Review Article

The Biological Roles of Urea: A Review of Preclinical Studies

Abstract

Urea is an organic compound that has been reported to be effective against many pathological conditions. However, many other studies have reported the toxic effects of urea. These controversies on the biological roles of urea remain unresolved. This review aims to evaluate the biological roles of urea in experimental animals from data published in peer-reviewed journals. A PubMed search was conducted using the phrase, "urea application in experimental animals." A total of 13 publications that met the inclusion criteria were evaluated. The test substance, animal model, number of animals, doses, duration of treatment, and effects were recorded. Regarding the toxic effect, urea caused decreased excretion of other nitrogenous compounds, increased oxidative stress, decreased insulin, and impairment of beta-cell glycolysis. Furthermore, it caused endothelial dysfunction, loss of synapsis, and decreased olfaction. Regarding the therapeutic effects, urea caused increased growth, increased digestion, and decreased hepatic dysfunction. It also induced apoptosis of tumor cells and exerted neuroprotective properties. Products containing urea should be used with caution, especially in individuals with symptoms of chronic kidney disease. However, more studies are needed to elucidate the mechanisms of its therapeutic effects.

Keywords: *Biological Roles, experimental animals, therapeutic effects, toxic effects, urea*

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Introduction

Urea is an organic compound with the chemical formula $CO(NH_2)_2$. It is produced in the liver and serves as the metabolic by-product of protein and nitrogen metabolism.[1] It helps in the excretion of most nitrogen-containing compounds and is highly soluble in water; it has a molecular weight of 60 g/mol. It is colorless, odorless, and neutral in solution. Exogenous urea is usually taken up by specific urea transporters (UT-A and UT-B).[2]

Dating back to the 19th century, the role of urea has been studied extensively. The findings from these studies have, however, remained controversial. The major issue surrounding the role of urea has been its effects on macromolecules within the cells, tissues, and organs of the body. Some studies reported that urea is toxic, especially at a high concentration,[3-5] while other studies claim that urea is nontoxic and even has therapeutic effects.[6-9]

When urea is produced, it is normally excreted into the urine through the kidneys. When urea gets to the kidneys (carried in the bloodstream), it plays an important role in urine formation and urine concentration, alongside with urea transporters. The first stage of urine formation is glomerular filtration. The other processes of urine formation are tubular reabsorption and secretion. In the renal tubules, 99% of the filtrate gets reabsorbed into the extracellular fluid, thus maintaining the fluid volume in the body. Urea filtered across the glomerulus enters the proximal tubule. Absorption depends on the permeability of different parts of the nephron, and absorption of some substances might be passive, actively transported, or co-transported.[10] In tubular secretion, urea and other waste products like uric acid, drugs, hydrogen, and bicarbonate ions are moved out of the peritubular capillaries to the filtrate. This is to maintain the pH and remove unwanted wastes.[11] After the filtrate has undergone the above processes of urine formation, the urine proceeds into the collecting duct, then to the calyces, and finally to the renal pelvis which joins with the ureter.

Principal and intercalated cells are located in the distal part of distal convoluted tubule and collecting duct and are responsible for reabsorption of sodium ions and

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water and the secretion of potassium ions. In addition, the intercalated cells are responsible for secretion of hydrogen and bicarbonate ions.[12] Tubular processes are mainly controlled by local factors such as starling forces and glomerulotubular balance. Tubular processes are also controlled by a central mechanism, that is, hormones such as vasopressin, aldosterone, natriuretic peptides, angiotensin II, parathyroid hormone, and sympathetic nervous system.[13] Urine is about 95% water and 5% waste products, in which urea constitutes 2% of the waste product excreted. In certain conditions, when the kidney functions are compromised, there is a buildup of urea in the blood. The concentration of urea in the blood could also increase when there is an increased or high consumption of protein.[14] The abundance of urea has customarily become a substitute marker of renal function, protein intake, and dialysis.[15] One major condition that affects the kidney function is chronic kidney disease (CKD), a major public health problem[16,17] Individuals with diabetes mellitus, hypertension, cardiovascular diseases, and many more conditions are more predisposed to having CKD.[18-20] Urea concentration has been shown to increase in CKD.[21-23] Although urea is generally believed to be nontoxic, studies have shown that the buildup of urea in CKD could cause devastating effects to cellular structures and functions.[24-27] Furthermore, CKD patients who undergo dialysis have shown great improvements and a decrease in the symptoms associated with renal functions.[28] Research has shown that urea is not the final end product of protein metabolism, and since it is soluble in water, it can release its nitrogen to form other compounds that are highly toxic (uremic toxins).[29,30] These derivatives of urea are as well toxic and have damaging effects on the tissues.[31]

