

QUALITATIVE ASSESSMENT OF RENAL BLOOD PRESSURE USING NANOTECHNOLOGY INTERVENTION

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Abstract

The renal system is one of the major organ systems present in the human body, it is responsible for maintaining electrolyte balance, secreting important hormones, and maintaining homeostasis in the body. Every individual has 2 kidneys which are located behind the peritoneum at the level of L2-L3 from the vertebral column. Broadly kidneys function to filter blood, purify it, and eliminate toxic waste. We propose a diagnostic test that evaluates the renal blood flow rate in the kidneys and assesses their functioning based on their hemodynamics in the given time frame. Here we aim to explore the application of nanoparticles to assess localized blood pressure in the kidney based on the illumination intensity/radioactivity. Connexin40(Cx40) is a transmembrane protein present in juxtaglomerular apparatus, Cx40 gold antibodies prepared can be conjugated with nanoparticles and introduced in renal circulation. Imaging technologies such as fluorescence/computed tomography can be used to detect these nanoparticles. Once injected they can be assessed and information on renal blood flow can be obtained. The intensity generated on screening can be used for the assessment of renal blood pressure. This information will be valuable for the assessment of kidney function.

Keywords: Kidney, Diagnostic test, Gold nanoparticles, Connexin 40, Antibodies, Fluorescence

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BACKGROUND

Kidneys are one of the major organ systems present. They mainly function in the elimination of toxins and maintaining electrolyte balance in the body. Nephrons are the functional units present that are responsible for the filtration of blood [1–3]. A single nephron contains a bowman's capsule (BC) containing the glomerulus

apparatus, proximal convoluted tubes (PCT), the loop of Henle (LH) and distal convoluted tubes (DCT), and the collecting ducts [1–3].

Blood first enters the glomerular apparatus via the afferent arteriole, here the blood gets rid of all the toxins, and purified blood is sent back out of the

nephron through the efferent arterioles [4–9]. All the toxins gathered in the bowman's capsule are transferred to PCT, LH, and DCT. Here via the counter-current mechanism between the nephron tubules and blood vessels. The urine formed gets more concentrated and excessive electrolytes are drained off in these tubules via the blood vessels. All the concentrated urine is then transferred to the collecting duct which transfers this urine to the urethra [4,7–14].

The afferent and efferent arterioles present in the bowman's capsule are essential in maintaining the local renal blood pressure [15–17]. The blood pressure is maintained with the help of the renin-angiotensin mechanism and connexins. The former is responsible for increasing blood pressure and the latter is responsible for reducing blood pressure [17,18]. Kidneys have specialized connexin 40 (Cx40) transmembrane channels that perform this function. Connexin 40 proteins are a part of the juxtaglomerular apparatus and directly influence the nuclear tractus solitarius structure of the brain, which is responsible for maintaining blood pressure throughout the body via the baroreceptors [12,13,19].

In the past few decades, extensive research has been dedicated all across the world, in light of the development of kidney diseases (chronic and acute), cancer, infections, etc. Disorders of the renal system place a severe burden on people and also on health care systems. Various research is underway in the hope to tackle these problems. Nanoparticles have provided an effective option for the treatment of this disorder. A pilot study conducted by [20] used lymphotropic nanoparticles for the assessment of renal cancer using MRI. Here, the cancer grade and stage were determined with the help of nanoparticle-guided MRI [20]. Another study exploited the use of triptolide mesoscale nanoparticles (TP-MNP) for the treatment of renal ischemic reperfusion injury (IRI). The toxic effects of triptolide were significantly reduced when encapsulated with nanoparticles as compared to its controls [21]. In another study based on histopathology, the toxicity profiles of gold nanoparticles were assessed in the liver, spleen, and kidney in mice. The study revealed negligible toxicity caused by gold particles in the kidney [22]. It has also been proposed that the smaller the size of nanoparticles, more the efficacy they tend to have. Based on this concept another research team developed polyethylene glycol-polylysine (PEG-PLL) gold nanoparticles and tested them for photoacoustic imaging and computed tomography [23]. This provided two advantages, firstly being large gold nanoparticles can be injected into the

circulation for efficient imaging studies and secondly the large gold nanoparticles will be broken down by the body into harmless products, giving small nanoparticles that could enter the renal tissues, studied and swiftly removed from the system [23]. Apart from nanoparticles a study also evaluated the use of black phosphorus quantum dots for photoacoustic imaging in renal tissues [24]. Hence nanoparticles have various applications in the treatment of renal disorders.

