β_2-adrenoreceptor-mediated airway vasodilation in asthmatics [94, 106]. In addition to impaired signal transduction or decreased expression of β-adrenergic receptors [102], the attenuation of the β_2-adrenergic agonist-induced vasodilation might be due to maximal vasodilation reached in response to airway inflammation [5].

Increased vascular permeability

Microvascular hyperpermeability and oedema formation are common features of inflammation. In patients with allergic asthma, inhalation of the allergen produces both immediate and late-phase inflammatory responses during which plasma extravasation occurs from the airway microcirculation [107–109]. Asthma-associated plasma leakage is believed to occur largely through the formation of intercellular gaps between otherwise tightly associated endothelial cells in postcapillary venules [110–112]. Extravasated plasma can pass the epithelium, collect in the airways, compromise epithelial integrity and ciliary function, and thereby contribute to excessive airway secretions to form luminal mucus plugs [113]. Plasma leakage can also lead to mucosal oedema and bronchial wall swelling, which could reduce airway calibre and cause airflow limitation [80]. In addition, increased microvascular permeability may contribute to bronchial hyperresponsiveness in asthma [8, 18].

Plasma extravasation is considered as a specific response to the asthma-associated inflammatory insult involving the release of inflammatory mediators, growth factors, neuropeptides, eosinophil granule proteins, cytokines, and proteases in the airway [109, 114]. Histamine, platelet-activating factor, leukotriene D_4 and bradykinin can increase microvascular permeability through the formation of intercellular gaps [115–118]. The induced sputum levels of vascular endothelial growth factor (VEGF), which has been shown to cause fenestration of endothelial cells [119], are increased [120] and correlate with airway vascular permeability in patients with asthma [8, 121]. In contrast to findings in rodents [122, 123], the phenomenon of ''neurogenic inflammation'' seems to have a minor role in increased permeability in asthma because inhalation of the irritant capsaicin that activates unmyelinated sensory nerves to release inflammatory neuropeptides (*i.e.* substance P and calcitonin gene-related peptide) evokes no exudation of plasma in the airway in humans [124]. In addition to inflammatory exudative agents, vasodilation and microvascular congestion have been shown to increase microvascular permeability [125]. Recently, blood vessels newly generated in chronic airway inflammation have been shown to be leaky, immature, and unstable [3], which may contribute to increased vascular permeability in asthma.

Inflammatory cell recruitment

The vascular manifestations of asthma include the influx of leukocytes from the airway microcirculation into the extravascular tissues [81, 82]. The recruitment of leukocytes is directed mainly by the inflammation-associated release of peptide chemokines [126] and lipid mediators, such as leukotriene B_4 and prostaglandin D_2 [127]. Specific subsets of cell adhesion molecules on both leukocytes and endothelial cells are responsible for the different steps of leukocyte extravasation [128]. The major steps of vascular transmigration of leukocytes are generally known as leukocyte rolling along the endothelial surface, activation by inflammatory chemoattractants, binding to endothelial intercellular adhesion molecules, extravasation, and chemotaxis to sites of inflammatory tissue damage [129, 130]. While inflammation causes endothelial gaps and plasma leakage in postcapillary venules, leukocyte migration mostly occurs in collecting venules in the airway [111].

Angiogenesis and microvascular remodelling

The airway circulation is known to proliferate in a variety of pathological conditions. In addition to pulmonary artery occlusion [131], lung abscess [132], pulmonary tuberculosis [133], and lung tumours [134], the airway circulation has been shown to undergo structural changes in chronic inflammatory airway diseases [135]. Chronic inflammation leads to the growth of new blood vessels from existing ones (angiogenesis) and the alterations of the existing blood vessels (microvascular remodelling). In patients with asthma, early histological studies showed that the abnormally thick bronchial mucosa contains enlarged and congested blood vessels [136, 137]. Since then, a number of studies have demonstrated increased vascularity (*i.e.* size and number of vessels, or cross-sectional vascular area) of the bronchial mucosa in patents with asthma [10–13, 15–17, 138–141]. Asthma-associated inflammation also results in functionally altered blood vessels as indicated by abnormalities of airway blood flow regulation [29, 94].

