EMBRYONIC LIFE OF HDAC INHIBITORS: – IN DIABETIC NEPHROPATHY

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ABSTRACT
HDAC are up-regulated in response to hyperglycaemia and contribute to development of DN. Inhibiting HDACs have shown to benefits in vitro and in vivo study. Based on study reports and evidence one can comment HDAC inhibitor (HDACI) are still in embryonic stage.

KEYWORDS: HDACs, HDAC Inhibitors, DN.

INTRODUCTION
Diabetic nephropathy (DN), a serious micro-vascular complication frequently accompanying with both T1 DM and T2 DM, is a foremost cause of renal failure. The ailment can also lead to augmented cardiovascular disease and macro-vascular complications. Presently available therapies have not been fully successful in the treatment of DN, suggesting that additional understanding of the molecular mechanisms underlying the pathogenesis of DN is needed for the improved management of this ailment. Although crucial signal transduction and gene regulation mechanisms have been known, especially those associated with effects of hyperglycaemia, transforming growth factor β1 and angiotensin II, progress in efficient genomics, high-throughput sequencing technology, epigenetics and systems biology tactics have greatly extended our understanding and exposed new molecular mechanisms and factors tangled in DN[1]

Histone units[30]
Gene transcription in eukaryotic cells is strongly responsible by the manner in which DNA is packaged.[10]
DNA is wrapped into chromatin, a highly structured and dynamic protein–DNA complex. A nucleosome, fundamental structural unit of chromatin, is defined as a histone octamer, made up of two each of H2A, H2B, H3, and H4 with 200 base couples of DNA warped on the outside.\[^{27}\]

Each histone is organized into compressed core of the nucleosome and an unstructured N-terminal tail, which extends outside the core.\[^{27}\]

Specific amino acids inside histone tails are targets of many number of post-translational alterations with acetylation, phosphorylation, methylation, and ubiquitination, all of which are tangled in gene-specific transcriptional regulation.\[^{28,29}\]

**HDACs**

Histone deacetylases (HDACs) are enzymes that balance the acetylation events of histone acetyltransferases on chromatin alteration and play essential part in regulating gene transcription.\[^{13}\]

**CLASSIFICATION OF HDACs\[^{2}\]**

Acetylation and deacetylation are structured by two groups of enzymes: HATs (histone acetyltransferases) and HDACs (histone deacetylases).

The reverse events of HATs and HDACs regulate gene expression through chromatin modification.\[^{3,4}\]

HDACs are a class of deacetylating enzymes that remove acetyl groups from ε-amino groups of lysine residues of histones, as well as non-histone proteins, triggering the condensation of chromatin structure and thus repressing gene expression.\[^{5,6}\]

**HDACs are classified into four groups\[^{2}\]**

Class I and II are referred to as ‘classical’ HDACs.

**Class I HDACs:** (HDAC1–3 and 8), which are connected to yeast Rpd3 (reduced potassium dependency 3).\[^{7}\]

**Class II HDACs:** Which are divided into two subclasses.

**Class IIA:** (HDAC4, 5, 7, and 9).

**Class IIB:** (HDAC6 and 10), both homologous with the yeast gene HDA1 (histone deacetylase 1)\[^{8}\]
**Class III:** Which comprise SIRT1–7, also known as Sirtuins, are homologous with yeast Sir2 (silent information regulator 2)\(^8\)

**Class IV (HDAC11):** Which comprises conserved residues in catalytic regions common to both class I and II HDAC enzymes.\(^9\)

Class I and II HDACs need Zn\(^{2+}\) for their enzymatic reaction. Class IV also has a Zn\(^{2+}\) based reaction mechanism. Nevertheless, class III HDACs do not require Zn\(^{2+}\) for their catalysis, but firmly depend on the cofactor NAD.\(^{13}\)

**MECHANISMS IN DN**

Mechanisms include DNA methylation, chromatin histone alterations\(^{[1,13]}\), novel transcripts and functional noncoding RNAs, such as microRNAs and long noncoding RNAs.\(^{[1]}\)

Post-translational alterations of histone H3, heat shock protein-27 (HSP-27) and mitogen-activated protein (MAP) kinase p38 expression.\(^{[18]}\)

As EGF signalling is changed by the acetylation status of histone proteins.\(^{[19]}\)

HDAC activity is required for snail protein, Hyperglycaemia activate HDAC which brings modifications in snail protein which causes Apoptosis.

