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**Targeting inflammation in diabetes: Newer therapeutic options**

Agrawal NK *et al.* Therapeutic targets in inflammation in diabetes

Neeraj Kumar Agrawal, Saket Kant

**Neeraj Kumar Agrawal, Saket Kant,** Department of Endocrinology and Metabolism, Institiute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

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**Correspondence to: Dr. Neeraj Kumar Agrawal,** **Associate Professor**, Department of Endocrinology and Metabolism, Institiute of Medical Sciences, Banaras Hindu University, Pandit Madan Mohan Malviya Rd,  Varanasi 221005, India. drnkavns@gmail.com

**Telephone:** +91-941-5224741 **Fax:** +91-542-2367568

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**Abstract**

Inflammation has been recognised to both decrease beta cell insulin secretion and increase insulin resistance. Circulating cytokines can affect beta cell function directly leading to secretory dysfunction and increased apoptosis; as also indirectly by increasing adipocyte inflammation. The resulting glucotoxicity and lipotoxicity further enhance the inflammatory process and these results in a vicious cycle. Weight reduction and drugs like metformin have been seen to decrease the levels of CRP by 31% and 13% respectively. Pioglitazone, insulin as also statins have anti-inflammatory effects. IL-1, TNFα antagonists are in trials and NSAIDs like salsalates have shown an improvement in insulin sensitivity. Inhibition of 12-Lipo oxygenase, Histone de-acetylases, activation of Sirtuin 1 are upcoming molecular targets to reduce inflammation. These therapies have also been shown to decrease the conversion of pre-diabetes states to diabetes. Drugs like glicazide, troglitazone, N-acetylcysteine and selective COX-2inhibitors have shown benefit in diabetes neuropathy by decreasing inflammatory markers. For retinopathy drugs are used to target VEGF, Angiopoietin 2, various proteinases and chemokines. Drugs targeting the proteinases and various chemokines as also pentoxifylline, inhibitors of NF-ĸB, mTor are in clinical trials for diabetic nephropathy. Commonly used drugs like insulin, metformin, PPARs, GLP-1 agonists and DPP-4 inhibitors also decrease inflammation. Anti-inflammatory therapies represent a potential approach for therapy of diabetes and its complications.

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**Key words:** Inflammation; Insulin resistance; Diabetes; Neuropathy; Retinopathy; Nephropathy

**Core tip:** The burden of diabetes and its complications is increasing worldwide. To control this pandemic drug targeting different areas of pathogenesis of diabetes and its complications are needed. Inflammation plays a key role in progression of pre-diabetes to diabetes, in the natural history of diabetes including decreased beta cell secretory capacity and insulin resistance. Insulin resistance is an important part of the metabolic syndrome and plays a role in the pathogenesis of various macrovascular complications. Drugs targeting inflammatory pathways represent a fresh approach for the treatment of diabetes and its complications.

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**INTRODUCTION**

The incidence of both diabetes and obesity is increasing worldwide and approaching epidemic proportions. Inflammation has been recognised as a common mechanism in the pathophysiology of both these conditions. Inflammation increases insulin resistance and islet cell inflammation, which leads to defects in beta cell secretion both of which lead to diabetes. Inflammation may also be the underlying mechanism in the increased risk of cardiovascular disease in subjects with diabetes and/or obesity. Targeting inflammation hence may be a new therapeutic target in the already expanding options for management of diabetes mellitus and its complications. There is a concern over many drugs used for diabetes increasing cardiovascular morbidity and/or mortality. Targeting inflammation in diabetes theoretically will lead to better glycemic control, decrease both micro and macrovascular complications including the cardiovascular complications. Most therapies for type 2 Diabetes Mellitus target insulin resistance and drugs targeting inflammation may be a paradigm shift wherein the earlier recognition of the inflammatory status of the predisposed individual with type 2 diabetes or at risk for the development of type 2 diabetes would be evaluated and appropriate therapy embarked upon. The aim of this review is to elaborate on the drugs targeting inflammation in diabetes and its complications. Both previous studies and upcoming targets including their molecular mechanisms will be discussed in the review.

***Inflammation in diabetes***

A number of studies have demonstrated that markers of inflammation correlate with incident diabetes. Total leucocyte count which is a surrogate marker of inflammation and more specifically the neutrophil count in the higher quartiles of the normal range correlates with worsening of insulin sensitivity, and incident diabetes[1] and cardiovascular disease[2]. This suggests that a simple surrogate marker as total leucocyte count may be a marker of insulin resistance.

Insulin resistance has been defined as a state of inflammation involving both innate and adaptive immunities[3]. Islet cell inflammation as a result of autoimmune phenomenon has been recognised in type 1 diabetes mellitus (T1 DM) already and has been increasingly implicated in the pathogenesis of type 2 diabetes mellitus (T2 DM). In fact obesity has been seen to modify the development of T1 DM also. Small human studies have demonstrated that anti-inflammatory therapy has improved glycemia and beta cell function in type 2 diabetes mellitus[4,5]. Inflammation thus is recognised as one of the important pathways in the pathogenesis of T2 DM as also its complications.

The major cell involved in inflammation and insulin resistance in T2 DM is the adipocyte. Insulin regulates glucose uptake and triglyceride storage by the adipocytes. The adipocytokines in turn also affect insulin secretion and insulin resistance[6,7]. The various adipocytokines specially leptin, adiponectin, omentin, resistin, and visfatin may contribute to beta cell dysfunction by increasing insulin resistance. Adipose tissue also secretes dipeptidyl peptidase-4 (DPP-IV) which enhances the degradation of glucagon like peptide-1 (GLP-1) which has an insulinotropic effect on the on beta cells[8].

