

Biodegradable Nanoparticles for Delivering Drugs and Silencing Multiple Genes or Gene activation in Diabetic Nephropathy

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ABSTRACT- Dialysis is the only mode of available palliative therapeutic modality to patient with end stage of renal disease. Diabetes is one of the foremost common causes of chronic renal disease and affecting large number of diabetic patients. By many theories, hypothesis and study are carried to understand the pathogenesis of Diabetic kidney disease or complication of diabetes i.e. diabetic nephropathy (DN) and based on pathophysiology many drugs and molecules are being developed to target enzymes, intracellular proteins, micro RNA, Receptor, channel etc. Genes (like NFE2L2, HD1, RPD3 etc.) responsible for synthesis of transcription factor, proteins, enzymes and cytokine factors, intracellular antioxidant factor all play vital role in pathophysiology of DN. Targeting multiple genes, which play important role in pathophysiology of DN with nanoparticle loaded with siRNA or drugs or combination will not only reduce multiple drug and medication burden but also mitigate the disease faster and with reversal of pathological changes with safety, if the challenges are met. It is possible to target multiple genes, which play vital role in fibrosis and extracellular matrix expansion which are key features of DN with biodegradable particle. Each drug by a unique mechanism specifically targeting protein, enzymes, receptor, channel etc. mitigates the progress of DN and multiple drugs are needed to inhibit the various mechanisms. The approach of silencing the multiple genes or delivering drugs inside the cell organelles with biodegradable nanoparticles is novel, versatile and target specific to inhibit the progress of DN and to reverse the pathological changes efficiently as compared to drugs/molecules, if the challenges of nanoparticle formulation are met. Based on the research till date and available resource suggest it is possible to target multiple genes or protein or enzymes or signaling molecules using biodegradable and biocompatible nanoparticles (NP) loaded with siRNA and drugs or combination of drug and siRNA.

Key-words- Diabetic Nephropathy (DN), Proteinuria, Nanoparticles (NP), siRNA, Pathogenesis

INTRODUCTION

Diabetes is common cause of end stage renal disease and no ideal therapeutic mode is available. Dialysis is a common therapeutic in patient with renal failure. DN is the most common complication of diabetes mellitus and is characterized by pathologically by proteinuria, fibrosis, podocyte injury, extracellular matrix expansion, thickening of basement membrane and biochemically raised levels of uric acid, serum urea and serum creatinine level and radiological finding on USG is small kidney and oxidative stress plays major role in pathogenesis.^[1,2]

Ideal Nanocarriers^[1]: Nanocarriers should be capable of safely and expeditiously transporting siRNA to the target organ or tissue.

It should be non-toxic, non-immunogenic to human tissue or cells. Competent in condensing siRNA and sustaining its integrity before to reaching the target organ or tissue site. Capable to evade fast clearance to reach the target organ or tissue site. It must internalize and dis-associated inside the cell to release its siRNA load, thus exposing the siRNA to the mRNA.

Advantage of using nanoparticle in Diabetic Nephropathy (DN)^[1]

Site specific or tissue/organ specific delivery, increased intracellular uptake can be done by using multiple drugs or combination of drugs with siRNA in nanoparticles. Functionalization and ligand attachment is possible, which provide unique site specific organ delivery or tissue delivery. Biodegradable and biocompatible nanoparticles decrease the cytotoxicity induced by them. Protects the degradation of drugs, phytochemical or siRNA loaded inside the nanoparticles thus increases the circulation time. Physiochemical property of nanomaterial helps in the unique release mechanism inside the cell. Metal nanoparticles loaded with drugs,

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antioxidants can be used to target cancer cells and cellular imaging.

Nanobiomaterial used [3]: Calcium phosphate [3,12] can be used as nanobiomaterial, also polymeric micelles have attracted the attention of many researchers for their role in drug delivery as they hold several biochemical and physicochemical advantages over other vehicles. These comprise their high stability, tissue-compatibility, ability to solubilize poorly soluble agents, and able to gather at poorly vascularized tissue or organ [3]. The carbon nano-tube can be also used to deliver siRNA to human T cells [4]. Chitosan Nanoparticles can be also used for siRNA delivery [5]. Novel Endosomolytic Diblock copolymer is also used for siRNA delivery [6]. Proton-sponge coated quantum dots is also used for siRNA delivery and intracellular imaging [7].

Targeting genes with calcium phosphate nanoparticles:

Very little toxicity to mammalian cells with calcium phosphate and so commonly used non-viral vector for trans-infection *in vitro* condition. [8] In acidic pH, calcium phosphates dissolve rapidly, which is responsible for its activity and the endocytosis of calcium phosphate in the endosome helps to liberate the consignment/load in the cytoplasm. [9]

Mode of Synthesis of calcium phosphate nanoparticles:

Calcium phosphate nanoparticles can be primed by condensed DNA in reverse micro environment. [9] Stabilized Calcium phosphate nanoparticles can be synthesized by rapid precipitation followed by speedy adsorption of DNA or siRNA [10]. Bovine serum coated calcium phosphate nanoparticle for DNA trans- infection [11].