**HYPERGLCEMIA ACTIVATES HDAC RETINOPATHY ROLE**

Hyperglycemia triggered HDAC and amplified HDAC1, 2, and 8 gene expressions in the retina and its capillary cells. The activity HAT was compromised and the acetylation of histone H3 was diminished. Termination of hyperglycaemia failed to provide any profits to diabetes-induced changes in retinal HDAC and HAT, and histone H3 remained subnormal. This suggests “in principle” the role of global acetylation of retinal histone H3 in the progress of diabetic retinopathy and in the metabolic memory phenomenon associated with its continued development.\(^{[12]}\)

**NEPHROPATHY AND FIBROSIS\(^{[13,40]}\)**

HDAC activity is also linked with the development and progression of some chronic diseases characterized by fibrosis, including chronic kidney disease, cardiac hypertrophy, and idiopathic pulmonary fibrosis.\(^{[13]}\)
Potential use of HDAC inhibitors against liver and kidney fibrosis using these kind of inhibitors in experimental animal models and in vitro models of fibrosis.[40]

**HDAC 2 PLAYS IMPORTANT ROLE IN DN**[10]

Among the six HDACs tested (HDAC-1 through -5 and HDAC-8), HDAC-2 activity significantly amplified in the kidneys of STZ-induced diabetic rats and db/db mice and TGF-β1-treated NRK52-E cells.

Interestingly, hydrogen peroxide amplified HDAC-2 activity, and the treatment with an antioxidant, N-acetylcystein, almost completely reduced TGF-β1-induced stimulation of HDAC-2. These findings propose that HDAC-2 plays vital role in the development of ECM accumulation and EMT in diabetic kidney and that ROS mediate TGF-β1-induced stimulation of HDAC-2.

**HDAC 4 PLAYS IMPORTANT ROLE IN DN**[11]

Zinc-dependent HDACs, HDAC2/4/5 were up regulated in the kidney from streptozotocin-induced diabetic rats, diabetic db/db mice, and in kidney biopsies from diabetic patients. Podocytes treated with high glucose, advanced glycation end products, or transforming growth factor-β (common detrimental factors in diabetic nephropathy) selectively amplified HDAC4 expression. The role of HDAC4 was assessed by in vivo gene silencing by intrarenal lentiviral gene delivery and found to decrease renal injury in diabetic rats. Podocyte injury was related with suppressing autophagy and exacerbating inflammation by HDAC4-STAT1 signaling in vitro. Thus, HDAC4 contributes to podocyte injury and is one of the dire components of a signal transduction pathway that links renal injury to autophagy in diabetic nephropathy.[11]

**HDAC9**[43]

HDAC9 and found that HDAC9 expression was significantly up-regulated in high glucose (HG)-treated mouse podocytes, as well as kidney tissues from diabetic db/db mice and patients with DN[43]

In diabetic db/db mice, silencing of HDAC9 diminished the glomerulosclerosis, inflammatory cytokine release, podocyte apoptosis and renal injury. Together, these data indicate that HDAC9 may be tangled in the process of DN, especially podocyte injury. The study suggest that inhibition of HDAC9 may have a therapeutic prospective in DN treatment.
**HDAC inhibitors**
The role of HDACs in cancer initiation and progression, as well as the therapeutic effects of HDAC inhibitors in several types of cancer, has been well studied.[17]

Currently available inhibitors are mostly non-selective inhibit multiple HDACs, and different HDACs serve very diverse functions. Therefore, it is essential to determine the role of individual HDACs in diabetic nephropathy and develop HDAC inhibitors with better specificity.[11]

**CLASSIFICATION OF HDAC INHIBITORS (HDACI)**[2]

**Chemical based classification**[2]

HDACIs are compounds that have the capacity to prevent the deacetylation of lysine residues inside the N-terminal tails of histone proteins.