Circulating cytokines can affect beta cell function directly as also indirectly by increasing adipocyte inflammation. Cytokines like tumour necrosis factor-alpha (TNF-α), interleukin beta (IL-1β), and interferon-gamma (IFN-γ) disrupt the regulation of intracellular calcium in the beta cells and hence insulin release. TNF-α in addition increases the expression of islet amyloid polypeptide (IAPP, amylin) in the beta cells leading to their accelerated death[9]. IAPP expression and deposition by itself induces and increases beta cell inflammation[10,11]. Glucotoxicity and especially lipotoxicity increase local level of free fatty acids (FFA) in the islets and long chain fatty acids specially palmitic acid causing oxidative stress and jun N-terminal kinase (JNK) activation[12]. This further leads to increased IL-1β, TNFα, chemokine (C-C motif) ligand 2 (CCL2), IL-6, chemokine (C-X-C motif) ligand 1 (CXCL1), and IL-8 production, and activated nuclear factor (NF)-kB in human islets leading to islet cell dysfunction[13]. This overall leads to a vicious cycle of inflammation induced beta cell dysfunction which in turn again increases inflammation.

Oxidative stress is another pathway that leads to inflammation through activation of jun N-terminal kinase (JNK), NF-kB, and p38 mitogen-activated protein kinase (p38MAPK)[14]. Palmitic acid causes endoplasmic reticulum (ER) stress, oxidative stress, ceramide production, and JNK activation, all of which provoke inflammatory responses. Pancreatic islets have low antioxidant defence and are hence vulnerable to oxidative stress. There is differential regulation of oxidative stress genes in T2DM donors compared with control subjects, implicating oxidative stress in islet dysfunction[15]. Divalent metal transporter 1 (DMT1) is another factor that increases IL-1β induced insulin resistance[16]. All this suggests that oxidative stress is an important factor in the pathogenesis of T2 DM.

Endoplasmic reticulum stress also leads to increased cytokine expression and NF-kB activation causing dysfunction of the beta cells[17]. Infact cyclopiazonic acid induced ER stress has been seen to cause beta cell dysfunction through increased levels of cytokines and NF Kβ expression[18]. The levels of thioredoxin-interacting protein (TXNIP) increases rapidly in islets during ER stress provoked by thapsigargin (depletes calcium stores in the ER). Up-regulation of TXNIP results in IL-1β and IL-6 production through initiation of the inflammasome[19,20]. TXNIP also leads to induction of oxidative stress through its interaction with thioredoxin, which is a critical redox protein in cells. TXNIP expression is regulated by glucose in human islets and plays a role in glucose-induced β cell death. Therefore, TXNIP may well be a key transducer of glucotoxicity, oxidative stress, and ER stress, feeding into various inflammatory pathways in islets.

The gut may also be involved in the development of diabetes mellitus. Obesity leads to increased lipopolysaccharide (LPS) from the gut which causes activation of toll like receptor 4 (TLR4) and NF-kB leading to decreased insulin gene expression and insulin secretion in rat and also human islets[21]. There is data to suggest that colonization of the gut by specific bacterial species alters the development of autoimmunity in NOD mice and can modify the cytokine and chemokine profile leading to islet cell inflammation[22].

With all this in mind, the search for anti-inflammatory therapies for diabetes was started. Life style modification and drugs already in use for the management of diabetes also have additional anti-inflammatory effects. In the Diabetes Prevention Program (DPP) weight reduction decreased the levels of C-Reactive Protein (CRP) by 31% whereas metformin decreased it only by 13%[23]. Similar results have been observed with surgical weight loss procedures[24]. This implies that life style interventions, even without drug therapy, can decrease insulin resistance; and decrease progression of pre-diabetes states to T2 DM as also can decrease the progression of DM and its complications by decreasing inflammation. Drugs like thiazolidinedione for the same degree of glucose reduction have been seen to reduce markers of inflammation to a greater extent as compared to other therapies[25]. This may be a result of peroxisome proliferator–activated receptor-γ (PPAR-γ) transrepression of inflammatory-response genes[26]. This points towards the fact that reduction of inflammation adds to the beneficial effects of these drugs which is independent of the effect on glucose levels and thus is a direct effect.

Insulin therapy by itself over the short term has been associated with a decrease in inflammation. This effect is mediated by the decreased activity of Nuclear Factor Kappa B (NF-kB) which is the master transcriptional regulator of the inflammatory response[27]. This effect of insulin is however temporary and/or requires higher doses of intravenous insulin[28]. This may be one of the additional advantages of adding insulin early in the course of T2 DM and may delay the progression of DM and its complications.

One class of drugs that are used widely in diabetes mellitus that also have anti-inflammatory effects are statins. Statins inhibit hydroxymethylglutaryl-CoA reductase and hence cause a reduction in cholesterol levels. In addition to this statins have also been seen to reduce the levels of CRP by 25%-30%[29].This is a class effect of all statins and is not dose dependent. The decrease in CRP levels does not correlate with the decrease in the lipid levels which implies this effect is a direct effect of statins. CRP is an independent predictor of cardiovascular events. The JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial assessed the effect of rosuvastatin on rates of primary cardiovascular events in people with high CRP concentrations but without hyperlipidemia (CRP > 2 mg/L; LDL < 130 mg/dL) [30]. The CRP concentration was reduced by 37%, however, the LDL concentration was reduced by 50%, so it is uncertain whether the effects of statins are truly mediated via the anti-inflammatory process, or are a result of just its lipid-lowering effect. In addition, incident type 2 diabetes increased in the statin-treated patients, an effect seen with other agents in the statin class[31]. This finding demonstrated a divide in the association of inflammation, diabetes, and cardiovascular disease that may be explained by the potent effects of statins on lipids. Apart from CRP, statins do not have any effect on any other markers of inflammation like fibrinogen.