Stabilized size of Nanoparticle: Nanoparticle, which is stabilized size range [12] 100–200 nm. Out of the many nanomaterial investigation reports suggested that polymer and calcium phosphate material can be used for delivery drug or to load multiple drug or load siRNA or use combination of drugs with siRNA or to induce activator drug or inhibitors of signaling pathway.

Discovery of RNAi (RNA interference): Discovery of RNAi (RNA interference) mechanism was done in the plant kingdom and practically later the method of gene silencing came into existence in free living nematode *Caenorhabditis elegans*. Andrew Z. Fire and Craig C. Mello received and jointly shared the Nobel Prize in Physiology or Medicine 2006 for their discovery of RNA interference - gene silencing by double-stranded RNA.

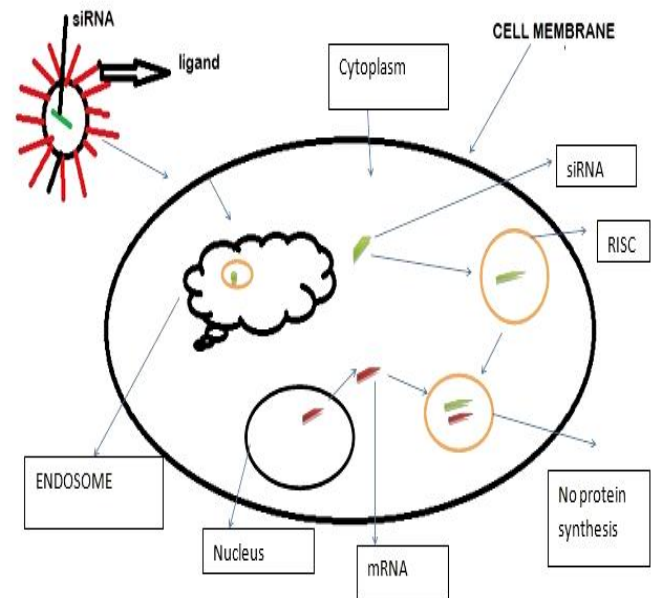


Fig. 1: Mechanism of action of siRNA loaded Nanoparticles inhibiting protein synthesis

Silencing mechanism [8-15]: Silencing mechanism can be done by using siRNA (small interfering RNA), which inhibited gene expression in several cells.

RNA stages and its interaction with RISC (RNA Induced Silencing Complex) [8-15]: Linking of RISC i.e. Multiprotein complex and siRNA Followed by activation of RISC. Integration of siRNA with RISC inside the cell followed by removal of sense strand. Using the antisense strand, the activated RISC directs cleavage of mRNA (messenger RNA).

Hurdles in delivering siRNA [8-15]: Nucleases cause rapid degradation of naked siRNA after *in vivo* condition of intravenous administration. The siRNA has short circulating life because it is rapidly excreted by the urinary excretory system and also intracellular uptake by phagocytes. Hydrophilic nature, negative charge and relative size 13kDa make the transportation difficult through the plasma membrane. The siRNA may stimulate the immune system causing off-target effect which may be dangerous. miRNA and siRNA may hamper the normal miRNA mediated gene expression due to competition amongst them. Naked siRNA is unstable. Principal hurdle in the entry of siRNA is bilayer lipid cell membrane of the cell. Some nanoparticles get disassemble in kidney glomerulus, which makes delivery more difficult and challenging [16]

Why the need to target genes and why to use nanoparticles: The following difference will make us clear:

Table 1: Difference between Drug and Gene Targeting

S. No	Points	Drug/Molecules	Genes targeting
1	Off Target effect	Drugs have off target effect	Each gene encodes specific proteins, enzymes etc.
2	Toxicity	Some drug or molecules with off target effect may have potential to damage other organ	Targeting specific genes in specific cells is superior as compared to drugs
3	Co-morbid condition	Some co-morbid condition along with the disease may limit physician from using drugs /molecules	Since specific genes are targeting using versatile and novel delivery system the co-morbid condition is overwhelmed
4	Multiple drugs	Since many disease involve multiple mechanism at molecular level add on therapy and multiple drug are needed for one condition thus increasing medication burden and cost	Many genes can be targeted in one specific organ or tissue with versatile novel tool using nanoparticle siRNA, drugs or combination can be delivered efficiently at target site
5	Pharmacokinetic and pharmacodynamics	Many problems with drug like solubility, bioavailability, plasma concentration and site specific action puts break on using E.g- resveratrol antioxidant useful in treatment of DN but poor solubility, poor stability and poor bioavailability such problems need to overcome by using nanoparticles (1)	Attaching specific ligands to nanoparticles make them novel versatile and gives specific target delivery of Gene, DNA, RNA, Drugs all can be added to nanoparticles to make more versatile. E.g- Resveratrol nanoparticles or resveratrol combine with curcumin both drugs increase bioavailability as well as have synergism with site specific delivery so novel and versatile with no off target effect (1) and problem of solubility bioavailability are overwhelmed.
6	Excipients /vehicles	Many excipients are in current use and safe. Safety data on use of excipients or many vehicles is available	Nanoparticle as vehicle -toxicity must be studied so biodegradable and biocompatible nanoparticles are needed (1), so nanoparticle formulation is challenging. Since concept of nanoparticle is new few study data and safety profile of particle is available
7	Cost	As compared to nanoparticles formulation cost is less	Costly and challenging and need future investigation