On the basis of their chemical structure, HDACIs are categorized into the six groups:
(i) Hydroxamates- examples- TSA (trichostatin A) and SAHA (suberoylanilide hydroxamic acid).
(ii) Short-chain fatty acids- eg- butyric acid and valproic acid.
(iii) Cyclic tetrapeptides - examples CHAP31 (cyclic hydroxamic-acid-containing peptide 31) and romidepsin (FK-228).
(iv) Benzamides- entinostat (MS-275), tacedinaline (CI-994) and chidamide (CS-055).
(v) Electrophilic ketones – trifluoromethylketone.
(vi) Miscellaneous compounds- example - MGCD0103.

**Classification on specificity**[2]

**CLASS I HDAC specific**

Romidepsin, MS-275 and mocetinostat are well-thought-out to be class I-specific.[21]

**Class I and class IIa HDACs Inhibitors**

SAHA, TSA, panobinostat, belinostat and resminostat are pan-deacetylase inhibitors. Butyrate and valproate.[2]

**HDAC6 INHIBITOR**

Tubacin is certain to inhibit HDAC6.[22]
MECHANISM OF ACTION OF HDAC INHIBITORS (HDACI)[2]

The mechanism of action of HDACIs involves inhibiting the deacetylation of histones. Hyperacetylation[2,24,25] results in an increase in the space between the nucleosome and the DNA that is wrapped around it. The opening of chromatin structure subsequently provides the access for gene transcription. HDACIs target gene expression without changing DNA sequence.[2]

Inhibitors of HDACs regulate gene transcription through hyperacetylation of nucleosomal histone and non-histone proteins.[24,25]

HDACIs also have effects on non-histone proteins which include proteins involved in the regulation of gene expression, pathways of extrinsic and intrinsic apoptosis, cell-cycle progression, redox pathways, mitotic division, DNA repair, cell migration and angiogenesis.[2,33-39]

In general, amplified levels of histone acetylation lead to de-condensation of chromatin allowing DNA accessible to transcription factors, and are thus related with increased transcriptional activity, whereas decreased acetylation levels are associated with transcriptional repression.[23]

A recent study, however, verified that structurally different HDAC inhibitors significantly activated or repressed more than 40% of genes on the array in cultured acute T-cell leukemia cell line.[26]

It is unclear as to in what way HDAC inhibitors determine to activate or repress gene transcription.[30]

HDACIs can induce acetylation of histone, as well as non-histone proteins, which affect a variety of physiological and pathological processes, controlling apoptosis/autophagy, cell cycle, fibrogenesis, immune response, inflammation and metabolism through its downstream molecular targets.[2]

HDACI USED IN DIABETIC KIDNEY MODEL STUDY

I] Sodium butyrate (NaB)[14]

Sodium butyrate (NaB) is a short chain fatty acid having HDAC inhibitory activity.
HDAC inhibitors stimulate beta-cell development, proliferation and function as well as improve glucose homeostasis.

NaB treatment diminished plasma glucose, HbA1c, beta-cell apoptosis and enhanced plasma insulin level and glucose homeostasis through HDAC inhibition and histone acetylation in diabetic animal as compared to control.

NaB treatment enhanced the beta-cell proliferation, function and glucose homeostasis as well as reduced beta-cell apoptosis in juvenile diabetic rat by the modulation of p38/ERK MAPK and apoptotic pathway.

II] VALPORIC ACID (VPA)\[^{15}\]
Decreased the expression of TGF-β1, CTGF, α-SMA, fibronectin, collagen I, COX-2, ICAM-1 and HDAC4/5/7. Further, VPA treatment significantly increased histone H3 acetylation and MMP-2 expression.\[^{15}\]

VPA treatment ameliorates the renal injury and fibrosis in diabetic kidney by preventing the myofibroblast activation and fibrogenesis by HDAC inhibition and associated mechanisms, thereby improving the profibrotic and anti-fibrotic protein balance.\[^{15}\]