On the other hand, urea and its derivatives are said to have beneficial effects.[32] Many publications, both old and recent, have supported the claim that urea and its derivatives have therapeutic actions.[33,34] It is also important to mention that urea products can be produced or synthesized in the laboratory for commercial purposes, and its role in plants has been widely studied and established.[35] It is used in fertilizers as a source of nitrogen and as an important material for chemical industries.[36]

Many studies have reported both the toxicological and therapeutic effects of urea and its derivatives. The need to have a clear understanding of the role of urea cannot be overemphasized. This would help the clinicians and other researchers to know when not to use urea for therapeutic purposes. In this article, we present a review to help elucidate the scientific relevance of urea in experimental animals. This would provide data to clinicians on how to recommend the use of these urea products for clinical research.

Biosynthesis of Urea (Urea Cycle)

The urea cycle is a series of reactions that result in the production of urea from ammonia. It was discovered in 1932 by Hans Krebs and Henseleit. Protein catabolism produces ammonia, which is highly toxic to the body organs. Most organisms eliminate ammonia directly to the external environment. However, in some animals including humans, ammonia is converted to urea before its clearance.[37] The production of urea is controlled by a series of enzymes within the cytosol and the mitochondria of cells.[38,39] In the mitochondria, ammonia combines with CO_2 using energy from adenosine triphosphate (ATP) to form carbamoyl phosphate. This combines with ornithine to form citrulline. Citrulline diffuses out of the mitochondria into the cytosol, where it combines with aspartate to form arginine succinate, which breaks down to form arginine and fumarate. Arginine receives water to form ornithine and urea. Ornithine re-enters the mitochondria to initiate another cycle, while urea is excreted in urine.

Uremia

This occurs when there is a high level of urea in the blood. The causes of elevated urea in the blood could be classified into three different categories, which include prerenal, renal, and postrenal causes. In prerenal causes, an increased production of urea in the liver through high-protein diets can lead to uremia. More so, a decreased blood pressure, shock, and dehydration could also reduce the clearance of urea, thereby increasing its blood concentration.[40] The renal causes may be due to a decrease in kidney functions, and the factors may include acute kidney disease and CKD, tubular necrosis, and many other related kidney diseases.[41] The post-renal causes occur due to the obstruction in the urinary outflow of urea. This could be due to enlarged prostate tumor of the bladder or severe infection of the urinary tract.[42]

Assessment of Urea

Urea is the primary metabolite of dietary protein and turnover of tissues. To determine its blood concentration, the whole urea is assayed and in some cases, only the nitrogen components of urea are measured. The normal range of urea nitrogen is between 5 and 20 mg/dL. This range varies due to several factors which include protein content of the diet, protein catabolism, water content of the body, liver urea biosynthesis, and urea excretion in the kidney.[43] Several methods exist for analyzing urea. The recent techniques are automated, and they produce reproducible and reliable results. Urea nitrogen can be measured using a diacetyl or Fearon reaction, which produces a yellow chromogen product when added to urea.[44] The quantification occurs through photometry procedure. Urea concentration can also be assayed using the enzymatic procedure which involves the breakdown of urea to ammonia and carbonic acid by the enzyme urease.[23] The absorbance is then measured at a specific wavelength. Blood urea nitrogen (BUN) test measures the amount of nitrogen in the blood that is derived from the

waste product urea; however, the assay could be performed using serum and not whole blood.[45]

Literature Search

A PubMed search was carried out for articles published before 2019 using the phrase, "urea application in experimental animals." A total number of 1142 articles were screened using the title and abstract. Thirteen articles met the inclusion criteria, while 1129 articles were excluded [Figure 1]. The search was mainly for research articles, and no clinical trials, case reports, and case series were reviewed. The information recorded from these publications included the test substance, amount injected, animal model, number of animals, duration of treatments, and effects. The research publications were categorized based on the following:

