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Review Article

A comparative review on *In-vivo* and *In-vitro* screening models for diuretic agents

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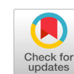

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ABSTRACT

Diuretics are medications that aid in the elimination of excess urine. Water, electrolytes, and metabolic waste are all found in urine. The anatomy and physiology of the renal excretion system are the topics of the current study. Along with the reasonable diuretic activity it also highlights the kidney's involvement in renal excretion. The mechanism of several diuretic classes is connected to their active target site in this overview. Diuretics have previously demonstrated their clinical efficacy in treating conditions including hypertension, renal failure, nephritic syndrome, and others. Here, several in-vitro and *in-vivo* screening models demonstrate their clinical relevance. The logical goal, experimental methods, and assessment criteria of diverse screening models of diuretic action are highlighted in the current study. It also highlights the value and significance of these screening methods for determining the efficacy and safety of various members of various types of diuretic medicines. This review also revealed how some screening model improvements were successful. Studying the diuretic potential of various drugs is therefore absolutely helpful. In the end, the current review establishes a robust framework for dosage modification and fatal impact research of diverse kinds of diuretics. Thus, this study provides some suggestions for choosing cutting-edge methods to check the activity of diuretics. It also exposes the effects of the creation and adjustment of these screening methods, making them more effective in the future for assessing the diuretic potential of various diuretics agents.

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1. Introduction

The name "diuretics" is derived from the prehistoric phrase "Diu oyr1ih" (Diu means through & oyr1lih means to urinate). Diuretics contain a variety of ingredients that speed up the production of urine and facilitate water excretion.[1] It mostly causes a rise in sodium, chlorine, bicarbonate, and water excretion. Additionally, it prevents the reabsorption of water, salt, chlorine, and bicarbonate. The result will thus be an increase in the rate of urine production.

The kidney is crucial for excretion. Nephrons are the primary functional unit of the kidney, and the renal excretion system has three main pathophysiological components: glomerular filtration, PCT, DCT mediated reabsorption and active secretion. 25% of cardiac output is directed toward glomerular filtration. It filters between 100 to 120 ml per minute.[2] The typical daily filtering capacity for a bowman's capsule is 180 lit. Glomerular filtration is primarily reabsorbed by the proximal and distal convoluted tubules. 60–70% of the sodium is recycled via the proximal tubules. It can pass through water. The substance is isotonic with urine. Proximal convoluted tubules are the main site of water absorption. Sodium reabsorption is shown by the Henle loop. It is not permeable to water. Distal tubule promotes the reabsorption of salt. The collecting duct is water impermeable.

Drug belonging to diuretics class show desired effects through the inhibition of reabsorption of several electrolytes

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like sodium, potassium, chlorine and bicarbonate etc. loop of Henle, early distal tubule, late distal tubule and collecting duct which are offers themselves as a prime target site for diuretics. Various *in-vivo* and in-vitro techniques are available to assess the diuretic's action. There are several ways for evaluating the activity of diuretics, including urine volume, electrolyte content.

2. In-vitro Methods

2.1. Isolated tubule preparation

2.1.1. Principle

Measuring changes in solute content in perfusion fluid. If the target location and mechanism of action of diuretics are known at that time, this is the preferred strategy. Examples of distinct tubule fractions include thin as seceding loop of Henle, and distal convoluted tubules. This method includes measuring the shift in solute concentration in the perfusion fluid.

2.1.2. Procedure

Scientist Burg and a colleague created the perfusion mode off isolated renal tubules in 1996. Following his creation, it has been successfully used to a variety of animal species, including the wrister albino rat, mice, rabbit, and others.[3] The thin (1 mm) kidney tubule fragment is extracted and then put through development assembly. A micropipette hole is drilled into a suitable tubule's end to allow for perfusion. The lumen of the kidney is filled using a perfusion pipette. The tubule's remaining end is drawn into the pipette used for collection. The collecting pipette's oil stops the evaporation. By submerging a thin gradual pipette in the collecting pipette, the entire collected fluid is collected at appropriate intervals. By placing a tubule in a rabbit serum bath, an isotonic rabbit serum sample is obtained to simulate an in-vivo environment.

