

# Finding novel treatment strategies for UTI through oestrogen receptors

Urinary tract infections (UTIs) are one of the most common bacterial infections and contribute to antibiotic use and the healthcare burden each year. Dr Rashmi Kaul and Dr Anil Kaul, of the Center for Health Sciences at Oklahoma State University, US, hope that by increasing understanding of the links between oestrogen and UTIs it may be possible to develop alternative therapies. Their research over the past few decades has shed important light on the role played by oestrogen receptors in the aetiology of numerous diseases. This research so far suggests that different subtypes of oestrogen receptor have varying impacts on bacterial clearance and immune response.

Urinary tract infections (UTIs) are one of the most common bacterial infections, affecting men and women and resulting in around a million hospital admissions each year in the US alone. Women are more susceptible, with UTIs occurring in up to 50% of women, with around 20–30% of these individuals having recurrent UTIs.

Risk factors for developing a UTI include age, gender, and having a catheter in place. Importantly, menopausal and pregnant women are more prone to UTIs, suggesting hormonal aetiology. In certain individuals, chronic infections can lead to cancer and persistent UTI in pregnant women can often lead to pre-term birth. Although many UTIs can be easily treated, complications can arise if the infection moves from the bladder to the kidneys, where it can cause illnesses such as pyelonephritis (inflammation of the kidney) or kidney failure.

Antibiotics are usually used for treating UTIs, but this is becoming more challenging as antibiotic resistance is increasing. This highlights the vital need for alternative treatments such as vaginal oestrogen suppositories, but these are not effective for all

*E. coli* cells attached to bladder epithelial cells.

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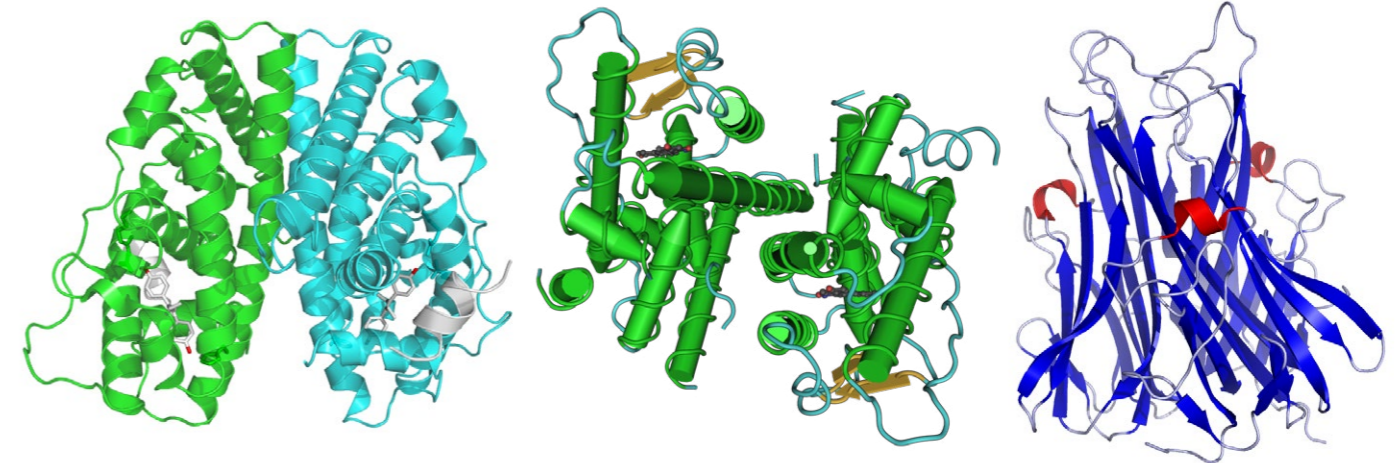
women. There are currently no viable uniform vaccines, and so alternative therapeutics are needed.

UTIs are caused by infectious bacteria, such as *Escherichia coli* (*E. coli*), which causes around 80% of infections. One particular strain of the bacteria, uropathogenic *E. coli*, causes recurrent UTIs by evading the immune system and entering bladder cells where it is able to hide and avoid destruction by antibiotics. This internalisation technique means that dormant bacteria can form reservoirs inside bladder cells. From here they can become active again when the bladder becomes immunocompromised, thus causing recurrent infection. It is then important for the body to either clear or to contain the infection.

## OESTROGEN AND ITS RECEPTORS

Previous studies have shown that when the body produces oestrogen, this can be protective against UTIs. For example, oestrogen levels drop during menopause causing women to become more susceptible to UTIs. A similar relationship is seen in pregnant women, who also have fluctuating oestrogen levels. Both pregnant and post-menopausal women also have the worst disease outcome, strengthening the prediction that there is a hormonal aspect to recurrent UTIs.

Immune responses in the bladder play a major role in protecting the urinary



ER $\alpha$  is expressed in tissues at different levels.

Studies have reported higher levels of ER $\beta$  in the kidney.