1. The toxicity of urea in experimental animals
2. The therapeutic effects of urea in experimental animals.

Urea Toxicity in CKD

Urea concentration increases during CKD. [46-48] Many studies administered urea in nephrectomized or CKD animals to further increase its concentration in order to study its effects.[46-52] Table 1 gives a list of seven articles which were reviewed, and the following observations were made. Different nitrogenous compounds (arginine, aspartic acid, and urea) were administered to experimental rats of different sample sizes. The results showed retention of nitrogen compounds in the blood, and this may likely imply a buildup of toxic products that should be excreted in urine.[46] However, using animals of the same sample size would have guaranteed more valid and reliable results. We observed that urea induces the production of intracellular reactive oxygen species (ROS) in cells such as renal tubular cells, vascular endothelial cells, adipocytes, vascular smooth muscle cells, and beta cells in CKD mice.[47,48] Most of the parameters were investigated using modern experimental techniques, which ensures validity of the studies. These ROS are highly reactive radicals that can damage cellular components. Increased ROS production in excess of antioxidants leads to oxidative stress, which has been reported in the etiology and pathogenesis of many diseases like diabetes, cancer, Alzheimer's disease, and many more.[53-55] Many of the effects reported in Table 1 are due to increased ROS production, which occurs in animals with chronic diseases. The high concentration of urea in the plasma (uremia), which occurs in CKD, could result in a defect in carbohydrate, protein, and lipid metabolism.[56] ROS production has been shown to increase modification of insulin-signaling molecules and reduce insulin-stimulated insulin receptor substrate (IRS) and Akt phosphorylation and glucose transport in uremic animals, which therefore creates a state of insulin resistance.[48,57] Uremia has also been reported to cause carbamylation of intracellular proteins which are responsible for the altered mitochondria ROS production. Trecherel *et al*.[58] studied *in vitro* expression of proapoptotic proteins (Bcl-2–associated death [BAD] promoter) in smooth muscle cells exposed to urea, and it was confirmed that urea has the potential to induce apoptosis and cell death in smooth muscle cells and plays a role in atherogenesis and progression of atherosclerosis, thus promoting cardiovascular disease. We observed that a high urea concentration administered to nephrectomized dogs was lethal and sufficient to cause death of animals. The symptoms exhibited by the animals included gastrointestinal disturbances like vomiting, diarrhea, nausea, weakness, and others.[49] There was also electrolyte imbalance, which has been reported to play a critical role in the pathogenesis of many diseases.[49] The hypernatremia, hyperkalemia, and hyperchloremia observed may be due to the inability of the kidney to excrete these ions. Although these ions are necessary for nerve and muscle functions, their excess concentration in the blood could be deleterious. In addition, the excess bicarbonate ions caused by urea could result in metabolic acidosis, which is characterized by increase in blood pH. In one of the studies conducted using CKD zebrafish, a decrease in the volume of the olfactory epithelium was shown to occur in uremic fish [Table 1], even though the mechanisms of this have not been ascertained.[50] The decrease may be due to olfactory cell degeneration or apoptosis induced by high urea level. The olfactory epithelium is the sensory system of smell, and therefore, any disruption to the olfactory cells may interfere with the ability to detect airborne substances in the environment.

Patients with CKD suffer from depression due to the accumulation of urea. This observation was further confirmed in mice and could be as a result of inhibition of the mTORC1-S6k signaling pathway and loss of synapses in the medial prefrontal cortex of the brain.[51] Furthermore,