HYPOTHESIS

Here we propose to use connexin transmembrane channels as a marker of localized blood pressure in the region. In this experiment, each nanoparticle is conjugated with a connexin 40 antibody (Cx-40 antibody) [25,26]. Nanoparticles are chosen due to their relatively reduced biotoxicity and increased surface area. The cx40 antibodies are also tagged with a complementary immunofluorescent molecule that emits fluorescence based on the expression of cx40. These transmembrane channels are highly efficient in reducing blood pressure and their activity can be accurately tracked by nanoparticles.

Nanoparticles conjugated with anti-Cx40 antibodies can be introduced into renal circulation via intravenous routes or a continuous infusion procedure could be followed. The individual is then subjected to immunofluorescent imaging techniques that can be used to determine the intensity of fluorescence. The blood pressure of that region will depend on the amount of Cx40 activity in the region [11,27,28], Cx40-antibody tagged nanoparticle will bind to the Cx40 transmembrane proteins present in the JGA cells, the Cx40 transmembrane proteins will be activated only when the patient is hypotensive to raise the blood pressure and bring them in normal parameters. The relative activity of Cx40 as detected by immunofluorescence can give estimates of time-dependent blood pressure in the region. A time-dependent curve could be drawn to assess the local renal blood pressure.

Fluorescent tomography can be used for accurate nanoparticle detection, it has been shown that fluorescence-guided surgeries have effectively been employed in determining tumor margins for surgical purposes [29,30]. Fluorophores have been used as molecules to detect these margins by using gold nanoparticles, this technique can be modified and applied here for diagnostic purposes. The imaging technologies can also be extended to humans in clinical trials for efficient diagnosis [31], the type of imaging technology used will depend on the type of nanoparticle

used and the feasibility of the experiment, many nanoparticle detection imaging technologies have been used in cancer therapies [32–34].

EVALUATION OF HYPOTHESES

The diagnostic technique proposed must be tested before translating it into clinics and making it available for mass use. Any diagnostic technique aims to provide individuals with efficient results to the clinician which will aid them in making an appropriate diagnosis, it is generally aimed that the diagnostic technique is safe and preferably non-invasive without causing any complications, cost-effective, and could be easily interpreted and which don't have many false positive results [35–37].

Here, we propose the preparation of lab-based gold nanoparticles. Gold nanoparticles are chosen because of their increased efficiency, biodegradability, and negligible toxic effects. These gold nanoparticles can be conjugated with connexin 40 antibodies. These antibodies can be purchased and prepared according to the manufacturer's guidelines. These connexin40 antibodies must be conjugated with gold nanoparticles for testing. The compatibility of antibodies with nanoparticles must be assessed before testing. The antibodies must also be conjugated with a radioactive molecule (like technetium-99) or a fluorophore based on the type of imaging used for experimentation. The binding efficiency of these Cx40-AuNP-FI/Rx must be assessed to determine the efficiency of the diagnostic testing. The type of imaging technology used will depend on the type of effector molecule used. Imaging technologies like fluorescence tomography for detecting fluorescence. To detect radioactivity computed tomography, X-ray, etc can be applied. We suggest the use of fluorescence to limit toxicity effects.

In vitro and in vivo testing must be performed before experimenting on humans. A clear picture of histological features, toxicity profiles, and receptor-antibody binding must be present. Dose modification must be done as per experimental data. The experimental framework can be proposed in which the population is matched with controls versus testing groups. The control group should provide information about healthy renal function. The testing group can provide information about which particular renal disorder is selected to assess blood flow to the kidneys for that particular group. The inclusion criteria can be age, sex, lifestyle habits, serum creatinine levels, presumed GFR, and rates in normal and testing populations. Other parameters can be included based on the renal disorder to assess the efficacy of the

diagnostic test. Exclusion criteria can involve people having multiple issues which might compromise renal function, and bad lifestyle habits such as alcohol consumption, smoking, etc. However, a separate study can be conducted in a population to study assess renal blood flow in healthy populations versus people with bad lifestyle habits.