A variety of growth factors, cytokines, chemokines, enzymes, and other factors (*e.g.* hypoxia) have been reported to induce angiogenesis [142], but their respective roles have not been clearly defined in asthma. Because the airway levels of VEGF are increased in asthma, directly correlate with airway mucosal vascularity, and inversely correlate with airway function and hyperreactivity [7, 15, 16, 120], VEGF may be a critical regulator of new vessel formation in asthma. Furthermore, in lung-targeted transgenic mice, selective overexpression of VEGF induced an asthma-like phenotype with vascular remodelling (angiogenesis, hyperpermeability, oedema formation) [143] and extravascular remodelling (mucus metaplasia, myocyte hyperplasia, airway hyperresponsiveness) in the airways [144]. In addition, tumour necrosis factor-α, transforming growth factor-α, interleukin-8 [145], matrix remodelling proteases [146], stromal cell-derived factor-1 [140], basic fibroblast growth factor [147], and angiogenin [16] have all been proposed to have some role in angiogenesis and microvascular remodelling in asthma.

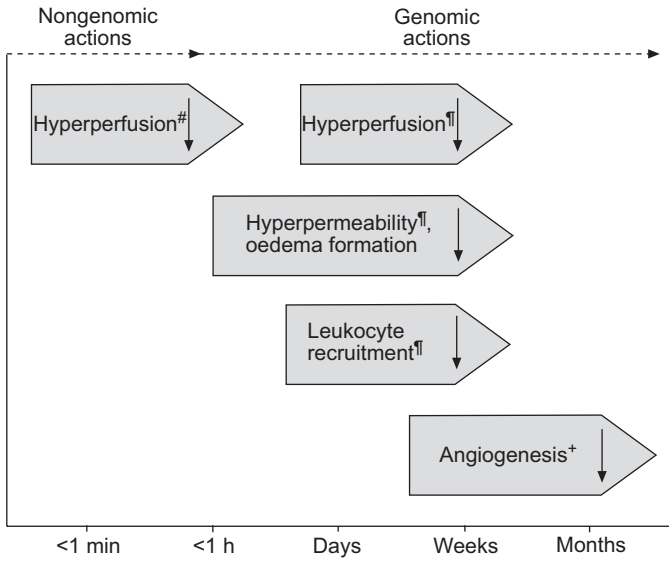

FIGURE 2. Rapid (#), delayed (¶), and long-term (+) vascular effects of inhaled corticosteroids in the airway of patients with asthma. Effects are spaced vertically on the y-axis simply to facilitate reading.

The enlarged and congested blood vessels of the airway mucosa contribute to increased airway wall thickness in asthma and, therefore, are potential contributors to airflow limitation and airway hyperresponsiveness [80]. A correlation between airway vascularity and airway hyperresponsiveness has been found in asthmatic subjects [11, 15, 16]; whether a cause–effect relationship exists remains to be demonstrated.

RAPID EFFECTS OF CORTICOSTEROIDS ON THE AIRWAY VASCULATURE

Corticosteroids exert rapid, delayed, and long-term effects on the airway vasculature in asthma (fig. 2). Among these effects, corticosteroids have been shown to acutely (within minutes) alter vascular tone through nongenomic cellular actions. In 1962, McKenzie and Stoughton were the first to report that corticosteroids applied on the skin cause blanching due to local vasoconstriction [148]. Although skin blanching after topical corticosteroids takes several hours to appear, intradermal injection of corticosteroids induces blanching within an hour, which suggests a nongenomic action. A similar phenomenon has been reported recently in the rat sciatic nerve [149] and the human bronchial mucosa [29]. Corticosteroids have also been shown to acutely enhance responses to vasoconstrictors in several systemic vascular beds, including radial [150], coronary [151], ophthalmic [152], and bronchial arteries [153]. Furthermore, corticosteroids may acutely (within 60 min) restore vasoconstrictor responses to norepinephrine in patients with medical conditions of impaired production of endogenous corticosteroids [154], and in experimental adrenalectomy [155].