**NEWER THERAPEUTIC TARGETS**

These drugs are in trials for targeting inflammation and are not yet available as prescription drugs for diabetes

***Etanercept***

Etanercept (934 amino acids,150 Kilo Dalton) is a dimeric fusion protein with an extracellular ligand binding domain of the Human Tumor Necrosis Factor Receptor (TNFR) linked to the Fc component of human IgG1.It is produced by recombinant DNA technique in Chinese Hamster Ovary (CHO).

Blockade of TNF-α receptor has been shown to decrease insulin resistance in obese rats[32]. A trial of etanercept failed to improve insulin sensitivity in subjects with the metabolic syndrome despite lowering C-reactive protein[33]. The possible explanation offered for the lack of effect was that TNF-α is present in about double concentration intracellularly than in the extracellular space and it is the intracellular TNF-α that is responsible for insulin resistance via the paracrine effects which could not be blocked by etanercept.

***Anakinra***

Anakinra (153 amino acids, 17.3 kilo Dalton) is a non glycosylated form of the Human Interleukin-1 Receptor antagonist (IL-1Ra) from which it differs only by an addition of a single methionine residue at the amino terminus. It is produced by recombinant DNA technique in E.Coli.

IL-1 contributes to impaired insulin secretion, decreased cell proliferation, and apoptosis of pancreatic β cells. The IL-1 receptor antagonist is endogenously produced, and its concentrations are reduced in the pancreatic islets of patients with type 2 diabetes mellitus. Anakinra was studied in type 2 diabetes mellitus and showed promise in increasing beta cell secretory function, reduced glycemia and markers of systemic inflammation[34]. Definitive conclusions on the possible clinical utility of IL-1 receptor antagonist in prevention of diabetes are awaited from the large ongoing Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) phase III clinical trial[35]. The study is being conducted in more than 40 countries around the world and is specifically testing whether blocking the pro-inflammatory cytokine interleukin -1β (IL-1β) with canakinumab, as compared to placebo, can reduce rates of recurrent myocardial infarction, stroke, and cardiovascular death among patients with history of myocardial infarction who remain at high risk due to a persistent elevation of the inflammatory biomarker hsCRP (≥ 2mg/L) despite getting best medical care.

***Salsalates***

Salasalates belong to the class of Nonsteroidal Anti-Inﬂammatory Drugs (NSAIDs) which exert their anti-inflammatory effect through inhibition of prostaglandin G/H synthase, or cyclooxygenase. These enzymes catalyse the transformation of arachidonic acid to prostaglandins and thromboxanes. NSAIDs also inhibit the expression of cell adhesion molecules which play a role in targeting circulating cells to inflammatory sites and also directly inhibit activation and function of neutrophils.

Trials with high dose salsalates in rodents[36] and in subjects with diabetes[37] have shown that salsalate by inhibiting Inhibitor of nuclear factor kappa-B kinase subunit beta (IKK β) decreases glucose intolerance and increases insulin sensitivity. In an open label study, salsalate, a prodrug form of salicylate, reduced fasting and post-challenge glucose levels and increased glucose utilizationin euglycemic, hyperinsulinemic clamp studies[37].Circulating FFAs were reduced and adiponectin levels were increased. In another study salsalate, when compared with placebo, reduced fasting glucose by 13% (*P* < 0.002), glycemic response after an oral glucose challenge 20% (*P* = 0.004), and glycated albumin by 17% (*P* < 0.0003). Although insulin levels were unchanged, fasting and oral glucose tolerance test C-peptide levels decreased in the salsalate-treated subjects compared with placebo (*P* < 0.03), consistent with improved insulin sensitivity and a known effect of salicylates to inhibit insulin clearance. Adiponectin increased by 57% after salsalate treatment compared with placebo (*P* < 0.003). Additionally, within the group of salsalate-treated subjects, circulating levels of C-reactive protein were reduced by 34% (*P <* 0.05)[38]. All this proves that salsalate reduces glycemia and may improve inflammatory cardiovascular risk indices in overweight individuals. These data support the hypothesis that sub-acute to chronic inflammation contributes to the pathogenesis of obesity-related dysglycemia and that targeting inflammation may provide a therapeutic option for diabetes prevention. The effects of salsalate on inflammation are however controversial as shown by another study in which salsalate did not change flow mediated dilatation (FMD) in peripheral conduit arteries in patients with T2DM despite lowering HbA1c. This finding suggests that salsalate does not have an effect on vascular inflammation[39].

**VITAMIN D**

Calcitriol exerts regulatory effect on molecular pathways involved in inflammation, such as inhibition of PG synthesis and actions, inhibition of stress-activated kinase signaling and the resultant production of inflammatory cytokines, as also inhibition of nuclear factor κB (NF-κB) signaling and the production of pro-angiogenic factors. Clinical trials investigating the effects of vitamin D supplementation on serum levels of inflammatory markers have provided inconsistent results, with no evidence of effects in most trials, or effects on selected markers in few others[40]. Similarly, available trials have shown no convincing benefits of vitamin D supplementation on plasma glucose levels and insulin resistance[41,42]. This systematic review and meta-analysis showed that vitamin D supplementation had a small improvement on fasting glucose and insulin resistance among people with diabetes or impaired glucose tolerance, but no effect on glycated haemoglobin among those with diabetes. The role of Vitamin D supplementation hence requires further well planned trials.