Overwhelming the hurdles of siRNA delivery

[8-15]: A few methodologies were suggested to cope up with these hurdles. Chemical adjustment of the bases, sugars, or phosphate linkages of siRNA may additionally lessen safe incitement and improve the solidness of siRNA. Changes of the opened/bolted nucleic acids and of the 2' sugar function may additionally lessen immune-stimulatory signal and enhance resistance towards endonucleases. The coupling power/specificity and C5-methylation of pyrimidines might be increased by way of utilising pseudouracil or 2-thiouracil. Refining the shape and arrangement of siRNA and confinement of the measure of exogenous RNA may additionally assist to keep a strategic distance from the off-target influences of siRNA and avoid overloading of RNAi apparatus. Inserting phosphorothioate bond in the backbone of siRNA at the 3' end Phosphate or sugar linkages are additional communal than base modifications thereby increasing strength and resistance against exonucleases.

Transportation of siRNA and its biological activity

[17,18]: Principal hurdle in the entry of siRNA is bilayer lipid cell membrane of the cell. The siRNA is transported inside the cell through endocytosis and is broken down by nucleases inside the endosome or lysosome vesicle. Inside pH in endosome and lysosome is acidic [5,6]. Rapid breakdown of endocytosed siRNA by lysosomal enzyme hydrolyases for instance like ribonuclease, deoxyribonuclease, phosphatases, phosphodiesterases, and pyrophosphatase. Artificial siRNA unable to enter the nucleus after transinfection and can be traced in the perinuclear area even after using liposomal Nanocarrier, while siRNA delivered directly into the cytoplasm through endocytic axis it efficiently moves inside the nucleus which is additional site for action. Transcription of encoded DNA occurs in the nucleus and delivery of siRNA at the site is difficult *in vivo* as compared to *in vitro* delivery.

Different molecular target, drugs, inhibitors in DN and targeting with nanoparticles: Many drugs, which have off-target effect right from antioxidants ^[2], vitamins ^[2], gene silencing and nano-drug delivery, which could ameliorate the symptoms of DN are available and also many molecular targets and drug molecules and signaling pathway and role in pathogenesis is reviewed. ^[2]

Nox 4: Nox 4 used as a molecular target ^[2] inhibitors drugs ^[2] and silencing Nox4 ^[19]. Nox inhibitor reduces symptoms and forestalls loss of podocyte and foot-process, prevents apoptotic death of podocyte, restores balance of oxidant and antioxidant and decreases oxidative stress. Nox 4 activates AMPK that is suppressor of oxidative stress. Furthermore 5-aminoimidazole-4-carboxamide-1-riboside is named as AICAR, which is a activator of AMPK and inhibit oxidative injury, podocyte injury both *in vitro* and *in vivo* study, metformin is AMPK activator and is used antidiabetic. High glucose up regulate the expression of Nox 4 in renal tubular cells, targeting with oligonucleosides reduces ROS produced in renal tubular cells other drug pitvastatin also inhibit Nox 4 subunits. Decreases in extracellular matrix and fibrosis by the Nox 4 inhibitors. The exact role of Nox4 in diabetic renal disorder stays questionable. Nox4 down regulation by antisense oligonucleotides, in period of two weeks, diminished glomerular hypertrophy and decreased fibronectin in the cortex and glomeruli of diabetic rats (streptozotocin-induced). ^[19]

Unfortunately, no study is available on targeting Nox 4 subunit with nanoparticles loaded with drug or activator of AMPK Category drug and only animal study of targeting Nox 4 subunit in animals have shown ameliorating results its promising area and needs further research and investigation and needs to be explored. If challenges are met it is likely possible to target with biodegradable nanoparticles either by drug or by siRNA or combination.

Nrf 2 ^[2,20]: Nrf 2 is the prospective molecular target for diabetes nephropathy it activation promotes antioxidant genes and restores the depleted antioxidant by ROS. Consequently intracellular anti-oxidant is restored back.

Natural sources, which contain potent Nrf2 chemicals:

Table 2: Natural Sources of Nrf2 activators

S. No.	Chemical found	Source
1	Sulforaphane	Cruciferous Vegetable
2	Cinnamic aldehyde	Cinnamon oil
3	Resveratrol	Grapes
4	Curcumin	turmeric
5	Sinomenine	Sinomenium Acutum
6	Rutin	Citrus fruit, black tea and peel of apple
7	Berberin	Bitter melon , Berberis vulgaris, garlic

Curcumin: Curcumin decreased urinary albumin excretion, lipid peroxidation, and inflammatory markers in T2DM patient.