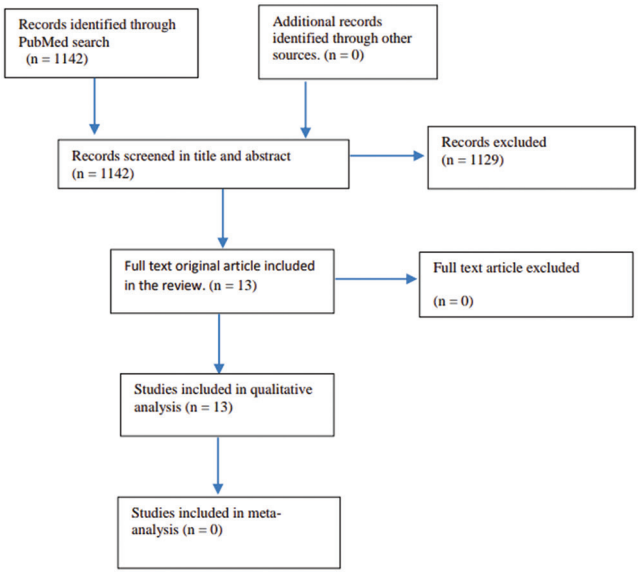

Figure 1: A systematic guide for the selection of articles

Table 1: Toxicity of urea in experimental animals

Test substance	Amount injected	Animal model	No. of animals	Duration	Effects	Ref.
Urea	25 mM	Rats	21	24 h	Decreased excretion of other nitrogenous compounds	Burton *et al*.[46]
Urea	10 mg/kg	C57BL/6J CKD mice	34	15 days	Increased oxidative stress Increased insulin resistance Glucose intolerance	D'Apolito *et al*.[47]
Urea	200 mg/kg	C57BL/BN CKD mice	9	3 weeks	Decreased insulin secretion and impairment of beta-cell glycolysis	Koppe *et al*.[48]
Urea	5-30 g/L	Nephrectomized dog	6	10 days	Weakness, anorexia, vomiting, comatous, hemorrhage, diarrhea, decreased tissue water content, hyperchloremia, hypernatremia	Grollman *et al*.[49]
Urea	7-20 g/L	CKD zebra fish	5	30 days	Thinning and decreased number of olfactory epithelial cells	Bettini *et al*.[50]
Urea	1 g/L	CKD mice C57BL/6J	4	7 days	Depression caused by impairment of medial prefrontal cortex, loss of synapses, inhibition of mTORC1-S6k pathway	Wang *et al*.[51]
Urea	72 mg/dL	Nephrectomized (C57BL/BJ) wild-type CKD mice	6	15 days	Endothelial dysfunction through increased ROS production	Vaziri *et al*.[52]

CKD: Chronic kidney disease, ROS: Reactive oxygen species

other studies on urea showed that it is capable of causing disruption of the intestinal barrier and proposed that the mechanism of this effect involves diffusion of urea into the gut and its conversion by microbial urease to ammonia.[53,59] The impaired integrity of the intestinal epithelia potentially supports leakage of bacterial intestinal toxins into the body fluid and systemic inflammation. The deposition of extracellular cell matrix (ECM) by increased ROS production and protein kinase C activation has widely been reported in CKD.[60] The ECM causes endothelial dysfunction and disturbances in the ultrafiltration processes of the renal tubular cells, as reported in the wild-type CKD mice exposed to urea.[52] This causes an increase in blood urea with increase in signs of toxicity. This elevated urea level may be because of carbamylation reaction, a posttranscriptional modification where urea derived from its catabolism (isocyanic acid) tampers with the function and structural integrity of proteins.

Therapeutic Actions of Urea in Experimental Animals

According to a study conducted by Sweeny *et al*.[61], urea was shown to improve animal feed supplementation. It was reported to increase nutritional value and aids the digestion of low-energy crop stubbles. Emmanuel *et al*.[62] also studied the effects of different levels of urea supplementation on nutrient intake and growth performance in growing camels fed roughage-based complete pellet diets and urea was confirmed to influence digestibility, feed intake, growth rate, feed efficiency, and economics.[63-65] Urea has been confirmed to have an effect on plasma sodium lower than 135 mEq/L (hyponatremia), according to a clinical research trial performed by Rondon-Berrios *et al*.[66] Patients who had syndrome of inappropriate ADH secretion (SIADH) as the only cause of hyponatremia were treated with oral urea as the sole drug therapy for hyponatremia[67] The mechanism of effectiveness of urea on hyponatremia caused by SIADH is based on the pharmacokinetics of urea; it is a partially effective due to its permeability into the muscle tissues, connecting tubule, cortical collecting duct, and medullary collecting duct. Urea has been shown to create a positive sodium balance, which contributes to improvement of plasma sodium.[68] This study was further supported by Van Reeth and Decaux[69] who reported that urea administration ameliorates hyponatremia and decreases brain damage in rats. Thiourea, a regulator of leptin hormone secretion from adipocytes, plays an important role in the progression of obesity and hepatic steatosis. In a study carried out by Liang *et al*.,[70] administration of thiourea (a similar compound to urea) in animals was observed to modulate triglyceride metabolism and decrease body weights, total cholesterol, high- and low-density lipoproteins.[70] Even though urea and its derivatives have been reported to have many therapeutic effects, their antitumor effects have also been widely reported [Table 2]. In a study carried out by Vedarethinam *et al*.,[71] it was reported that 1,3-bis-((3-hydroxynaphthalen-2-yl) phenylmethyl) urea (1,3-BPMU) has cytotoxic and growth inhibitory effects on hepatocellular carcinoma cells (HEP-G2).[71] They also stated that a flow cytometry analysis shows that 1,3-BPMU causes early and late apoptosis. This compound is a derivative of urea, and the effects are mediated