**CHLOROQUINE**

Chloroquine is a weak base and carries a positive charge at acidic pH. It is this property of the drug that makes it to selectively accumulate in lysosomes and generate a concentration gradient of a high order. This lysosomatotrophic action is responsible for the hepatic retention of insulin. Another action of the drug is decreased degradation of insulin in the muscle tissue.

A retrospective study suggested that the use of chloroquine to treat rheumatoid arthritis is associated with a lower incidence of type 2 diabetes[43]. This however is a study in a specific group of patients who required the drug for another indication. Prospective studies of chloroquine are ongoing and the results are awaited.

**DIACERIN**

Diacerin a semi-synthetic anthraquinone derivativedirectly inhibits IL-1 synthesis and release in vitro and downregulates IL-1 induced activities. It has been shown to possess disease modifying effect in osteoarthritis.

In a randomized double-blind, placebo-controlled trial, 2-mo treatment of drug-naive T2DM patients with diacerin increased insulin secretion without changes in insulin sensitivity[44]. This implies a direct effect of the drug on Beta cell function.

***Other emerging therapies***

**Inhibition of 12-Lipo oxygenase:** Twelve-Lipo oxygenase (12-LO) produces pro-inflammatory arachidonic acid products and is upregulated in islets of both type 1 and type 2 diabetes mellitus[45] to the pro inflammatory products lead to insulin resistance and islet cell dysfunction. Hyperglycemia as also inflammatory cytokines increase expression of 12-LO[45,46].The activation of 12-LO has also been implicated in causing adipose tissue inflammation and insulin resistance. In NOD mice (T1DM model), Zucker diabetic fatty (ZDF) rats (T2DM model), and diet-induced obese mice (T2DM model) gene deletion and pharmacological suppression of 12-LO prevented the development of diabetes[47,48]. All this points towards inhibition of 12- LO being a promising target in both Type 1 and Type 2 Diabetes Mellitus for decreasing insulin resistance, β cell dysfunctionand cardiovascular complications.

**Histone de-acetylases inhibition:** Histone de-acetylases (HDAC) I, IIA, IIB, III and IV are involved in inflammatory responses in a variety of conditions including diabetes. HDAC inhibitors cause acetylation of the p65 subunit of NF-kB leading to its inhibition and hence the decrease the inflammatory response. As of today there is no human data, but animal data supports the role of HDAC inhibition in β cell preservation. Linkage analysis also has revealed that a locus in 6q21, associated with both T1DM and T2DM, lie near HDAC2. Beta cell mass expansion has been observed with HDACIIA. In streptozotocin (STZ)-induced diabetes, ITF2357 an orally active inhibitor against class I and II HDAC lead to prevention of diabetes[49].

**Sirtuin 1:** Sirtuin 1 (Sirt1) is a NAD+- dependent HDAC class III deacetylase. Some of the SIRT1 deacetylation substrates (PGc1a, FoXo, p53, and the p65 subunit of nuclear factor-kb (nF-kb)10,41–43 proteins) are central regulators of cellular metabolism, energy expenditure, inflammation and stress response pathways in the cell. These may be an additional target in reducing inflammation. Activation of Sirt1 may have an antiinflammatory role to play in the islets. Sirt1 over expression prevents NF-kB mediated cytokine-induced β cell damage and its expression has been seen to be reduced in pancreatic islets after cytokine exposure[50]. Nicotinamide mononucleotide (NMN), a metabolite that augments sirtuin action, rescues islets from reduced insulin secretion after IL-1β and TNF α exposure[51].

Identification of targets of each class of HDACs in human islets under inflammatory conditions will aid in therapeutic application of this emerging class of agents.

**FAT-1 transgene:** Long-chain n−3 PUFAs act directly by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism as also indirectly by altering the expression of inflammatory genes through effects on transcription factor activation In addition they increase anti-inflammatory mediators like resolvins. Thus, n−3 PUFAs are potent anti-inflammatory agents. The FAT-1 transgenic mouse, which expresses the Caenorhabditis elegans fat-1 gene encoding an n-3 fatty acid desaturase that converts n-6 to n-3 fatty acids (which is absent in mammals) shows augmented production of n-3 polyunsaturated fatty acids. This has been shown to be protective against development of diabetes after multiple low dose STZ injections, and displays lower levels of IL-1β, TNF α, NF-kβ and 12-HETE[52]. This can be an additional target for inflammation in type 2 diabetes.

Recent studies have indicated that elF5A a ancient and poorly understood protein is an important regulator of cytokine release and signalling. This protein is the only protein which contains the unique amino acid hypusine, which is a modified amino acid lysine residue. Hypusine modification by inhibitable enzymes deoxyhypusine synthase and deoxyhypusine hydroxylase is required for eIF5A action in cytokine signalling. This modification therefore may well be a new therapeutic target for preventing beta cell decline in the setting of diabetes inflammation[53]. Anti inflammatory therapeutic targets has been used for decreasing the conversion of prediabetes to diabetes as also for the progression of T2 DM. Anti-inflammatory therapies have also been used as treatment modalities for the complications of T2 DM and are detailed as follows.