Resveratrol: It has now arrived phases I trial in combination with losartan. Antioxidant, scavenges free radical, acts on NADPH and improves epithelial mesenchymal transition [EMT] induced by NADPH oxidase.

Bardoxolone methyl: It is an oral antioxidant inflammation modulator, it activates Nrf-2 but it produces hypomagnesemia.

Silybin: It is an antioxidant and activator of Nrf 2.

Sodium butyrate: Activates Nrf2 to improve diabetes nephropathy (DN) perhaps via inhibition of Histone Deacetylase written as HDAC. Suppresses smooth muscle cell growth *in vitro* and decrease in oxidative stress in rabbit aorta *in vivo* additionally antioxidant enzymes are induced after Nrf2 gene transfer. ^[20] Unfortunately, no study is available for activation of Nrf 2 using nanoparticles loaded with drug or activator of Nrf 2 Category drug in DN and only animal study of transferring a gene in animals have shown to induce antioxidant enzymes, however no study is available for DN using nanoparticle for activation of Nrf2 with drugs or gene. It's promising area and needs further research and investigation and needs to be explored. If challenges are met it is likely possible to target with biodegradable nanoparticles either by drug or by gene or combination. Recently nano-formulation of delivery of resveratrol by nanoparticles in DN is reviewed.

Targeting PKC ^[2,21]: PKC activation is well recognized in the pathogenesis of diabetic nephropathy. A various isoform of PKC are alpha, beta, zeta, which can be targeted. PKC beta is prominently activated in kidney tissue, studies till date done and report suggested that targeting it can offer kidney protection against diabetes induced renal hypertrophy, glomerular infiltration, reactive oxygen species and pro-fibrotic factors. Furthermore, targeting PKC alpha can decrease albuminuria and also decrease the up regulated VEGF. Ruboxistaurin well-known PKC inhibitors can decrease retinal damage and kidney injury in diabetic's patients.

PKC Gene silencing: It can restore the vascular function in diabetic rat model. ^[21] Unfortunately, no study is available on targeting of PKC using nanoparticles loaded with drug Ruboxistaurin in DN and only animal study of silencing gene in animals have shown to restore the vascular function in diabetic rat model, however no study is available for DN using nanoparticle with drugs or gene loaded. Its promising area and needs further research and investigation and needs to be explored. If challenges are met it is likely possible to target with biodegradable nanoparticles either by drug or by silencing gene or combination.

NF- κ B Transcription factor as molecular target [2,22-23]: Oxidative stress, Growth factors, Cytokines, Chemokine's and adhesion molecules all activates NF- κ B it is evident from study activated NF- κ B is seen cells of diabetic human kidney and diabetic rodent. Type 2 DM patient proximal tubular cells of the kidney also display Activated NF- κ B. While, activated NF- κ B is regarded as pro-inflammatory.

NF- κ B is inhibited by Type 2DM, commonly used drug Thiazolidinedione [2]. Hyperglycaemia induced NF- κ B activation is suppressed by 1, 25 dihydroxy vitamin D3. Kidney injury of diabetic rats is improved by Thiazolidinedione drugs. NF- κ B is down regulated by statins and fenofibrate. NF- κ B and ICAM-1 in diabetic rats is inhibited by Berberine a plant alkaloid and improve renal function. Berberis nano formulation has been primed and investigated in diabetes [22,23], but no further investigation is carried out to see the effects of Berberine on NF- κ B using nanoparticle in DN. Specific NF- κ B inhibitor incorporated in biodegradable, nontoxic and non-immunogenic nanoparticle should be investigated since the pathological role of NF- κ B is very clear in the development and progress of DN.

Targeting ICAM [2]: It is linked with Diabetes and Diabetic Nephropathy. It is induced by Elevated level of TNF α , Hyperinsulinemia, Hyperlipidemia, Oxidative stress, Hyperglycaemia, AGEs. ICAM-1 can be reduced by the GLP-1 agonist, Calcium channel blocker Nifedipine, Plant derived alkaloid Berberine, Taurine. All drugs have shown effects in reducing the expression of ICAM-1, using anti ICAM-1 monoclonal antibody prevented infiltration of mononuclear cells in the glomerulus of the Diabetic animal model. Accordingly ICAM-1 can reduce albuminuria, hypertrophy, infiltration, mesangial expansion and AGEs induced damage to cells of the kidney tubule. Berberis nano formulation has been primed and investigated in diabetes [22,23], but no further investigation is carried out to see the effects of Berberine on ICAM-1 using nanoparticle in DN. Specific ICAM-1 inhibitor incorporated in biodegradable, nontoxic and non-immunogenic nanoparticle should be investigated since the pathological role ICAM-1 of is very clear in the development and progress of DN. No study is available with taurine loaded nanoparticles in DN, while taurine is well known in DN.