2.1.3. Evaluation

The change in concentration of an impermeable marker, such as (3H) insulin or (125I) iothalamate in the collecting fluid, is used to calculate the absolute volume of reabsorption. From the appearance of the marker in the external bath, leaks near the perfusion pipette may be identified.

2.2. Carbonic anhydrase inhibition

Acetazolamide (Diamox®) is the first member of this class to have received recognition. It inhibits the carbonic anhydrase enzyme's activity.[4] It is a synthetic derivative that shows zinc presence in its structure from a structural point of view. It is primarily in charge of how CO_2 and water combine to generate carbonic acid. Additionally, it transports reabsorption of water, sodium, and bicarbonate in the proximal convoluted tubule. By inhibiting the action of the enzyme carbonic anhydrase, these inhibitors prevent the reabsorption of salt, bicarbonate, and water. Scientist Maren was given a description of the micro technique in 1960, which seems simple and effective. It is fully enriched in red blood cells. Enzymes, which are also found in the eye, are a key source of the same.[5]

2.2.1. Procedure

Reaction Vessel (Monostat bench mounted flowmeter) is used. CO2 flow rate is.maintained on. 30- 45 .ml/min. In this model, following parameters are evaluated in duplicate samples: Tu (Uncatalyzed time) stands for required time.to occur color change in the absence of enzyme, The (Catalyzed time) stands for required time to occur color change in. the presence of.the enzyme. Tu – Te elucidates enzyme rate and Ti stands for enzyme rate with the presence.of assorted concentrations.of inhibitor.

2.2.2. Calculation

Percentage reduction in activity of carbonic anhydrase.enzyme is measured by employing below formula.

$$percentage\ inhibition = \frac{1-(Tu-Te)-(Ti-Te)}{Tu-Te}$$

Tu =. (Uncatalyzed time). = required time.to occur color.change in the absence of enzyme.

T_e =. (Catalyzed time) = required time to occur color change in. the presence of .the enzyme.

Tu – Te. = enzyme rate.

Ti =. enzyme rate with the presence.of assorted concentrations.of inhibitor.

Measurement of Percent inhibition of CA - inhibitors is effective tool to access the diuretic potential of several sulfonamides. There are several implementations have been reported for this procedure by Landolfi et al. (1997).[6] It measures time which is required to for pH alternation between 8 to 7.5. further alternation in time period and PH can be achieved with use of such CA enzyme inhibitors.

2.3. Patch clamp technique

2.3.1. Principle

The numerous kidney segments, including the Loop of Henle, early and late convulated tubules, etc., play a significant part in fluid reabsorption throughout the whole excretion process. In that instance, there would either be active secretion or a flow of material into the bloodstream from the tubular lumen. Numerous connected transport technologies are also available in addition to active transportation. Ion channels stand out among them as having a significant impact on renal cell activity. Regarding the usage of single and entire cell ion channels, many variations of this technology considerably differ from one another. Use of patch electrodes with reasonably big tips (greater than 1 mm) and smooth surfaces is permitted. There are various

technique models for patch clamp as attached mode with cell, excised mode with cell and whole- cell mode.[7]

A cell membrane can be inserted within the tip of an electrode by applying vacuum and compressing a patch-clamp electrode on the other side of the membrane. Because of this vacuum pressure, the cell produces an electrode that is tightly sealed and has a high resistance (>10 giga ohms).

Cell-attached mode: This method involves enclosing the patch electrode within the cell membrane, allowing currents from the membrane patch to flow through nearby single-ion channels that are covered by the electrode tip.[8]

Whole-cell mode: In contrast to the prior cell-attached mode, more suction is used to produce cell membrane rupture and increase access to the inner cell channel. The electrodes content takes a place of cell's soluble content this method only allows for the single operation of current passage from all of the ion channels in the membrane.