A standardized scale should be prepared based on the results of multiple experiments which will enable qualitative assessment of renal blood pressure, further analysis can be done to mark the intensity of fluorescence, determine renal blood pressure, and direct clinical treatments which could be administered to the patients.

It must be noted that various parameters may affect the experiment like the type of nanoparticles used, fluorescence emission, imaging techniques, and chemical, physical, and biological properties of nanoparticles. All these parameters must be assessed before applying this procedure in experimental use.

Based on the imaging technology and standardized scale, data can be obtained about the localized blood pressure of kidneys at a given particular time. Graphical charts could be prepared with a function of emission of fluorescence with the function of time, renal blood pressure could be estimated by estimation of slope value and exact blood flow can be determined at that particular time.

It can be speculated that a stable constant graph representing a horizontal line indicates that blood pressure has been stable for a given period, Fluctuations of graphical charts can indicate the existence of any pathology or changes in renal function in the given time frame, hence this technique can prove to be a tool used for early detection of any underlying disease affecting kidney functioning. Many pathological conditions which affect the renal vasculature and increase or decrease the blood flow to the organ can be detected.

All experimentations must be conducted with approval from proper ethical authorities. Ethical approval for conducting experiments on animal subjects permissions must be taken from the institution. If the diagnostic test is performed in humans, ethical consent must be taken from patients and required approvals from the institutional ethical committee. The hypotheses presented here will provide additional clinical information that will guide doctors to better plan treatments for their patients.

CONSEQUENCE OF HYPOTHESES AND DISCUSSION

The above-given technique is hoped to give clinical assessment values for the qualitative estimation of blood pressure, nanoparticles are suggested to be used in this experiment. Nanoparticles have a high surface area which will allow more antibody-cx40 to be attached to the renin cells for more accurate diagnosis and reduced toxicity, Nanoparticles can also be PEGylated to enhance their circulation time in blood for prolonged analysis and acquire sufficient pictures for analysis.

When nanoparticles reach the target site, the Cx40 antibodies will recognize the transmembrane proteins attached to it, the antibody will bind to the protein & fluorescence will be emitted from the nanoparticles. Higher fluorescence emitted by the nanoparticles, hence higher renal blood pressure indicating a reduced efficiency of connexins accounting for fluctuations in blood pressure. The emitted fluorescence can be measured by fluorescence tomography, nanoparticles can also be designed in a way that can be detected in imaging technologies. Using imaging pictures, standardized scales can be developed to make an assessment and give qualitatively estimated blood pressure.

This piece of clinical information will prove valuable to clinicians and will allow them to more accurately assess their patients suffering from chronic kidney disease (CKD), acute kidney failure, renal cell carcinoma, and other diseases that heavily rely on the vasculature of the renal system if an estimate of the renal blood pressure mechanism is known, medications can be developed for accurate targeting of underlying mechanisms and more accurately monitoring and controlling the renal blood pressure and protect the kidneys from further damage.

Thorough insights into the pharmacokinetics of these nanoparticles must be studied to determine their stability and half-lives in the body for accurately analyzing renal blood pressure, and a study for determining the accurate dosage of nanoparticles must be administered to assess their toxicity profiles.

Kidney disorders are very prevalent in many countries, especially in Central Asia. Diseases like CKD are highly prevalent in eastern, southern, and southeastern Asian countries [38,39]. These diseases are usually diagnosed in higher grades and treatment options become very limited. IgA nephropathy is also highly prevalent in Asia and treatments must be given for

better grade outcomes [40]. Today's sedentary lifestyle, changes in food habits, alcohol and tobacco consumption, etc. have significantly increased the healthcare burden and caused severe morbidity due to kidney disorders. There is an urgent need to promote research to provide the treatment of renal disorders and make the latest treatments available for everyone.