Targeting Chemokine MCP-1[2]: MCP-1 is induced by TGF β , Cytokines, AGEs, High glucose concentration, and RAAS. Renal MCP-1 production- kidney cells like podocyte and mesangial cell produce MCP-1. It is also produced by epithelial, endothelial and smooth muscle. Signaling mechanism, autocrine, and paracrine activation by MCP-1 with its interaction with CCR2, which is a chief receptor of MCP-1.

Pathological role: Tcells, macrophages ,monocytes,and dendritic cells are recruited at site of inflammation, Injury and also infection. Mesangial expression of TYPE IV collagen, fibronectin, cytoskeleton

reorganization, motility are due to involvement of MCP-1/CCR2. High glucose produces injury in the cells human diabetic kidney with up-regulation of MCP-1.

Targeting MCP-1/CCR2 system by drugs [2]: Emapticap pegol (NOX-E36) is a direct inhibitor of MCP-1, while RO 5234444, CCX-140 B is CCR2 ANTAGONIST. Bindarit (AF-2838) is in clinical trials with RAAS blockade therapy in Type 2DM patients with micro-albuminuria and macro albuminuria. Unfortunately, no investigational report is available on targeting MCP-1/CCR2 with nanoparticles loaded with Bindarit. Molecular target is unique; if the challenges of nanoformulation are met it is possible to deliver Bindarit to a specific site.

PARP [2]: In diabetes PARP enzyme activity is increased and has revealed to contribute in pathogenesis of diabetic complication. PARP is activated by oxidative stress and high glucose levels. PJ-34 AND INO-1001 are Inhibitors of PARP enzyme.

Advantages of PARP inhibition: Due to reductions in diabetes induced podocyte loss, blocks hyperglycaemia induced apoptosis, blocks hyperglycaemia induced ROS generation, blocks hyperglycaemia induced NF- κ B in podocytes , reduces hypertrophy of kidney in diabetic mice, decrease nuclear translocation of NF- κ B p50, NF- κ B/AP-1 binding at MMP-9 promoter is inhibited by PARP-1. Hyperglycaemia induced PARP activation plays significant role in pathogenesis of glomerulopathy associated with Type 2DM and could work as novel therapeutic target. PARP inhibitor is still in embryonic stage and its long way to develop nanoparticle loaded with such inhibitors. Currently till date no study is available for DN using nanoparticle with PARP inhibitors.

SOCS mimetic [2]: JAK stat pathway is regulated by diverse mechanism Suppressors of cytokine signaling. (SOCS), Protein inhibitors of activated STAT, Protein tyrosine phosphatases, Internalization of Receptor. SOCS have inhibitory effect on JAK-STAT and has appeared as probable target. SOCS protein expression is amplified in patient of Diabetic Nephropathy and in animal model of Diabetes. Reduced JAK/STAT activation *in vivo* with gene therapy with SOCS expressing adenovirus and thus improved the early changes in kidney of diabetic rats.

Budding target for Research: SOCS mimetic or SOCS inducer is forthcoming therapeutics to retard the progression of diabetic nephropathy. However, only animal study with SOCS mimetic is reported intracellular delivery of SOCS mimetic or inducers with nanoparticle is more ideal and unique, if the challenges of formulation and delivery are met.

Rho Kinase [2,24-26]: In diabetic condition Rho A-ROCK is activated in kidney cells. Progrowth, pro fibrotic /prosclerotic signaling is major contribution of Rho A-ROCK Pathway. Increased Extra-cellular matrix

production and plays significant role in in pathogenesis Kidney haemodynamic. High glucose level and Angiotensin II activate Rho A-ROCK Rho A-ROCK can be targeted by Fasudil, ROCK iY 27632 and siRNA Inhibitor drugs [2] simvastatin inhibits Rho A-ROCK in mesangial cells' action like fluvastatin.

Investigation report with fasudil and statin suggest benefits

Important benefits of Rho A-ROCK inhibition: Reductions in fibrosis and sclerosis, Decreases extracellular matrix, Reduces VEGF, Increases microcirculation, Modulate kidney hemodynamic, Decreases collagen, Blocks the effect of Angiotensin II Intervened signaling, Decreases expression of Nox 4, Reduces urinary albumin excretion, GFR gets Stabilization, Causes vasodilation in renal blood vessels, Reduces resistance in renal blood vessels. Rho A-ROCK can be targeted by Fasudil, nanoparticle loaded with Rho Kinase inhibitor are investigation for pulmonary [24-26] however nanoparticle loaded with siRNA can be versatile tool for targeting Rho A-ROCK and prevent the progression of DN.

Drugs with Antioxidant property used in DN [2]:

N-acetyl cysteine (NAC), Probuco, Resveratrol Rhein, Coenzyme Q10, Silybin, Bardoxolone methyl, Vitamin C and E, Nox4 inhibitors example GKT 137831 GKT 136901 [2] all have antioxidant property and tried in DN some have entered into clinical trials. Resveratrol phase I clinical trial in combination with Losartan free radical scavenger, antioxidant, acts on NADPH oxidase and improves epithelial mesenchymal transition induced by NADPH oxidase. Vitamin C and Vitamin E, Alpha lipoic acid, Probuco, Silybin are antioxidant phase II clinical trial.