Reduction of airway blood flow

Inhaled corticosteroids have been shown to acutely suppress airway hyperperfusion associated with asthma. A single dose of inhaled fluticasone propionate has been shown to decrease airway mucosal blood flow in healthy and asthmatic subjects with a maximal effect ~30 min after inhalation, and a return to baseline at 90 min [29]. The blood flow effect increased in a dose-dependent manner up to 880 μg of fluticasone propionate, with a significantly greater effect in asthmatics than in healthy controls. The acute vasoconstrictor action has also been demonstrated after inhalation of beclomethasone dipropionate and budesonide [44].

To date, evidence suggests that corticosteroids decrease airway blood flow by modulating norepinephrine-mediated (sympathetic) control of vascular tone. Pretreatment with 5 mg terazosin, which is a selective α_1-adrenoreceptor antagonist, inhibited the effect of fluticasone propionate on blood flow [153], suggesting that corticosteroids facilitate noradrenergic signal transmission to induce neurogenic vasoconstriction. There are many possible mechanisms to facilitate noradrenergic signal transmission [156]. In essential hypertension, impaired neuronal uptake of norepinephrine has been proposed to be responsible for the increased sympathetic tone [157]. Conversely, corticosteroids are known to acutely inhibit the extraneuronal uptake of norepinephrine [158], thereby increasing sympathetic signal transmission [159] and vascular tone [160]. In the airway, corticosteroids inhibit the extraneuronal monoamine transporter-mediated uptake of norepinephrine by bronchial arterial smooth muscle cells [58, 60]. This rapid effect reduces the removal of norepinephrine released from airway nerves and, therefore, may be responsible for the α_1-adrenoreceptor-dependent vasoconstrictor action of corticosteroids (fig. 3). Furthermore, the impaired extraneuronal uptake of norepinephrine in atopy, as demonstrated in ovalbumin-sensitised rabbits [103], might result in increased vasoconstrictor responsiveness to corticosteroids as seen in asthmatic subjects [29]. Corticosteroids are also expected to rapidly increase the pharmacological actions of cationic drugs inactivated by the extraneuronal uptake [161]. This notion is supported by the finding that inhaled fluticasone propionate acutely potentiates the vasoconstrictor effect of an inhaled α_1-adrenergic agonist [153].

Alternatively, the acute vascular effects of corticosteroids may involve other nongenomic actions, such as the rapid stimulation of phosphoinositide breakdown to increase intracellular inositol 1,4,5-triphosphate levels [162], the protein kinase C-dependent activation of the cell membrane Na^+/H^+ exchanger [163], and calcium mobilisation in the cytoplasm of vascular smooth muscle cells [164].

Other rapid effects

Corticosteroids could disrupt the inflammatory process through rapid inhibitory actions on cellular processes or mediators related to inflammation [65, 165, 166]. For instance, corticosteroids have been shown to rapidly inhibit the release of proteolytic enzymes by stabilising the rat liver lysosomal membranes [167], the epidermal growth factor-stimulated phospholipase A_2 activity and arachidonic acid release in A549 human adenocarcinoma cell line [67, 168]. They also inhibit cation cycling across the cell membrane and respiration in lymphocytes activated by the mitogen concanavalin A [169], phagocytosis and superoxide anion production in macrophages [170, 171], endothelial cell activation and leukocyte–endothelial interactions [165], and degranulation of peripheral blood neutrophils [172]. The rapid inhibition of the inflammatory reactions could reduce asthma-associated microvascular hyperpermeability, oedema formation, and inflammatory cell

TABLE 2 Potentially vasoactive genes and their regulation by corticosteroids in vascular smooth muscle and endothelial cells

	Upregulation	Downregulation
Vascular smooth muscle cells	α_{1B}-Adrenergic receptor [177] AT_1 receptor [179] Vasopressin V_{1A} receptor [181] NPR-A [182] ACE [183] Preproendothelin-1 [184] Na^+/K^+ ATPase [185]	Na^+/Ca^{2+} ATPase [178] Ca^{2+}-activated K^+ channel [180]
Endothelial cells	ACE [186]	eNOS [187] CAT-1 [188] Phospholipase A_2 [189] COX-2 [190]

AT_1: angiotensin II type 1; NPR: natriuretic peptide receptor; ACE: angiotensin-converting enzyme; eNOS: endothelial nitric oxide synthase; CAT-1: cationic amino acid transporter-1; COX-2: cyclooxygenase type 2.