III] Trichostatin A (TSA)
An HDAC inhibitor, barred TGF-β1–induced EMT in cultured human renal proximal tubular epithelial cells. Treatment with TGF-β1 induced morphologic changes such as EMT in human renal proximal tubular epithelial cells. However, co-treatment with TSA completely prevented TGF-β1–induced morphologic changes and significantly prevented TGF-β1–induced down-regulation of E-cadherin and up-regulation of collagen type I. Treatment with TSA did not alter TGF-β1–induced phosphorylation of Smad2 and Smad3 but induced numerous inhibitory factors of TGF-β1 signals, such as inhibitors of DNA binding/differentiation 2 (Id2) and BMP-7.\[^{16}\]

Administration of TSA suppressed the expression of α-SMA and fibronectin and diminished the accumulation of renal interstitial fibroblasts in the kidney after the obstructive injury. Activation of renal interstitial fibroblasts was accompanied by phosphorylation of signal transducer and activator of transcription 3 (STAT3), and TSA treatment also stopped these responses.\[^{13}\]
TSA treatment inhibited tubular cell apoptosis and caspase-3 activation in the obstructive kidney. Together, the data suggest that pharmacological HDAC inhibition may induce antifibrotic activity by inactivation of renal interstitial fibroblasts and inhibition of renal tubular cell death. STAT3 may mediate those actions of HDACs.[13]

Trichostatin A, a nonselective HDAC inhibitor, reduced mRNA and protein expressions of ECM modules and prevented EMT.[10]

IV] CURCUMIN[18]
Increased levels of HSP-27 and MAP kinase (p38) in diabetic kidney. However, curcumin treatment prevented this increase in HSP-27 and p38 expression. Furthermore, at nuclear level curcumin prevented the reduction in dephosphorylation and increases acetylation of histone H3. Curcumin treatment tangled changes in post-translational modifications of histone H3, expression of HSP-27 and MAP kinase p38 in diabetic kidney.

V] VORINOSTAT
In cultured proximal tubule (normal rat kidney) cells, vorinostat treatment reduced EGFR protein and mRNA, and decreased cellular proliferation. Within 72 h of diabetes induction with streptozotocin, urinary EGF excretion was increased approximately threefold and was unaffected by vorinostat, even though the kidneys of vorinostat-treated diabetic rats had reduced tubular epithelial cell proliferation. Everyday treatment of diabetic rats with vorinostat for 4 weeks diminished renal growth and glomerular hypertrophy. Therefore, early renal changes in diabetes are amenable to epigenetic intervention. Attenuating effects of HDAC inhibition, although multifactorial, are possible to be mediated in part through down-regulation of the EGFR.[19]

Control and streptozotocin-diabetic wild-type and eNOS−/− mice were treated with vorinostat by daily oral dosing for 18 weeks. Poorly affecting either blood glucose concentration or blood pressure, vorinostat diminished albuminuria, mesangial collagen IV deposition, and oxidative-nitrosative stress in streptozotocin–wild-type mice. These attenuating effects were related with a >50% reduction in eNOS expression in mouse kidneys and in cultured human umbilical vein endothelial cells. These observations demonstrate the therapeutic efficiency of long-term HDAC inhibition in diabetic nephropathy and highlight the importance of the relationship between eNOS activity and oxidative stress in mediating these effects.[20]
The histone deacetylase inhibitor (HDACI), vorinostat (suberoylanilide hydroxamic acid), fast regulatory approval for the treatment of advanced cutaneous T-cell lymphoma in 2006. While investigation into the potential beneficial effects of inhibitors of HDACs has principally focused on neoplastic and neurodegenerative disorders, their capability to modulate the expression of genes tangled in the physiological and pathophysiological conditions of the cardiovascular system proposes their broader applicability.\(^{[20]}\)

VI] **SK-7041\(^{[30,31]}\)**
A novel hydroxamic acid-based class I HDAC inhibitor, brought a qualitatively similar effect to TSA on TGF-β1-induced expression of fibronectin, collagen I, α-SMA, and E-cadherin in these cells, signifying that class I HDAC is involved in ECM growth and epithelial–mesenchymal transition.