Bardoxolone Methyl is in phase III clinical trial antioxidant, activator of Nrf-2 but adverse effect is hypomagnesemia. Nanoparticle loaded with single or multiple antioxidant having intracellular delivery is possible, depending on physiochemical property suitable nanoparticle can be prepared already Resveratrol Nanoparticle formulation is prepared, however no investigation report are available of using such nanoparticle formulation in DN.

NADPH oxidase [2]: NADPH oxidase plays vital role in oxidative stress induced by hyperglycaemic condition in diabetic person backing the development of diabetic nephropathy. NADPH OXIDASES existing in all cells. Nox is the subunit of the NADPH Oxidase and has played vital role in the development of cancer, cardiovascular, renal, neuronal, organ failure and oxidative damage.

Inhibitors NADPH oxidase [2]: Individual NADPH subunit or precise drug to inhibit Nox 1/ Nox 4, can be done directly or indirectly or by directing oligonucleoside. Many natural compounds are discovered and investigated to be Inhibitors of NADPH Oxidases and synthetic compounds are also developed.

Specific: specific inhibitor Nox 4 isoform

Non specific: Apocynin, Diphenyleneiodonium chloride

Inhibiting NADPH oxidase subunit: p22 (phox)

Selective NADPH subunit Nox 1 inhibitor: ML 171, NoxA1ds

Other natural compounds

Emodin: Active compound from rhubarb: Inhibit ROS generation.

Flavonoids: Kaempferol, morin, quercetin etc inhibits outburst from neutrophil cells.

Ginkgo biloba: Inhibit ROS generation.

Magnolia officinalis: Magnolol and honokiol both inhibit superoxide generation by inhibiting NADPH oxidase.

Resveratrol: It can inhibit high glucose induce NADPH oxidase pathway and ROS generation.

Plumbagin inhibits: NADPH dependent superoxide generation in cell lines that express NADPH oxidase 4 (Nox 4) enzyme.

Celastrol: Derived from *Tripterygium wilfordii* is a potent inhibitor of several Nox enzymes and is used as anti-inflammatory, anticancer and in arthritis.

Apocynin: Apocynin is orally active and blocks NADPH oxidase assembly requires peroxidase for reaction reduces ROS, Prevents Endothelial Dysfunction, Increases Glutathione Synthesis, Direct ROS Scavenger, Activate AP-1 Transcription factor, Limits the Generation of NO.

Disadvantage: Does not work immediately, Not specifically, It also interferes with arachidonic acid metabolism.

Anti-sense Oligonucleotide Directed to p22

(phox): Vascular Endothelial growth factor is associated with progression of proteinuria in diabetic nephropathy. Overexpression of p22 is associated with enhanced VEGF in Diabetes. High glucose level increased VEGF Expression is significantly increased in mesangial cells. Pathogenesis of increased level of VEGF is unclear. High glucose induced VEGF can be blocked by antisense oligonucleotide directed towards p22 (phox). Unfortunately, in spite of several investigations and study of various compounds could not make much progress in DN. Nanoparticle loaded with single or multiple NADPH oxidase inhibitor, intracellular delivery is possible, depending on physiochemical property of drug suitable nanoparticle can be formulated for targeting NADPH oxidase.

Endogenous Apelin and Protein C [2,27-29]: Apelin hinders the progression of diabetes nephropathy. Apelin stimulates diabetic nephropathy by inducing podocyte dysfunction via inhibiting proteasome. Studies have created confusion apelin retard progress of diabetic nephropathy or promote podocyte dysfunction need to study further.

Activated Protein C study reported following action of activated protein: It inhibits redox sensitive transcription factor NF- κ B. SOD-1 is blocked. Protein C exerts direct Antioxidant effect. Suppress glucose induced release of cytochrome c and smac/Diablo from mitochondria and mitochondrial apoptosis. p66Shc is potential target of activated protein C. Decline ROS production in kidney podocytes. In the diabetic mice inhibition of glomerular p66Shc expression occurs. *In vitro* inhibits glucose induced expression p66Shc in kidney podocyte via PAR-1 and PAR-3. p66Shc expression in kidney podocyte is epigenetically inhibited by activated protein C. Maintains mitochondrial membrane potential and inhibit ROS generation. This study as given insight of cytoprotective effect *in vitro* and *in vivo* further trial need to be done to prove the efficacy in diabetic patients with nephropathy. Apelin nanoparticles are formulated [27,28] but no study reports are available on using such nanoparticles in DN. In near future, if the role of apelin becomes clear and challenges of nanoformulation are met delivery of Apelin at target site will be unique. Also Protein C nanoparticle are formulated [29] yet no specific study is available for DN but such polymer nanoparticle loaded with protein C can be used to target DN.