***Therapeutic treatments targeting inflammatory mediators in diabetic neuropathy***

The various proposed mechanisms for diabetic neuropathy include increased reactive oxygen species production, increased protein glycosylation, neurovascular disturbances, and decreased neurotrophic support. Mice models have shown that NF-ĸb activation is associated with diabetes neuropathy. Toll like receptors can also activate NF-ĸb and lead to increased expression of cytokines and chemokines. The levels of pro-inflammatory cytokines, chemokines and TNF-α have been shown to be increased in mouse and human models although the pathogenesis is not yet clear. Rodent studies revealed that increased COX 2 expression lead to a decrease in sensory and motor nerve conduction velocities (NCV), endoneurial blood flow, and intraepidermal nerve fiber density compared to non-diabetic mice. This led to the trials of COX-2 inhibitors and other anti-inflammatory drugs in diabetic neuropathy.

Monocytes from type 2 diabetic patients demonstrated increased expression of TNF-α, IL-1, IL-6, and IL-8 as compared to healthy controls and type 1 diabetic patients; treatment of these monocytes with 1,25-dihydroxyvitamin D3 has been seen to down regulate the mRNAs of these cytokines[54]. The natural flavonoid, curcumin lead to a dose-dependent decrease in the serum TNF-α levels and attenuated thermal hyperalgesia in STZ-treated mice[55,56]. The beneficial effect of this treatment was further enhanced with the use of insulin[57]. Other agents capable of preventing inflammatory mediated events in rodent models include glicazide and troglitazone both of which attenuate TNF α levels. Both these treatments also prevented decreases in myelinated fiber area, fiber density, and axon/myelin ratio in the tibial nerve of diabetic rats[58,59].

The anti-oxidant N-acetylcysteine, in a dose dependent manner decreases TNF-α levels[60] which translates into decreased incidence or severity of neuropathy.

The expression of COX-2 is increased in the peripheral tissues of diabetic neuropathy models. Piroxicam statistically improved STZ-induced decreases in sensory neuron action potential amplitude[61]. The non-selective inhibitors, sulindac and indomethacin, decreased losses in sural and caudal sensory nerve conduction velocity of diabetic rodents compared to control mice[62,63]. Some non-selective COX inhibitors are effective treatment options but flurbiprofen alone decreased motor nerve conduction velocity (MNCV). In fact Flurbiprofen treatment mimicked Streptozocin(STZ) induced changes and did not reverse/alter STZ-induced changes on MNCV[64]. All these point towards the fact that COX-1 maintains neural function in rodents. After this observation, studies were planned to assess the efficacy of COX-2 inhibitors. It was found that Celecoxib treatment prevents decrease of MNCV and sensory nerve conduction velocity (SNCV slowing)[65], and meloxicam was shown to protect against MNCV slowing and endoneurial blood flow deficits in diabetic rodents. Intrathecal administration of COX-2 inhibitors lead to a dose dependent attenuation of mechanical behaviour[66]. Selective inhibition of COX-2 via pharmacological or gene inactivation played a preventive role in the increased TNF-α expression in the sciatic nerve of STZ-induced diabetic rodents[67]. However clinical studies with the drugs are lacking. Only one study evaluating NSAID treatment in diabetic patients has been done which demonstrated an improvement of neuropathy score with ibruprofen and sulindac treatment compared to placebo[68]. These results should however be interpreted with caution considering that there were no healthy age matched controls. The study only compared responders with the non-responders. NSAIDS are a double edged sword in that they have to be very cautiously used for the long term considering their well-known side effects. Although selective COX-2 inhibitors do not have the gastrointestinal side effects but the cardiovascular side effects are of concern especially in the high risk for cardiovascular disease population of which subjects with DM form a part. It is however clear that the agents targeting inflammation in diabetic neuropathy are effective only if targeted very early in the course of neuropathy. Evidence demonstrating their effectiveness after the development of DN in reversing any of the symptoms of DN such as reductions in nerve conduction velocities or nociceptive behaviour is however lacking. Larger studies investigating the time course of anti-inflammatory therapeutics should be planned. Current studies have demonstrated no reversal of DN and the benefits observed also are only have a treatment period of at least 12 wk[69,70]. Overall, more studies are needed to validate these findings.

***Therapeutic treatments targeting inflammatory mediators in diabetic retinopathy***

Hyperglycemia increases AGE formation, reactive oxygen species and leads to Nitric oxide synthatase dysregulation leading to activation of NF-ĸb followed by increased cytokines (IL-1,6,TNF-α), chemokines like CCL-2,58,10,12 and adhesion molecules like Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1).This leads to activation of endothelial cells, recruitment of inflammatory cells, increased levels of *vascular* endothelial growth factor (VEGF) and Angiopoietin 2. .All these are involved in the pathogenesis of increased capillary permeability, capillary dropouts and neo-vascularization.

The various therapies used as anti inflammatory therapies in diabetes retinopathy hence target VEGF, Angiopoietin 2, various proteinases and chemokines.

The most important factor investigated extensively in alteration of the blood retinal barrier (BRB) is VEGF. Levels of VEGF are elevated significantly in patients with diabetic macular edema (DME) as compared to non-diabetic eye diseases[71,72]. VEGF is a potent vasoactive cytokine which increases vascular permeability. The major effect of VEGF is on endothelial tight junction proteins, leading to extravasation of fluid and hence retinal edema. It also induces the phosphorylation of VE-cadherin, occludin, and ZO-1, causing a disruption of the barrier[73].

In addition it also stimulates increased leukostasis in the microvasculature of retina, which also leads to breakdown of the BRB[74,75].