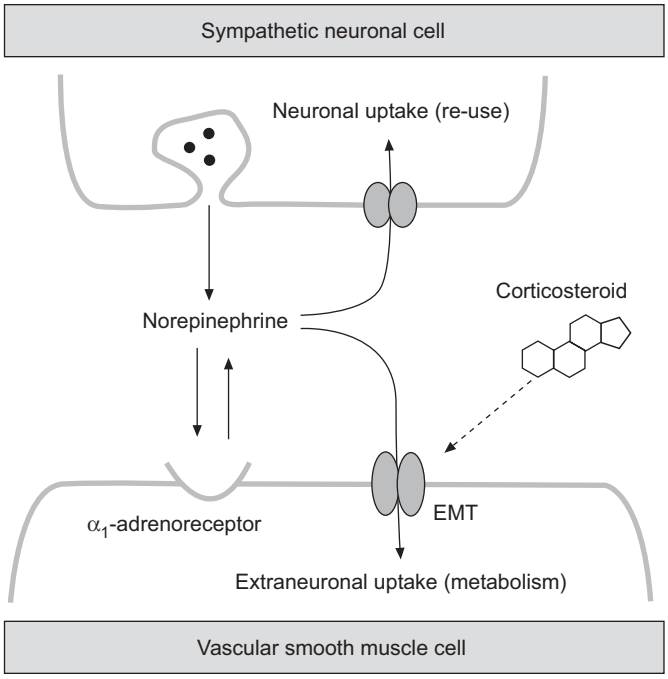

FIGURE 3. Proposed mechanism of the acute vasoconstrictor effect of inhaled corticosteroids in the airway. Corticosteroids facilitate the noradrenergic (sympathetic) neuromuscular signal transmission by rapidly (within 5 min) inhibiting the extraneuronal monoamine transporter (EMT) in vascular smooth muscle cells.

recruitment in the airways; however, these acute effects of corticosteroids are poorly documented in patients with asthma.

DELAYED EFFECTS OF CORTICOSTEROIDS ON THE AIRWAY VASCULATURE

Corticosteroids interact with the vasculature to maintain and, in excess, enhance vascular tone [173]. Impaired production of endogenous corticosteroids in various medical conditions (*e.g.* acute adrenal insufficiency) is commonly associated with systemic hypotension. In contrast, corticosteroid administration or overproduction of endogenous corticosteroids (*e.g.* Cushing's syndrome) is thought to induce systemic hypertension, partially by increasing peripheral vascular tone. The vascular effects are largely mediated through genomic mechanisms, and thus occur with a delay of hours or days.

In asthma, inhaled corticosteroids have been shown to counteract all the vascular manifestations of airway inflammation by acting directly on vascular smooth muscle or endothelium, or indirectly by inhibiting the release of vasoactive inflammatory mediators [174, 175]. Because disease severity seems to correlate with airway vascular changes in patients with asthma, these genomic vascular actions of inhaled corticosteroids may be of therapeutic value and have recently received increased interest [176].

Reduction of airway blood flow

A number of studies have demonstrated that inhaled corticosteroid therapy decreases airway mucosal blood flow in patients with asthma. In a cross-sectional study, airway mucosal blood flow was higher by ~23% in corticosteroid-naïve than in corticosteroid-treated asthmatics [5]. In interventional studies, inhaled fluticasone propionate (440 $\mu g \cdot day^{-1}$) therapy for 2 weeks reduced blood flow by ~12% and 21% in two different groups of asthma patients [6, 93], and additionally showed that airway blood returns to the pretreatment level 2 weeks after cessation of therapy.

Corticosteroids are believed to decrease blow flow in the airway mucosa by suppressing inflammation and the release of vasodilator mediators. This was supported by the fact that the effect of inhaled fluticasone propionate (440 $\mu g \cdot day^{-1}$) was similar to the leukotriene modifier montelukast (10 $mg \cdot day^{-1}$), to decrease airway blood flow in a 2-week treatment period in asthma patients [6]. Corticosteroids may also act directly on both vascular smooth muscle and endothelial cells (table 2), thereby increasing vascular tone and reducing blow flow in the airway vasculature. In endothelial cells, corticosteroids have been shown to decrease the availability of the potent vasodilator NO [187], possibly by inhibiting the endothelial NO synthase activity [188] or by enhanced NO elimination through the overproduction of reactive oxygen species [191].