TNF- $\alpha$ , which has been found in greater amounts in patients with UTI.

tract from infection through first line defences that inhibit binding of bacteria to bladder cells. Also, antimicrobial and pro-inflammatory molecules are released from bladder cells to combat bacterial infection. There is also evidence that oestrogen can regulate the immune response, and marked differences in immune markers, such as CD55 (which activates immune cells called CD4 T-cells) can be seen corresponding with increases in oestrogen levels. This molecule is particularly relevant because uropathogenic *E. coli* binds to CD55 to help them cross cell membranes and enter into the bladder cells.

While there is a clear link between hormone levels and the chance of developing a UTI, the mechanisms are less well known. As oestrogen and oestrogen receptors may influence immune responses to bacterial infections, Dr Rashmi Kaul and Dr Anil Kaul, at the Center for Health Sciences, Oklahoma State University, US, aim to explore how these immune responses are regulated in the bladder and the kidney, and how oestrogen and its receptors are involved.

Early studies by Dr Anil Kaul led to the discovery of hormone regulation of UTI by *E. coli* and gestational UTI. These findings paved the way for further research into oestrogen and oestrogen receptor regulation of immunity against UTI.

Dr Anil Kaul also identified that there were changes in receptors for bacteria, such as *E. coli*, at different times. For example, expression is modulated with changes in hormone levels and different individuals also show these changes in receptor expression over time. Leading on from this, Dr Anil Kaul postulated that changes in receptor expression may help explain why young, healthy individuals were sometimes more susceptible to urogenital infections than other populations.

Further, animal studies emphasised the importance of understanding the complications and mechanisms behind UTIs. Kaul's group showed that

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90% of pregnant mice infected with one form of *E. coli*, which contained proteins that target immune receptors upregulated during pregnancy, gave birth prematurely, compared to 10% of mice infected with an alternative form of the bacteria that did not contain the receptor binding protein.

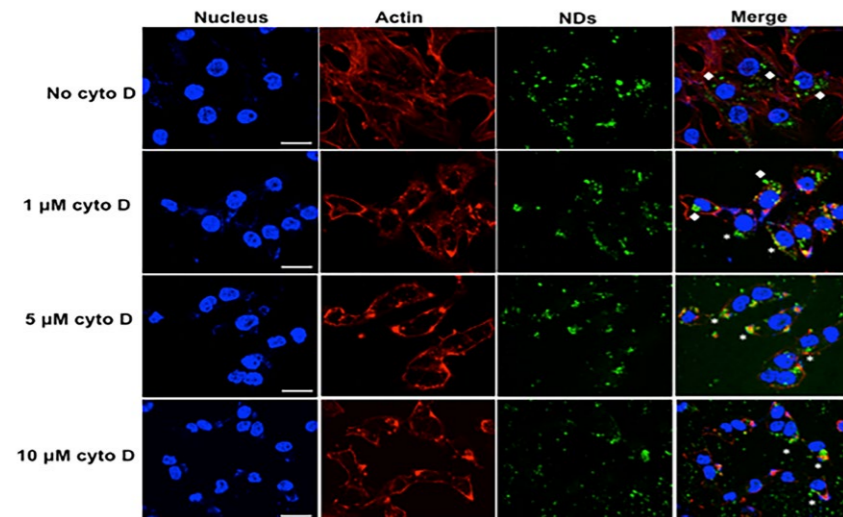
Oestrogen works by binding to receptors on cells, such as oestrogen receptor  $\alpha$  (ER $\alpha$ ), oestrogen receptor  $\beta$  (ER $\beta$ ) or membrane oestrogen receptor (GPR30). Dr Rashmi Kaul explains that oestrogen receptors are expressed in

tissues at different levels. For example, several studies have reported higher levels of ER $\beta$  in the kidney. Once bound to its receptors, oestrogen causes a change in how certain genes are read, which in turn influences which molecules get activated by the body, including those that are part of the innate/early immune system. This means that oestrogen receptors have an important role in regulating innate/early immune responses, possible during urogenital infections.

## UTI PATHOGENESIS

One study, carried out by PhD student Ayantika Sen and Dr Rashmi and Anil Kaul, used a rodent model to show that an agonist (a drug that binds to ER $\alpha$  receptors and mimics the action of the oestrogen that would usually bind there) called propyl pyrazole triol (PPT) is able to reduce bacterial load in the kidney. Despite this, there was no significant observed change in immune response overall.

The researchers then used an antagonist (a drug which binds to the same ER $\alpha$  receptor but this time blocks its usual response), called methyl-piperidino-pyrazole (MPP). This time, they found the opposite: bacterial load was lower in the bladder rather than the kidney, and immune markers such as CD55 were also decreased. In addition, adding MPP to uninfected cells showed an increase of a pro-inflammatory marker



Nanodiamonds internalised via cellular actin into the bladder cells. Cells were treated with different concentrations of actin inhibitor cytochalasin D for one hour followed by treatment with 100 μg/mL of nanodiamonds for two hours.