2.3.2. Evaluation

Drug concentration versus ion channel inhibition is presented on a graph. With the aid of a split cell from the Assen ending loop of Henle, whole cell operational mode from patch clamp technique gives superior and effective measurement of sodium- Alanine co transport. It is possible to record the apparent Km values for sodium and L-alanine.[9]

3. *In-vivo* Methods

3.1. Lipchitz test

3.1.1. Principle

Based on water and sodium excretion in test animal and compared to rats treated with std. drug. Male Wistar albino rats weighing 100-200 gm are used and placed in metabolic cages. Animals are divided into three group like test, control and standard.[10]

3.1.2. Procedure

There are 5 groups of animals in this technique. Each group has six different creatures. Animals from each group are put into metabolic cages that have a wire mesh bottom and a funnel for simple urine collection. Only pee travels through the funnel, which is kept clean by SS sieves to limit excrement. Group I is given the control (for example, normal saline solution), Group II is given the reference standard (in a standard dose as per reference), and Groups III, IV, and V are given the test sample in a dose in accordance with an acute toxicity study (mild, moderate, high). Animals are examined for obvious illness symptoms prior to research, and only healthy animals are used in the experiment. Before the trial lasts 17 to 24 hours, food and drink are removed. A typical room temperature of 25 2 OC is maintained throughout the investigation. Prior to administering the sample or controls, great care must be taken to ensure that the rats' bladders are empty by pressing on the pelvic region and extending their tails.[11] It makes sure that each animal receives the identical dosage, which is typically prepared in an equal volume of normal saline. For the administration of reference/tests, the i.p. method is typically favoured since it is simple and safe for administering higher fluid dosages. Animals are placed in a metabolic cage after treatment that is specifically made to collect urine. The volume of urine excretion is measured after 5 and 24 hours. Na concentration in urine is determined using flame photometry. It is preferable to test an active chemical at lower dosages.[11]

3.1.3. Evaluation

Following formula is employed to measure this index.

$$Lipchitz\ value = \frac{Urine\ output\ in\ test}{Urine\ output\ in\ Standard}$$

3.2. Saluretic activity test in rates

3.2.1. Principle

The maintenance of electrolyte and water excretion is used to treat peripheral oedema, congestive heart failure, and hypertension. However, in that situation, potassium (K+) loss must be prevented, leading to the creation of saluretics and potassium-sparing diuretics. This diuretic test for rats has undergone a number of improvements that allow for the detection of potassium and chlorine (Cl-) levels in addition to sodium and water content. Osmolality determination is also part of this test.[12]

Electrolyte ratio calculations are used to quantify the effects of potassium sparring and carbonic anhydrase inhibition.

3.2.2. Procedure

Only male Wister rat species (weighing 100–200g) can be used for this test. The three groups of animals are Control, Standard, and Test. There are three creatures in each group. Male Altromin pellets are a common staple of the usual diet for Wistar rats., plus water. Prior to the 15-hour trial, just food is removed. Test chemicals are given orally at a dosage of 50 mg per kilograms in 0.5 ml per 100 g of body weight. Suspension of starch weight. Each metabolic cage should only hold 3 animals, together with wire mesh and a funnel assembly at the bottom, for the intended function of urine collecting. Up to 5 hours' worth of urine excretion volume is monitored every hour. After 5 hours, Flame Photometry is used to assess the sodium (Na+) and potassium (K+) content of the collected urine sample. Urine samples that are taken up to 24 hours after the test are analysed to determine the test sample's prolonged impact. As a standard, furosemide or hydrochlorothiazide are chosen in this procedure.[13]

3.2.3. Evaluation

The total of sodium and chloride excretion (Na+ excretion + Cl- excretion) is used to quantify the saluretic activity. The ratio of sodium excretion to potassium excretion (Na+ excretion / K+ excretion) is used to quantify natriuretic activity. The ratio of chloride excretion to the total of sodium and potassium excretion (Cl- excretion / Na+ excretion + K+ excretion) is used to quantify carbonic anhydrase inhibition. Ion quotient is the name for this ratio.[14]