VII] **APELIN\(^{[41]}\)**
Apelin-13 treatment diminished diabetes-induced glomerular filtration rate, proteinuria, glomerular hypertrophy, mesangial expansion and renal inflammation. The inflammatory factors, activation of NF-κB, histone acetylation and the enzymes tangled in histone acetylation.

Apelin-13 treatment inhibited diabetes-, high glucose- and NaB-induced rise of inflammatory factors, and histone hyper-acetylation by up-regulation of histone deacetylase.

VIII] **MS-275\(^{[42]}\)**
**ANIMAL STUDY**
MS-275, a selective class I HDAC inhibitor, on the growth of renal fibrosis in a murine model of unilateral ureteral obstruction (UUO).

Class I HDACs are censoriously tangled in renal fibrogenesis and renal fibroblast stimulation through modulating TGF-beta and EGFR signalling and propose that blockade of class I HDAC may be a useful treatment for renal fibrosis.

MS-275 inhibited all these fibrotic responses and suppressed UUO-induced production of transforming growth factor-beta1 (TGF-beta), amplified expression of TGF-beta receptor I, and phosphorylation of Smad-3. MS-275 was also actually effective in suppressing phosphorylation and expression of epidermal growth factor receptor (EGFR) and its downstream signalling molecule, signal transducer and activator of transcription-3.
MS-275 treatment repressed TGF-beta induced phosphorylation of Smad-3, differentiation of renal fibroblasts to myofibroblasts and proliferation of myofibroblasts.

FIGURE FROM SOURCE[2]

DISCUSSION AND CRITICAL COMMENTS
1. The mechanism of HDAC in activation or repression is unclear. HDACs are dire enzymes involved not only in the development of cancer, but also other diseases such as interstitial fibrosis, autoimmune, inflammatory diseases, and metabolic disorders. HDACIs have been tested for their therapeutic properties in treating these diseases in clinical trials and/or animal models. However, the underlying mechanism (s) by which HDACIs play a role in inhibiting cancer and other disease onset and progress remains incompletely understood.[2]

2. Many isoform of HDAC are up-regulated in diabetic Nephropathy, many study suggest HDAC 2/4/9[10,11,43] plays important role in development of diabetic nephropathy which makes more difficult in interpretation and coming to conclusion which isoform should be targeted in vivo study.

3. Most of study done till date are still inadequate to come to a conclusion and unfortunately no more study data is available in area of Diabetic nephropathy.

4. HDACI are used in treatment of cancer since its targets downstream signalling molecules and provides cell cycle arrest.

5. Based on evidences and study till date provides us insight of HDACs vital role in pathogenesis of Diabetic Nephropathy (DN).
6. Recent studies revealed that pharmacological inhibition of histone deacetylase (HDAC) reduced early tubular epithelial cell proliferation in diabetic rats and diminished renal growth, which may be mediated in part through down-regulation of the EGF receptor.\textsuperscript{[19]} Other studies implicated HDAC-2 in the development of ECM growth in the diabetic kidney and showed that ROS mediate TGF-\(\beta\)-induced activation of HDAC-2.\textsuperscript{[10]}

7. HDACIs are novel agents for Diabetic Nephropathy.\textsuperscript{[30]}

8. HDACIs can improve the DN by inhibiting fibrosis\textsuperscript{[2]}, inhibiting transcription factors\textsuperscript{[2]}, may prevent Apoptosis\textsuperscript{[2]}, anti-inflammatory\textsuperscript{[2]}, immunomodulation\textsuperscript{[2]} correct renal metabolism. Improve Beta cell proliferation and function\textsuperscript{[14]} and glucose homoeostasis.\textsuperscript{[14]}

However its long way to go and one say HDACIs are still in initial stage in treatment of Diabetic Nephropathy.

Conflict Of Interest
The Author declares there is no conflict of interest regarding publication of this paper.

Declaration
The author has not conducted any animal study or Human Trial, the matter in the article is which comprises animal or human trial are the study done by researcher /scientist listed in the Reference section and cited appropriately and are taken up in the article to support the Hypothesis.

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