## This suggests that novel approaches, based on subtypes of oestrogen receptor, may offer an attractive alternative to antibiotic treatment of UTIs.

called TNF- $\alpha$ . TNF- $\alpha$  has previously been shown to be found in greater amounts in urine from patients with UTI and reduced after antibiotic treatment eliminated the infection. It has also been suggested that different subtypes of oestrogen receptors are able to regulate TNF- $\alpha$  production.

As well as in an animal model, the study looked at human bladder cells grown in the laboratory (a cell line) and again showed that using the ER $\alpha$  agonist drug helped cells clear bacterial infection quicker. Overall, different subtypes of oestrogen receptor may have protective effects, with ER $\alpha$  having a protective role in the kidney.

The researchers suggest that drugs targeting specific oestrogen receptors may help boost immune responses in the urogenital tract for more effective bacterial clearance. Their work is likely to be the first to indicate that varying levels of involvement of ER $\alpha$  in the kidney and bladder in maintaining immunity and response to experimental infection. However, this has only been studied in cell and rodent models so far, and further research is still required to explore how these findings may translate to a real-world

setting, as well as the mechanisms at play in the bladder.

A more recent study published by the same group expanded on these findings and demonstrated the protective role of ER $\alpha$  and ER $\beta$ , but not the membrane-bound GPR30 oestrogen receptor against *E. coli* infection in a bladder cancer cell line. They also showed that mice without ER $\alpha$  lost the protective effect linked to this receptor.

In contrast to the other oestrogen receptors, GPR30 activation led to increased bacterial colonisation which was reversed when the activity of the receptor was inhibited by an antagonist molecule. This suggests that novel approaches, based on subtypes of oestrogen receptor, may offer an attractive alternative to antibiotic treatment of UTIs.

### THE POWER OF NANODIAMONDS AS THERAPEUTICS

Understanding more about the underlying mechanisms and pathogenesis of UTIs and the relationship with oestrogen and oestrogen receptors may help to develop novel therapeutic ideas to treat recurrent UTIs. This is vital to combat

antibiotic resistance and contribute to a move towards alternative ways to treat disease.

One example of a novel therapy is the use of nanodiamonds, carbon-based nanoparticles that can be used to deliver drugs to target areas inside the body. Nanodiamonds are less harmful to living tissue than some nanoparticles (because they do not alert the immune system and they are easily cleared by the body), and they can be internalised by cells, meaning that even bacteria such as uropathogenic *E. coli* can be targeted by the nanoparticles and their cargo. The fact that nanodiamonds can deliver antimicrobials locally means less resistance with lower doses, and also prevents the need for oral antibiotics.

Dr Rashmi and Dr Anil Kaul investigated whether nanodiamonds were able to effectively kill uropathogenic *E. coli* in human bladder cells grown in the laboratory. The results showed that smaller nanodiamonds were better at reducing the number of bacteria that were able to hide in bladder tissue, as well as displaying greater antimicrobial properties than larger nanodiamonds.

### FUTURE DIRECTIONS

Understanding the roles of oestrogen receptor subtypes and their impact on bacterial infection and immune responses can help to develop potential new therapies for recurrent UTIs, as well as offering possible diagnostic biomarkers for recurrent infection.

It is also important to consider that oestrogen receptors may have subtle differences when two patients are compared, and this may partly explain why some people are more susceptible to urogenital infections and why they respond in different ways to infection.

Dr Rashmi and Dr Anil Kaul's work offers new insight into the links between oestrogen receptors, bacterial clearance, and immune responses, but further work is required to explore this in more detail. New therapeutics based on the knowledge expounded by Rashmi and Anil Kaul will usher in a new era beyond antibiotics, and their work is yielding vital insights into the role played by oestrogen receptors.

# Behind the Research



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## Research Objectives

Dr Rashmi and Dr Anil Kaul research oestrogen receptors and urinary tract infection treatments.

## Detail

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### Bio

**Rashmi Kaul** received her PhD (microbiology and immunology) from Delhi University, India, in 1989. She currently serves as a professor of immunology at Oklahoma State University, Center for Health Sciences in Tulsa, Oklahoma. Dr Kaul's research involves understanding the hormonal regulation of inflammation and innate immunity during infectious disease

pathogenesis and how these responses are influenced by gender specific host factors.

**Dr Anil Kaul** formally trained as a physician, a dentist, and in public health, and currently serves as Clinical Professor & Clinical Laboratory Director at Oklahoma State University. At the start of the current pandemic, he volunteered to direct the COVID-19 testing laboratory for Oklahoma. With more than 80 scientific papers, he has received multiple awards, including six US patents.

### Funding

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### Collaborators

- Ayantika Sen PhD Stanford University School of Medicine, California, US
- Janaki Iyer PhD Department of Natural Sciences, Northeastern State University, Oklahoma, US
- Raj Narayan Singh PhD Materials Science and Engineering, Oklahoma State University, Oklahoma, US

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## Personal Response

### Why did you choose to use an agonist instead of oestrogen itself in the studies?

It is highly important that agonists selectively target receptors, giving researchers ability to control the effects of the specific receptor. Using oestrogen itself, on the other hand, would affect numerous receptors, lessening the researchers' control.