Most of the clinical trials in retinopathy have hence targeted VEGF. Direct VEGF inhibitors include the anti-VEGF aptamer, pegaptanib, the monoclonal antibody fragment Ranibizumab and the full length antibody bevacizumab. Other drugs include soluble VEGF receptor analogs, VEGF-Trap, small interfering RNAs (siRNAs) bevasiranib, and rapamycin (Sirolimus). Some studies have shown that after two years, the mean change in the visual acuity letter score from the baseline was 3.7 letters greater in the ranibizumab and prompt laser group, 5.8 letters greater in the ranibizumab and deferred laser group, and 1.5 letters worse in the triamcinolone and prompt laser group[76]. It is however important that response to the anti-VEGF treatments in DME is variable, and is not as robust as in proliferative diabetic retinopathy (PDR) or neovascular glaucoma. This implies that the pathogenesis of DME is multifactorial and anti VEGF therapy is only but one player in the overall pathogenesis.

Angiopoietins are another class of inflammatory growth factors that are important modulators of angiogenesis. The levels of angiopoietin-2 (Ang-2) are significantly elevated in patients with clinically significant macular edema (CSME)[77] indicating that it alters the blood-retinal barrier. In another study increased expression of Ang-2 mRNA and proteins has been demonstrated in the retinas of diabetic animals[78]. Even in non-diabetic rats intra-vitreal injection of Ang-2 lead to a three-fold increase in retinal vascular permeability. Ang-2 also induces phosphorhylation and loss of VEcadherin[78]. Recent data has suggested that Ang-2 sensitizes the endothelial cell to TNF α induced ICAM 1 expression and hence monocyte adhesion. This implies that Ang-2 is an autocrine regulator of endothelial cell inflammatory responses. Ang-2 therefore plays a permissive role for the augmentation of pro-inflammatory cytokines[79]. This molecule maybe an important therapeutic target in DME. Ang-2 inhibitors in various tumor models have been found to be effective in preventing tumor growth through the modulation of monocyte infiltration and angiogenesis[80]. Matrix metalloproteinases (MMPs) are a major regulator of innate and acquired immunity[81]. Knockout mice models have shown that these molecules play an important role in both acute and chronic inflammation[82]. It has also been shown that MMPs are important for the proteolytic alteration and hence activation of chemokines. They cleave many members of the CCL / monocyte chemoattractant protein (MCP) family of chemokines rendering them proactive that amplifies the inflammatory response. Furthermore, MMPs organise the recruitment of leukocytes as an essential component of tumor-associated inflammation[83].It is now evident that MMPs also play an important role in the pathogenesis of diabetic retinopathy. The vitreous level of proteinases, like MMP9,are higher in diabetic subjects with DR thanwithout DR[84]. Both MMP2 and MMP9 are elevated in the retina of animal models with early DR[85]. The retinal vascular permeability in diabetic animals is significantly increased which is a result of a decrease of cell–cell junctional protein, VE-cadherin. MMP inhibitors can decrease this vascular permeability[86]. This implies that the proteolytic degradation of VE-cadherin contributes to the BRB breakdown. This is an evidence for the role of extracellular proteinases in the alteration of the BRB seen in diabetic retinopathy[87]. Hyperglycemia can activate many soluble mediators such as advanced glycation endproduct (AGE), reactive oxygen species (ROS), and inflammatory cytokines, which can increase MMP levels and activity in a diabetic state. Retinal inflammation leads to increased leukocyte infiltration in the retina, which by binding to the endothelial cells activates cellular proteinases such as elastase, followed by removal of VE-cadherin and its associated protein from the cell surface, resulting in alterations in endothelial monolayer[88]. All these studies point towards an important role for these proteinases in DR.

The levels of many chemokines have been seen to be elevated in various studies.The most common chemokine that is elevated in serum and vitreous Chemokine Ligand 2 (CCL2) [89,90]. CCL2 also known as the monocyte chemotactic protein-1 (MCP-1) plays an important role in vascular inflammation by inducing leukocyte recruitment and activation. Hyperglycemia increases the CCL2 / MCP-1 generation from retinal vascular endothelial cells, pigmented epithelial cells and Muller’s glial cells[91]. Furthermore the gene polymorphism of CCL2 has been indicated as a potential risk factor for diabetic retinopathy[92].

Studies have shown that a genetic knock out of the CCL2 gene in diabetic mice plays a preventive role in the alteration of blood retinal barrier[93] and that selective inhibition of the CCL2 gene can prevent the alteration of the BRB in diabetes. Further studies using selective inhibitors of CCL2 and CCR2 are in progress.

Genistein, a tyrosine kinase inhibitor has been shown to be effective in dampening diabetes-induced retinal inflammation by interfering with inflammatory signaling (ERK and P38 MAPKs) that occurs in the activated microglia. This beneficial effect of genistein may represent a new intervention therapy to modulate early pathological pathways long before the occurrence of vision loss among diabetics[94].

**THERAPEUTIC TREATMENTS TARGETING INFLAMMATORY MEDIATORS IN DIABETIC NEPHROPATHY**

Inflammation activated by the metabolic, biochemical and haemodynamic derangements may play a key role in the development and progression of diabetic nephropathty. Cytokines like Il-1,6 and TNF-α stimulates expression of cell adhesion molecules and profibrotic growth factors, increase endothelial permeability, promotes mesangial proliferation, glomerular hypertrophy and promote production of ROS. Chemokines like Protein kinase C (PKC) dependant ICAM-1, VCAM-1 and MCP-1 facilitate leukocyte-endothelial adhesion and infiltration into diabetic kidneys. Adiponectin is protective in that it reduces oxidative stress, production of TNF-α, and leukocyte-endothelial adhesion. Adiponectin has also been seen to interfere with receptor activation for platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Increased mTOR activity has been seen to cause glomerular hypertrophy and hyperfiltration in diabetes subjects.