3.3. Stop flow technique

3.3.1. Principle

The Stop flow approach significantly localises the renal tubular fluid passageways that linn with nephron structure. Glomerular filtration rate is dramatically decreased when the ureter is clamped. When the intracellular renal sample specimen's electrolyte concentration reaches the state of absolute static equilibrium-head, the contact time between the tubular fluid and the corresponding nephron segments increases. When the clamp is removed, the fluid in the tubing changes somewhat in composition. The distal nephron segment serves as the site for the first sample, and glomeluar fluid serves as the site for the last sample.[15]

In compared to the micropuncture approach, the stop-flow method is now thought to be the least recommended method.

3.3.2. Procedure

Various animal species can be used for the stop flow procedure. This method enables the animal's ureter to be clamped for a number of minutes, producing strong osmotic dieresis. Because of this, equilibrium renal column pressure allows for longer than usual periods of intact exposure between various renal segments. As a result, the experimental approach is expanded on every segment of tubular fluid. Following the release of the clamp, a urine sample is taken. Smaller samples are quickly taken in this set of activities, whereas prior samples expressed tubular fluid that was in touch with the distal region of the nephron.[16] Test samples are given out along with inulin before urethral occlusion is done. When a nephron enlarges, the downstream content of tubules, particularly from the proximal section of the nephron, may change the fluid content of the tubules.

3.3.3. Evaluation

Stop flow approach examines the concentration level of test samples as well as globular marker samples like inulin. The fractional excretion volume of the marker and test sample is then plotted against the total volume of the urine.

3.4. Micro puncture technique

3.4.1. Principle

Micropuncture methods found that diuretics affected nephron activity. Electrolyte concentrations and variations in tubular fluid reabsorption rate are primarily employed as assessing parameters to establish this technique's efficacy. Rats are used most frequently in this procedure. The thick ascending loop of the Henle, as well as the distal convoluted tubules and collecting ducts, are the primary target locations for micropuncture activity.[17]

3.4.2. Procedure

Rats of any sex weighing 250g are used for this procedure. Rats are given intraperitoneal injections of thiopentone for anaesthetic purposes. Only food is taken out before the experiment begins, but water is still available. The rats are tracheotomized only after the animals have been given anaesthesia and placed on a table with a thermostat. By cannulating the carotid artery and jugular vein, B.P. measurement, blood sample collection, and compound cure such duties are carried out. The retroperitoneum (excretory organ) can be accessed by a flank incision. This area is then encapsulated in a tiny plastic item with cotton and afterwards bathed in liquid paraffin oil at room temperature. Body temperature is continuously monitored when a cannula is put into the ureter. After three hours, a single big dose of inulin injection produced in sodium chloride solution is given straight into a bolus. Then, 0.85% sodium chloride solution is given at a flow rate of 2.5 ml/min, and the dose is appropriately calculated as per 100 g of body weight. (9) After 45 minutes of intravenous administration, a thin, elongated channel begins to gradually leak. Renal cortex and medulla intracellular liquid samples are directly collected using glass capillaries (8 to 10 m external diameter). A micromanipulator and for that, microscopic observation is employed. Lissamine green is administered intravenously to detect the distal tubule. Only after the control sample is provided are the test samples. Only after a half-hour equilibration interval, which is reached together with the sample material, is micropuncture done once again before tubular fluid is collected. Blood and urethral urine samples are taken between clearing intervals.

3.4.3. Evaluation

The evaluating parameters are as follow: Inulin. Clearance. (GFR), Single. nephron. GFR, Fractional. delivery of. water, sodium and potassium electrolyte concentration in renal tubules and in urine also.

4. Conclusion

To assess the diuretic effectiveness of diuretic drugs, a variety of effective screening procedures are now available. It is the best method for evaluating the efficiency and

potency of various diuretic drugs. Additionally, it offers helpful details on the amount of dose regimen requirements for a certain class of diuretic drugs. Modification highlights current developments in the used method.

5. Conflicts of Interests

The authors have no financial interests or conflicts of interests.

6. Source of Funding

None.

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