CONCLUSIONS

Renal blood pressure assessment can prove to be an important diagnostic parameter indicating the condition of the kidney and how diseases have reduced the efficiency of their functioning. More accurate and precise treatments can be given to the patients based on their kidney functioning and provide hope for more research for precise targeting, which will help preserve the leftover functioning of the kidney. To develop the technique in a clinical setting, a thorough study of the type of nanoparticles to be used and nanoparticle detection imaging technologies must be assessed. The test can only be accurate in adults due to well-formed and compacted JGA. The main challenge of the study is the half-life of nanoparticles introduced in the patient and also it is important to identify suitable and cost-efficient imaging technology which can be used for the experimental procedure. Pharmacological studies must be conducted to generate standardized dosage to be given to patients, a thorough study based on the elimination of these nanoparticles must also be addressed. Thorough research must be done to recognize which imaging technologies would be suitable for this study.

DISCLAIMER

The manuscript including the figure is an original work contributed by all authors and has not been published anywhere

CONFLICTS OF INTEREST

None

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AUTHOR CONTRIBUTIONS

Parth Shah: Framing of hypotheses, manuscript designing, and writing.

Dr. Roja Rani Pallavali: Verification of content and suggestions for improvement of the text.

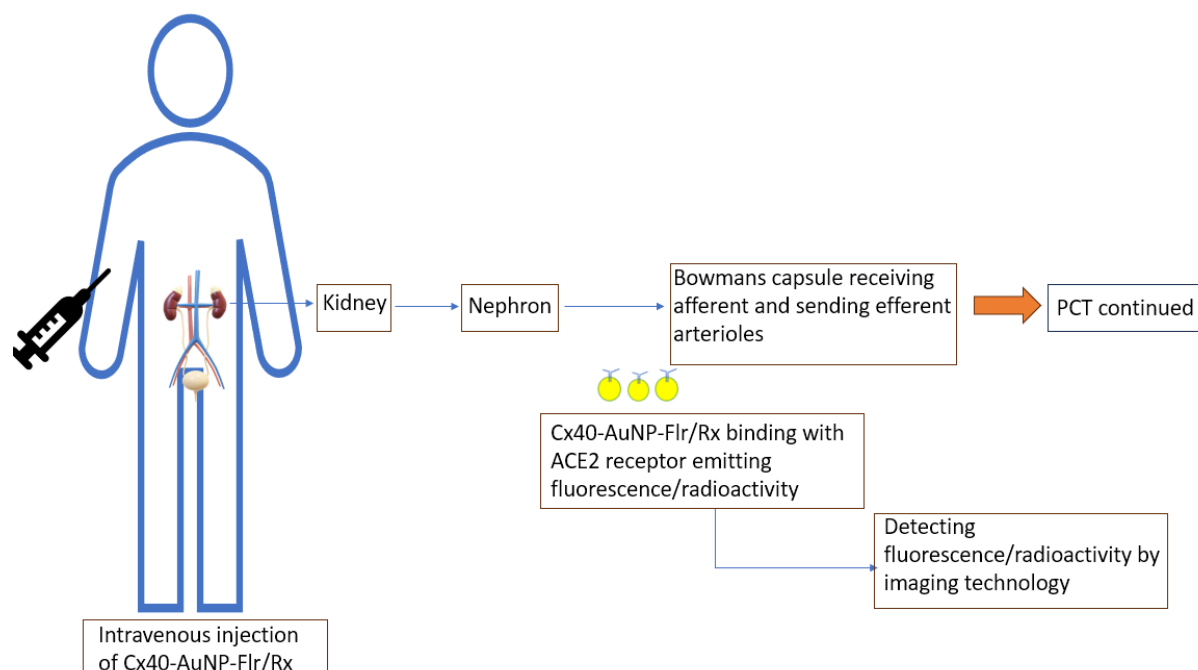
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Figure 1. The figure shows intravenous injection of nanoparticles tagged with Cx40 antibodies in the bloodstream, it also shows a schematic representation of the experimentation and live imaging of nanoparticles in the renal circulation. Abbreviations: Cx40-AuNP-Flr/Rx:connexin40-gold nanoparticle-fluorescence/radioactive), ACE2: angiotensin converter enzyme-2 , PCT: proximal convoluted tube, Cx40: Connexin 40.