In vascular smooth muscle cells, corticosteroids enhance responses to endogenous vasoconstrictors through multiple mechanisms. Among others, these may include the increases in receptor expression [179], transmembrane signalling by receptor–G protein coupling [192], calcium influx [193], and calcium sensitivity of contractile proteins [194].

Effects on blood flow regulation

Initial reports indicate that corticosteroids can restore vascular responsiveness to adrenergic agonists [94], in keeping with the observations on impaired adrenergic responsiveness of bronchial smooth muscle. Whereas the β_2-adrenoreceptor-mediated airway vasodilation is blunted in corticosteroid-naive asthmatics [94, 106], the vasodilator effect of inhaled albuterol was present in asthmatic patients on inhaled corticosteroid therapy [5]. The vasodilator response to inhaled albuterol was also restored by a 2-week treatment with inhaled fluticasone propionate (440 $\mu g \cdot day^{-1}$) [93]. Currently, there are no reports available on the effects of inhaled corticosteroid therapy on α_1-adrenergic vascular hyperresponsiveness seen in asthma patients.

Inhibition of oedema formation

Corticosteroids suppress increased microvascular permeability and oedema formation associated with airway inflammation. In the rat trachea, dexamethasone treatment reduced plasma extravasation produced by mediators of the neurogenic inflammation, such as tachykinins and substance P [195]. In ovalbumin-sensitised rats, dexamethasone treatment reduced protein extravasation in response to allergic inflammation caused by exposure to ovalbumin [118]. In patients with asthma, inhaled corticosteroid therapy has been shown to suppress the increased microvascular permeability and plasma leakage into the airway lumen, as determined by measuring concentrations of high molecular weight proteins (*e.g.* α_2-macroglobulin) in induced sputum [121, 196] and bronchoalveolar lavage fluid [14, 197]. Because increased microvascular permeability has a role in asthma-associated airflow obstruction, the anti-permeability actions of corticosteroids may be of therapeutic value.

In neurogenic inflammation, the inhibitory effects of corticosteroids are likely to be mediated by neutral endopeptidase and angiotensin-converting enzyme, since dexamethasone's action was completely reversed by inhibiting these tachykinin-degrading enzymes [198]. To inhibit airway extravasation of plasma proteins, dexamethasone seems to block mast cell degranulation, and thus the release of leukotrienes B_4 and C_4 in the airways of ovalbumin-sensitised rats in response to antigen challenge [118]. In a recent study of asthmatic subjects treated with inhaled beclomethasone dipropionate (800 $\mu g \cdot day^{-1}$), a significant correlation has been found between the reduction of airway vascular permeability and induced sputum VEGF levels [8]; this is consistent with the notion, but not definite proof, that inhaled corticosteroids might reduce airway microvascular hyperpermeability by decreasing airway levels of VEGF, a potent stimulator of vascular permeability.

Inhibition of inflammatory cell recruitment

Leukocyte and endothelial cell adhesion molecules are critically involved in leukocyte extravasation from the bloodstream into the surrounding peribronchial tissue in inflammatory airway diseases and, therefore, these molecules are generally considered as targets for anti-inflammatory therapeutic interventions [128]. By interfering with these key molecules of the recruitment cascade [199], corticosteroids inhibit leukocyte emigration from post-capillary venules into the extravascular matrix, thereby reducing inflammatory cell infiltration in the airway wall [24]. Corticosteroids are known to interfere with leukocyte and endothelial cell adhesion molecules through multiple mechanisms [165]; however, these are incompletely elucidated in asthma. Genomic actions have been shown decrease the expression of a variety of adhesion molecules (*e.g.* intracellular adhesion molecule 1 and 3, E-selectin, vascular cell adhesion molecule 1, lymphocyte function-associated antigen) involved inflammatory cell recruitment in the airways in asthma [22, 199, 200]. Corticosteroids can also suppress the production of cytokines and chemokines responsible for increased expression of adhesion molecules associated with inflammatory conditions [201, 202]. Additionally, corticosteroids have been proposed to induce the synthesis of such anti-inflammatory mediators (*e.g.* annexin I), which inhibit the functions of cell adhesion molecules [165].