Targeting VEGF [2,30]: VEGF-plays an important role in development of diabetic nephropathy, inducer of vasopermeability and angiogenesis. High blood glucose level, Polyol pathway-AGEs products, Endothelin 1, RAAS-Angiotensin II, Stretch, TGF β modulates the expression of VEGF and its receptors. Serum level of VEGF correlates with albuminuria and increase with Diabetic nephropathy stage in patients with type 1 and type 2 Diabetes. VEGF affects podocyte function and takes part in influx of macrophage.

Targeting VEGF: Anti-bodies directed to VEGF improves renal dysfunction, improvement of early and late structural changes, in experimental diabetic animals. Pan -VEGF TYROSINE KINASE INHIBITOR SU 5416- Improve albuminuria in Diabetic. TEMPOL can block the effect of VEGF. Directing antisense oligonucleotide will decrease the level of VEGF. Overexpression p22 (phox) augments VEGF.

Multidrug can be loaded nanoparticles can be targeted in DN. [30]

Targeting AGEs and RAGE [2]: Hyperglycaemia derived advanced end product of glycation (AGEs) are known to play vital roles in the pathogenesis of diabetic

complication and the AGEs interaction with receptor RAGE causing altered protein and altered collagen turnover. AGE also activate NAPDH OXIDASE and also help in ROS generation. Targeting RAGE Inhibiting RAGE with neutralizing antibodies reversed pathogenic effect. Several molecules known to inhibit age formation like pimgedine or pyridoxamine showed benefit in animal models. Pimgedine stopped due to adverse effect and pyridoxamine is ineffective in many study setup. Berberine exerts renoprotective effects by regulating the AGEs-RAGE Signaling pathway in mesangial cells during diabetic nephropathy. Nanoparticle loaded with AGEs inhibitor could be excellent if challenges are met with meticulous study.

Targeting aldose reductase [2]: This enzyme plays crucial role in the development of diabetic nephropathy as well as neuropathy. *In vitro* study inhibition of Aldose Reductase- Epalrestat inhibited the signaling and kidney cell injury induced by high glucose, thus Aldose treatment of diabetic nephropathy. Further, investigation in animal models and human subject is necessary. Aldose reductase inhibitors are in budding stage and currently no study data using nanoparticle is available.

Targeting PLC γ in DN [2]: Increase the level of angiotensin II will cause podocyte dysfunction through PLC γ mediation causing changes in α actinin distribution. PLC γ INHIBITOR U-73122 prevented actin changes, Podocyte dysfunction, Preserve PIP2 and regulate the function of filtrations slits. Unfortunately no more progress is made on such inhibitors. LC γ inhibitors are in budding stage and currently no study data using nanoparticle is available. Nanoparticle loaded with PLC γ inhibitor could be excellent if challenges are met with meticulous study.

Targeting NLRP-3 [2]: In investigational study Curcumin reduced renal hypertrophy, Reduced mesangial matrix expansion, Decreased collagen IV and fibronectin level, Reduction in interleukin 1 β and NLRP 3 level, Antifibrotic activity, Curcumin inhibited the NLRP3 Inflammasome activity.

Role of NLRP 3: Hyperuricemia activate NLRP 3 of macrophages leads to evolution of diabetic nephropathy. Tubulointerstitial inflammation in Diabetic kidney takes place due to ATP-P2X4 signaling mediates high glucose-brings activation of the NLRP3 inflammasome, controls IL-1 family cytokine secretion. NLRP 3 is activated in the numerous inflammatory and autoimmune diseases.

Inhibitors of NLRP 3: Inhibitors like MCC 950, β Hydroxybutyrate, Type I interferon (IFN) and IFN β , CB2R agonist like HU 308 for handling NLRP3 inflammasome linked disease by bringing autophagy. Plant polyphenolic compound Resveratrol induces autophagy, inhibit mitochondrial damage in macrophage and subsequently prevent NLRP3 inflammasome

activation and mediated by IL-1 β secretion. Further targeting NLRP-3 would be hopeful approach for diabetic nephropathy. Curcumin nanoparticle are formulated, however the difference between curcumin and nanoparticle delivered curcumin on NLRP3 is not yet evaluated.

Currently specific NLRP 3 inhibitor loaded nanoparticle is not available since such inhibitors are yet in the budding stage. Nanoparticle loaded with NLRP3 inhibitor could be excellent if challenges are met with meticulous study.

Inhibitors of p38MAPK signaling Pathway [2,31]:

Animal model study

Beraprost Sodium: In investigational study Beraprost sodium enhanced lipid profile, blood glucose, 24 hour urinary protein p38MAPK signaling pathway is activated in the diabetic kidney.

Type 2DM: It prevented kidney dysfunction and pathological change the protective mechanism is complicated but credit goes to inhibition of p38MAPK signaling pathway. Further study is needed to understand the beneficial effect of inhibiting pathway. Currently nanoparticles of Beraprost are tested in animal models with pulmonary hypertension [31] on similar lines it could be tested in DN.

mTOR as molecular Target [2,32-34]: The mTOR activation can occur from hyperglycaemia via PI3K OR AKT.