Table 2: Therapeutic actions of urea in experimental animals

Test substance	Amount administered	Animal model	No. of animals	Duration	Effects	Ref.
Urea	10-30 mg/kg	Lamb	70	9 weeks	Increased growth, performance and fattening Increased digestibility	Xu *et al*.[63]
Urea	6-9 g/kg	Lamb	36	9 weeks	High growth rates Fattening of the animals	Saro *et al*.[64]
Urea	1.0 l/kg	Camel	18	7 days	Increased nutrient intake Increased digestibility Increased growth performance	Pan *et al*.[65]
Urea	2 mg/kg	Rats	6	48 h	Ameliorated hyponatremia and SIADH Decreased brain damage	Van Reeth and Decaux[69]
Thiourea	50 mg/kg	C57BL/6J mice	20	5 weeks	Ameliorated hepatic steatosis Decreased leptin expression Modulated adiponectin, TC, TG, LDL, HDL synthesis Decreased hepatic dysfunction	Liang *et al*.[70]
1,3-bis-((3-hydroxynaphthalen-2-yl) phenylmethyl) urea	50 mg/kg	Albino rats	6	-	Inhibited cell growth of HEP-G2 hepatoma cells Enhanced apoptosis by upregulating caspase 3 and caspase 9 Downregulated Bcl-2 and Bcl-XL mRNA expression	Vedarethinam *et al*.[71]

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, SIADH: Symptoms of inappropriate antidiuretic hormone secretion, TC: Total cholesterol, TG: Triglyceride

by urease. Another urea derivative, URD12, has been confirmed to exhibit cytotoxic activity against the K562 human leukemia and KB human mouth epidermal carcinoma cell lines. Wang *et al*.[72] further studied URD12 in *in vivo* and *in vitro* assays using BGC-823 human gastric carcinoma, SMMC-7721 human hepatoma, and HepG2 human hepatocellular carcinoma cell lines [Figure 2]. URD12 was confirmed to inhibit the growth of tested tumor cell lines with no effects on the weight, spleen, and thymus. Several other studies have also reported that urea has therapeutic applications. Evidence suggest that topical urea may impact epidermal permeability and barrier function. Rawlings *et al*.[73] Susanne *et al*.[73] confirmed this myth by conducting a placebo-controlled, double-blinded study in healthy human volunteers, after which urea significantly improved cutaneous barrier function as there was a significant decrease in transepidermal water loss. Dermal therapists have shown considerable interest in urea due to its properties. Urea has been confirmed to have a great effect on xerosis.[74] It has a good hydrating effect by improving the water-holding capacity of the stratum corneum and the skin barrier function and regulates skin surface pH. With appropriate urea concentration, reduction of stratum corneum integrity together with decreased protease activity was not observed.[75] According to a comparative study conducted by Kiane de Kleijne *et al*., Caduff *et al*.,[76] urea showed great environmental benefits. Urea production from basic oxygen furnace gas (BOFG)

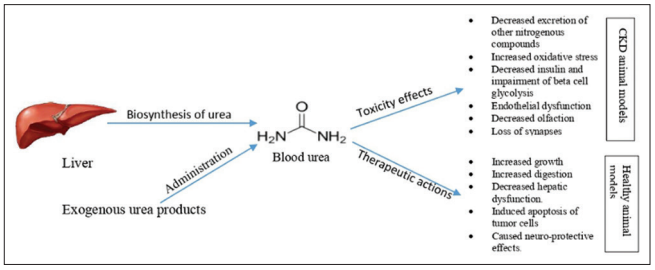

Figure 2: Summary of findings on the biological roles of urea

avoids greenhouse gas emissions, and net emission savings could be achieved especially when combined with excess carbon-dioxide transport and storage.

Conclusions

The scientific role of urea remains controversial; however, we have been able to analyze some of the toxicological and therapeutic effects of urea in this review. In most of the articles reviewed, we observed that the toxicity of urea was mainly reported in CKD and other related pathological conditions. We also observed that urea plays a significant role in inhibiting the growth and causing apoptosis of cancerous cells, even though it has several other therapeutic functions.

Ethical approval and consent to participate

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Conflicts of interest

There are no conflicts of interest.

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