LONG-TERM EFFECTS OF CORTICOSTEROIDS ON THE AIRWAY VASCULATURE

Current therapeutic approaches to suppress airway inflammation may not control all symptoms of asthma. Thus, attention has focused on inflammation-associated structural changes in the airway (collectively termed airway remodelling) in recent years. The morphological changes have been implicated in accelerated respiratory function deterioration, irreversible or partially reversible airflow obstruction, and persistent bronchial hyperreactivity seen in some patients with asthma [203, 204]. However, which elements of airway remodelling cause airflow obstruction and airway hyperreactivity and the magnitude of these effects remain subject to debate.

Among other asthma-associated structural alterations in the airway mucosa, quantitative (angiogenesis) and qualitative (microvascular remodelling) changes of airway blood vessels seem to correlate with disease severity, including lung function [12, 13] and bronchial hyperreactivity [11, 16, 18]. Thus, morphological changes of the airway vasculature are now being considered as potential targets for therapeutic intervention; however, their roles in the pathophysiology of asthma are still speculative.

Regression of airway blood vessels

Corticosteroids have a potential therapeutic role of reversing components of airway remodelling in asthma. This has been tested in some recent studies using monoclonal antibodies against type IV collagen to identify blood vessel in bronchial specimens obtained from asthmatic patients. In a cross-sectional study, therapy with inhaled beclomethasone dipropionate has been shown to be associated with a reduced area of the lamina propria occupied by vessels; however, the number of vessels decreased only in patients on high doses ($\geqslant$800 $\mu g \cdot day^{-1}$) of the inhaled corticosteroid [11]. Based on interventional studies, the inhaled daily doses and the length of therapy seem to be the critical determinants of the vascular

effects of inhaled corticosteroids. Furthermore, the inhibitory effects on the remodelling process seem to occur only with long-term therapy with corticosteroids. Whereas a 6-month treatment with a daily dose of 800 μg beclomethasone dipropionate reduced the number of blood vessels and the vascular area [139], a 6-week treatment with fluticasone propionate was only effective at a daily inhaled dose of 1000 μg, and not at 200 μg, to reduce significantly the number of blood vessels and the vascular area [17]. Moreover, in patients with asthma receiving a daily dose of 400–1000 μg of inhaled corticosteroid (beclomethasone dipropionate or budesonide), adding of a daily dose of 200 μg fluticasone propionate has not shown any effect on airway vascularity [205].

The inhibitory effects of long-term therapy with inhaled corticosteroids on airway angiogenesis presumably involve the suppression of angiogenetic factors associated with airway inflammation; however, these still need to be explored.

CLINICAL IMPLICATIONS

Recent studies suggest that inhaled corticosteroids have important roles in the regulation of airway vascular tone, permeability, and structure in patients with asthma. These vascular effects of corticosteroids on airway blood vessels can be expected to have diagnostic and therapeutic implications in the management of asthma [176].

Despite the growing appreciation of the genomic and nongenomic mechanisms that underlie vascular actions, there remain significant gaps in the understanding of the patterns of corticosteroid actions and their effects on the pathophysiology of asthma, including disease severity and persistence. Furthermore, in asthmatic patients who do no respond well to corticosteroid therapy [206], the insensitivity to both genomic and nongenomic corticosteroid actions has yet to be established.

Airway blood flow as a biomarker of inflammation

There is an ongoing search for markers of airway inflammation that can be assessed independently of lung function as an index of disease severity and used to quantitate the response to anti-inflammatory therapy in patients with asthma [207]. Several invasive and noninvasive methods have been used, including the analysis of cellular and acellular components of induced sputum and bronchoalveolar lavage fluid, histological examination of bronchial biopsy specimens, assays of inflammatory serum markers, measurements of the concentrations of exhaled gases (*e.g.* NO), and inflammatory mediators in exhaled breath condensate. With the advent of a noninvasive method to measure airway blood flow [5, 92], airway hyperperfusion has recently been proposed as a biomarker of asthma-associated inflammation, given that vascular hyperperfusion is a consistent feature of tissue inflammation. Reported reduction of airway blood flow by anti-inflammatory therapy in asthmatics lends support to this concept [6, 93].