Adenosine is a potent autocrine anti-inflammatory and immunosuppressive molecule that is released from cells into the extracellular space at sites of inflammation and tissue injury. The levels of adenosine, an endogenous purine nucleoside released from various tissues and organs are decreased in diabetes nephropathy (DN)[95]. Diabetic nephropathy is more severe in A2A receptor Knockout mice than WT mice, which suggests that endogenous adenosine might contribute to kidney protection from diabetes in a similar manner as it does in kidney ischemia-reperfusion injury[96]. MCP-1/CCL2 Inhibition using propagermanium ameliorated diabetic glomerulosclerosis and is another targets for DN[97]. However clinical inhibitors of CCL2 have shown only partial effects[98]. Even with CCL2 knockout, only a reduction in albuminuria was observed[99].

Pentoxifylline inhibits the expression of mRNA levels of TNF-α[100]. In combination with angiotensin-converting enzyme inhibitors (ACEI) and AT1 receptor blockers (ARB), pentoxifylline decreased albuminuria in diabetic nephropathy[101,102].

In a prospective, randomized, double-blind, placebo- controlled study pentoxifylline (1200 mg daily) for 12 mo, in 34 patients with incipient or established DN had a reno-protective effect determined by a significant reduction in the urinary albumin excretion (UAE) in both incipient and established (*P* < 0.01) DN patient. This effect was attributed to a reduction in the C-reactive protein, interleukin-6, tumor necrosis factor-alpha and serum leptin levels (*P* < 0.01)[103].

The results from 7 animal studies, 13 randomized controlled trials on diabetic kidney disease consistently demonstrate that short-term use of pentoxifylline produced a significant reduction of proteinuria and microalbuminuria in patients with diabetic and also non-diabetic kidney diseases. The reports of long-term studies also show that urinary protein excretion exhibits a considerable reduction in patients treated with PTF; however, since these results are mostly based on small clinical trialsit is not clear whether the additive anti-proteinuric effect of pentoxifylline is sustained over time. Large scale clinical trials are needed to establish the long-term use of pentoxifyllineas a pharmacological alternative for delaying or preventing the development of endstage renal disease (ESRD)

Adiponectin has been seen to suppress inflammatory markers including TNF-α, receptor activation for platelet-derived growth factor (PDGF), epidermal growth factor (EGF) or fibroblast growth factor (FGF). Adiponectin has been seen to preserve nephrin, decrease expression levels of TGF-β, and reduce albuminuria .

Inhibition of NF-κB in kidney using peroxisome proliferator-activated receptor-γ (PPAR-γ)[104], ARB[105], or pentosan polysulfate (PPS)[106] has been shown to ameliorate DN in animal models. However efficacy of inhibition of NF-κB in delaying progression of DN has not been reported.

HMG-CoA reductase inhibitors (statins) in DN have a controversial role.In a subanalysis of the Treating to New Targets (TNT) study, treatment with 10 mg and 80 mg atorvastatin was found to increase estimated glomerular filtration rate (eGFR)[107], while in the Prevention of Renal and Vascular End-Stage Disease Intervention Trial (PREVEND-IT), treatment with 40 mg pravastatin did not result in any increase in eGFR[108].

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that mediates cell proliferation, survival, size, and mass[109]. Rapamycin decreases hyperglycemia induced increase in mTOR activity and thus decrease the renal changes in DN, including mesangial expansion and glomerular basement thickness[110]. Rapamycin also significantly reduces the influx of monocytes and macrophages which is associated with progression of DN[111,112]. It also has been seen to decrease the release of pro-inflammatory cytokines or chemokines including MCP-1, regulated and normal T cell expressed and secreted (RANTES), IL-8, and fractalkine[111,112] Rapamycin thus represents a new and valuable anti inflammatory target in DN.

A recent study has shown that aspirin could decrease albuminuria in patients with DN[113]. In combination with AT1 receptor blockers (ARB) it lead to a further decrease in the progression of DN and inflammatory markers compared to when used alone[114]. This effect of COX-2 inhibitors is postulated to occur as a result of the effects on renal hemodynamics and decrease in profibrotic cytokines[115]. In another study however, treatment with 200 mg/day COX-2 inhibitor for six weeks did not decrease DN[116]. The overall data for COX-2 inhibitors in DN thus remains controversial.

Protein Kinase C (PKC) is induced by hyperglycemia and insulin resistance. This PKC activation then alters cell signaling molecules including inflammatory cytokines such as NF-κB, IL-6, TNF-α, and plasminogen activator-1 (PAI-1) in endothelial and mesangial cells[117-119]. Ruboxistaurin (RBX), a PKCβ isoform selective inhibitor, has been shown to prevent DN in rodent DN models through inhibition of mediators of extracellular matrix accumulation, TGF-β and amelioration of insulin signalling[120]. Diabetic PKCβ null mice have shown decreased albuminuria and mesangial expansion[121]. A phase II clinical trial with RBX significantly decreased albuminuria and maintained a stable eGFR[122]. Recently, it has been seen that hyperglycemia itself can activate PKCβ isoforms, which increases the detrimental effects of angiotensin II (Ang II) on glomerular endothelial cells and decrease glucagon-like peptide-1 (GLP-1) receptor, leading to resistance to GLP-1 treatment in DN[123]. Some recent findings suggest that hyperglycemia also activates PKCδ and p38 mitogen-activated protein (MAPK) to increase Src homology-2 domain-containing phosphatase-1 (SHP-1) and causes VEGF resistance and independent NF-κB activation to induce podocyte apoptosis in DN[124] which can be new targets of treatment.