НАНОТЕХНОЛОГИЯЛЫҚ АРАЛАСУДЫҢ ҚОЛДАНУЫМЕН БҮЙРЕКТІК ҚАН ҚЫСЫМЫН САПАЛЫ БАҒАЛАУ

Түйіндеме

Бүйрек жүйесі адам ағзасындағы негізгі мүшелер жүйесінің бірі. Ол электролит тепе-теңдігін сақтауға, маңызды гормондардың бөлінуіне және ағзадағы гомеостазды сақтауға жауап береді. Әр адамның омыртқа бағанынан L2-L3 деңгейінде перитонийдің артында орналасқан 2 бүйрегі бар. Кең мағынада бүйрек қанды сүзеді, тазартады және улы қалдықтарды кетіреді. Біз бүйректегі бүйрек қан ағымының жылдамдығын бағалауға және олардың гемодинамикасы негізінде олардың жұмысын белгіленген мерзімде бағалауға мүмкіндік беретін диагностикалық тест ұсынамыз. Мұнда біз жарық қарқындылығы/радиоактивтілік негізінде бүйректегі ошақталған қан қысымын бағалау үшін нанобөлшектерді қолдануды зерттеуге тырысамыз. Коннексин40 (Cx40) - юкстагломерулярлық аппаратта болатын трансмембраналық ақуыз болып саналады. Алынған Cx40 алтын антиденелерін нанобөлшектермен біріктіріп, бүйрек қанына енгізуге болады. Бұл нанобөлшектерді анықтау үшін флуоресценция/компьютерлік томография сияқты бейнелеу технологияларын қолдануға болады. Инъекциядан кейін оларды бағалауға және бүйрек қан ағымы туралы ақпарат алуға болады. Скрининг кезінде пайда болатын қарқындылықты бүйрек қан қысымын бағалау үшін пайдалануға болады. Бұл ақпарат бүйрек қызметін бағалау үшін пайдалы болады.

Түйінді сөздер: бүйрек, диагностикалық сынақ, алтын нанобөлшектері, коннексин 40, антиденелер, флуоресценция.

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КАЧЕСТВЕННАЯ ОЦЕНКА ПОЧЕЧНОГО КРОВЯНОГО ДАВЛЕНИЯ С ИСПОЛЬЗОВАНИЕМ НАНОТЕХНОЛОГИЧЕСКОГО ВМЕШАТЕЛЬСТВА

Резюме

Почечная система является одной из основных систем органов в организме человека. Она отвечает за поддержание электролитного баланса, секрецию важных гормонов и поддержание гомеостаза в организме. У каждого человека имеется 2 почки, расположенные позади брюшины на уровне L2-L3 от позвоночного столба. В широком смысле почки фильтруют кровь, очищают ее и удаляют токсичные отходы. Мы предлагаем диагностический тест, позволяющий оценить скорость почечного кровотока в почках и оценить их функционирование на основании их гемодинамики в заданные сроки. Здесь мы стремимся изучить применение наночастиц для оценки локализованного артериального давления в почках на основе интенсивности освещения/радиоактивности. Коннексин40 (Cx40) представляет собой трансмембранный белок, присутствующий в юкстагломерулярном аппарате. Полученные золотые антитела Cx40 можно конъюгировать с наночастицами и вводить в почечный кровоток. Для обнаружения этих наночастиц можно использовать такие технологии визуализации, как флуоресценция/компьютерная томография. После инъекции их можно оценить и получить информацию о почечном кровотоке. Интенсивность, генерируемая при скрининге, может быть использована для оценки почечного артериального давления. Эта информация будет полезна для оценки функции почек.

Ключевые слова: почка, диагностический тест, наночастицы золота, коннексин 40, антитела, флуоресценция.

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