Tissue bioavailability of inhaled corticosteroids

The standard *in vivo* screening test to rank the relative anti-inflammatory potencies and tissue bioavailabilities of topical corticosteroids is the McKenzie ''skin blanching'' test [148]. The test is based on the ability of corticosteroids to cause relatively rapid (within a few hours) vasoconstriction in the skin after topical application [208]. Despite its good correlation with GR binding affinities [209], the McKenzie test is far from ideal to predict the anti-inflammatory potencies of inhaled corticosteroids in asthma. This was demonstrated in a recent study showing no significant correlation between the effects of a 3-week therapy with inhaled budesonide (400 $\mu g \cdot day^{-1}$) on bronchial hyperreactivity to metacholine, exhaled NO levels, blood eosinophil counts and budesonide's effects on the McKenzie test in patients with asthma [210].

In vivo activity of a corticosteroid is known to depend on the rate and extent to which the drug is absorbed and becomes available at the site of action [211]. This can be tested by measuring airway blood flow responsiveness to inhaled corticosteroids, which seems to be ideal in assessing how these drugs gain access to their sites of actions in the bronchial wall [29, 44]. Thus, analysis of the rapid vasoconstrictor effect of inhaled corticosteroids can provide detailed information on their airway tissue bioavailability, which depends on the aerosolised drug's physicochemical characteristics and the aerosol-generating device. However, further studies are required to establish a link between the rapid nongenomic vasoconstrictor and the anti-inflammatory effects of inhaled corticosteroids.

Airway conductance

Airway vascular engorgement, submucosal oedema, and luminal fluid accumulation have all been proposed to contribute to the excessive airway narrowing and airway hyperreactivity in asthmatic subjects. Although the airway vasculature is known to occupy a significant portion of the inner bronchial wall [212, 213], with respect to airway blood and vascular engorgement the reports are conflicting [78, 213–215]. There is less controversy concerning the effect of airway oedema, which narrows the airways by increasing the thickness of the inner bronchial wall and contributes to airway hyperreactivity to the same degree that airway muscle shortening causes greater luminal narrowing compared with the normal airway [9, 216].

The immediate impact of inhaled corticosteroids on lung function appears to be marginal in patients with acute asthma [29, 52, 53]. The delayed inhibitory actions on airway hyperaemia, hyperpermeability, oedema formation, and angiogenesis suggest that long-term therapy with inhaled corticosteroids may significantly reduce the size of the vascular compartment, and consequently decrease airflow obstruction. However, these findings have to be confirmed by future studies.

Interactions with inhaled bronchodilators

The airway circulation has a critical role in the clearance of inhaled drugs from the respiratory tract, and the distribution of systemic drugs to the airway. In asthma, the inflammatory increase in airway blood flow is expected to enhance the clearance and, therefore, decrease the magnitude and duration of the effects of inhaled bronchoactive drugs. In contrast, increased airway blood flow could favour the distribution of systemically administered drugs to the airways. Thus, the

inflammatory increase in airway blood flow appears to have clinically beneficial, as well as undesirable effects, in the pharmacotherapy of asthma [87].

The corticosteroid-induced decrease in airway blood flow is likely to enhance the action of inhaled bronchodilators by diminishing their clearance from the airway. Although this hypothesis has not yet been directly verified in asthma patients, there is evidence supporting the enhancement of the biological effects of compounds delivered to airways with impaired blood flow [83]. Furthermore, the rapid airway vasoconstriction induced by inhaled budesonide [44] might be responsible for enhancing the bronchodilator action of inhaled formoterol as seen in patients with chronic obstructive pulmonary disease [50]. Since the corticosteroid-induced vasoconstriction peaks rapidly (~30 min after drug inhalation), simultaneous administration of inhaled corticosteroids and bronchodilators is likely to be of clinical significance.

CONCLUSIONS

The complex vascular actions of corticosteroids suggest that asthma-associated angiogenesis, hyperperfusion, hyperpermeability, and leukocyte recruitment are anti-inflammatory targets. The inhibitory effects involve genomic drug actions. The recently demonstrated rapid nongenomic actions of corticosteroids on airway vascular smooth muscle open new avenues for additional interventions in the pharmacotherapy of asthma.

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