Exogenous insulin has been shown to inhibit the activation of TNF-α in animal models[125]. Furthermore insulin inhibits MCP-1 expression and activation of NF-κB in endothelial cells[126]. Recent studies in patients with type 2 diabetes have shown that insulin treatment decreases expressions of inflammatory cytokines, such as MCP-1, ICAM-1, soluble VCAM-1 (sVCAM-1), TNF-α, and IL-6[127,128].

Insulin can increase endothelial nitric oxide (NO) production by rapid post-translational mechanisms, mediated by the PI3K/Akt signaling pathway, leading to vasodilatation, antithrombotic effect, and anti-inflammatory actions[129-131]. Insulin not only stimulates NO production but also increases the expression of endothelial NO synthase (eNOS) [132]. Recent data indicates that vascular endothelial cell specific insulin receptor knockout mice had decreased eNOS expression in aorta[133]. Thus insulin resistance in vascular tissue could contribute to DN. However, till date the efficacy of exogenous NO donor remains unclear. Insulin and metformin were studied in a trial for 14 wk. Despite substantially improving glucose control, neither insulin nor metformin reduced inflammatory biomarker levels including hsCRP, IL-6, and sTNFr2 for the main effects evaluated or in comparisons between the individual treatment groups (placebo metformin only; placebo metformin and insulin; active metformin only; or active metformin and insulin)[28].

Peroxisome proliferator-activated receptors (PPARs) regulate insulin sensitivity, lipid metabolism,adipogenesis and cell growth[134-137]. Recent studies indicated that PPAR-γ agonist decrease expression of inflammatory markers such as PAI-1, ICAM-1, and NF-κB in the kidney in DN and ameliorate renal function[138].

Analysis of GLP-1 receptor (GLP-1R) has revealed its expression in endothelial cells and kidney[139,140]. In endothelial cells, GLP-1 inhibits the expression of TNF-α and VCAM-1[141]. GLP-1 acts on the glomerular endothelial cells and decreases the signaling pathway of AngII at phospho-c-Raf (Ser338)/phospho-Erk1/2 via phospho-c-Raf(Ser259) activated by cAMP/PKA pathway. Administration of GLP-1 in DN decreases inflammatory markers including PAI-1, CD68, IL-6, TNF-α, NF-κB, and CXCL2 in the kidney[117].

Dipeptidyl peptidase-4 (DPP-4) inhibitors provide vascular protection by increasing GLP-1’s bioavailability and its action. They also have been reported to decrease the levels of MCP-1. In addition to this they have vasotropic actions and the possibility of an actual reduction in DN[142]. A recent large phase III data shows that linagliptin significantly reduces albuminuria by 30% in DN[143]. However, the role of DPP-4 inhibitors in the regulation of inflammatory cytokines and vasotropic actions remains largely unexplored and open to further trials.

**DIABETES, THE METABOLIC SYNDROME AND NON ALCOHOLIC FATTY LIVER DISEASE**

Type 2 Diabetes mellitus is a part of metabolic syndrome and Non Alcoholic fatty liver disease(NAFLD) shares insulin resistance as a common pathophysiology with Type 2 Diabetes Mellitus. More recently NAFLD has been proposed but not yet accepted as a criteria to define metabolic syndrome[144]. Hepatic insulin resistance has a key role to play in the pathogenesis of NAFLD and adiponectin the most abundant adipocytokine decreases both hepatic and systemic insulin resistance by decreasing inflammation[145]. Adiponectin and its agonists hence may be promising targets to reduce both hepatic and systemic insulin resistance[146,147]. Exercise in addition to the benefit of reducing weight and insulin resistance also reduces the levels of inflammatory cytokines incriminated in diabetes associated NAFLD[148]. Omega-3 polyunsaturated fatty acids(n-3 PUFAs) have been used in NAFLD and lead to a significant reduction of the expression of pro-inflammatory molecules (tumour necrosis factor-α and interleukin-6) and of reactive oxygen species[149]. Inhibition of Bcl-2 (B-cell lymphoma 2) the founding member of the Bcl-2 family of apoptosis regulator proteins encoded by the BCL2 gene leads to intensification of inflammation in NAFLD[150]. Serum Bcl-2 concentrations in overweight-obese subjects with nonalcoholic fatty liver disease have been seen to be reduced and may represent an additional target for therapy[151]. Jun amino-terminal kinase(JNK), insulin resistance and inflammation represent possible link between NAFLD and coronary artery disease. There are not many studies on anti-inflammatory drugs such as aspirin, anti-interleukin-6 receptors, immune-modulators (calcineurin inhibitors), substances enhancing the expression of heat shock proteins (which protect cells from endoplasmic reticulum stress-induced apoptosis), and anti- c-Jun amino-terminal kinases in NAFLD and needs to be studied further[152]. NAFLD is thus a chronic low grade inflammation that leads to insulin resistance because of the increased levels of cytokines[153,154] and anti-inflammatory therapies may help decrease the burden of NAFLD as also Type 2 diabetes mellitus.

Thus inflammation has a role to play both in pathogenesis of diabetes and its complications and it represents a potential target for treatment of both diabetes and its complications.

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