Increase expression mTOR causes: Tubular injury and apoptosis, Mesangial cell expansion, Thickening of basement membrane, Epithelial mesenchymal transition (EMT), Fibroblast Proliferation, Increase Ccn2 activity leading to fibrosis.

Targeting mTOR would be beneficial: At present no clinical study with mTOR inhibitor in diabetic nephropathy is available. Nanoparticle using PAMAM, zinc oxide and silica [32-34] are formulated and target mTOR pathway, however no study data is available for biodegradable non nephrotoxic nanoparticle loaded with mTOR inhibitors.

Targeting Notch-1 signaling: Notch-1 signaling is up-regulated due to high glucose in podocyte and induces VEGF expression and following suppression and apoptosis. Modulation of NOTCH-1 signaling may hold potential as a unique therapeutic target for treatment of diabetic nephropathy. Currently specific Notch-1 modulators loaded nanoparticle is not available since such modulators are yet in budding stage. Nanoparticle loaded with Notch-1 modulators could be excellent if challenges are met with meticulous study. Several investigation and study have been done and reported by researcher about nano particle methods to increase bioavailability, methods to stabilize nanoparticle, prevent degradation, methods to overcome solubility issues, control release from particle

[35-39] and use of biodegradable, non-toxic, biocompatible nanomaterial in drug delivery [3,5,7,9,11-13] one such drug molecules which is well reported in nanoparticle formulation is Resveratrol. So based on the application of nanotechnology advances and identification of molecular target, cell signaling pathways in DN is possible to deliver drug or silence gene or deliver gene activator or drug with siRNA combination [40] using Nanoparticle. However, very few studies, especially in DN are available. In Nutshell to say versatile drug delivery will overcome all hurdles of treating DN.

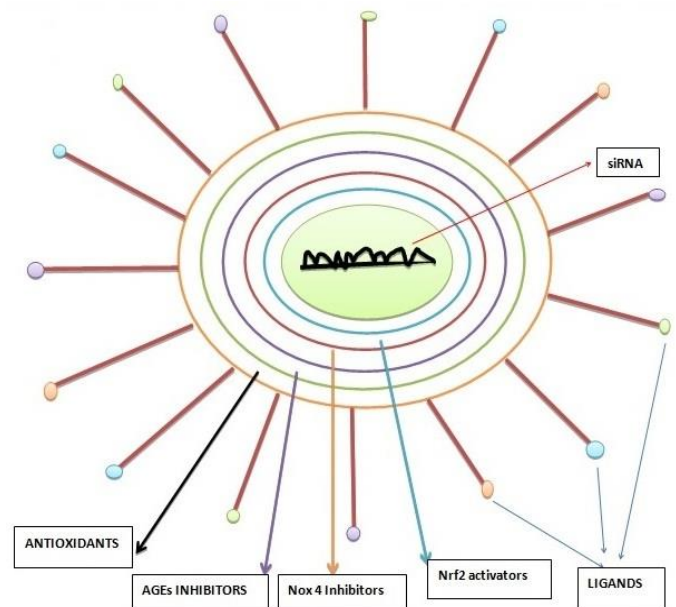


Fig. 2: Multidrug layered with siRNA loaded nanoparticle and ligands attached at surface for specific site delivery- Schema of versatile nanoparticle delivery different drug and silencing gene a unique combination

Limitation: Drug, which is unstable in acidic pH and which are broken down from enzymes in endosome or gets ionized in acidic pH limits the use of nanoparticles delivery through endosome pathway. Physicochemical property or drug solubility, stability in acidic media is hurdle in drug delivery through nanoparticles.

CONCLUSIONS

Many drugs targeting unique molecular target have been identified, some have low bioavailability, some have off-target effect, which can be overwhelmed by using versatile drug delivery system or deliver siRNA to silence the gene or induce gene to restore the balance. It is possible to load multidrug or combine drugs with siRNA also old drugs with new tactic can be delivered at target site with the use of nanoparticles. Nanoparticles can increase bioavailability, controlled release provide stability to unstable compounds/drugs, prevent degradation, site specific targeted delivery. So calcium phosphate nanoparticles, which are nontoxic and biodegradable can be used to deliver drug, genes activators or siRNA or combination or multidrug if the challenges are met. However, several further

investigations must done using drug targeting precise molecular pathway in DN.

If the experiments are encountered in manipulative, preparation, production of biodegradable nanoparticles one can load multiple drugs and siRNA and deliver at specific site maintain good intracellular concentration of drugs and block multiple mechanism responsible for pathogenesis in cell line study and animal models of diabetic nephropathy such type of delivery will dispose of oral solid drug delivery system and improve the quality of life and survival rate in patient with diabetic kidney failure and may prolong the need for dialysis or delay the dialysis.

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