

A NEW AETIOLOGY FOR OAB: INTRACELLULAR BACTERIAL COLONISATION OF UROTHELIAL CELLS

Hypothesis / aims of study

There is a very significant problem, that has to be acknowledged and faced, in relation to overactive bladder OAB symptoms. It appears that errors in the routine urinalysis methods for screening for cystitis have resulted in a systematic failure to diagnose urine infection when it was surely present. From the time that this problem was first identified an increasing body of experiments have served to confirm the facts of the situation.

It is therefore apparent that the role of urinary tract infection (UTI) in the aetiology of OAB symptoms has been seriously neglected. At first it was extremely perplexing that the conventional urine tests should have proved to be so misleading and inappropriately reassuring. However, more recent research has come to the rescue and led to the proposition that bacteria may be evading detection by concealment through intracellular colonisation of urothelial cells.

A murine model of urine infection, has shown that populations of *E. coli* can persist in the bladder for months on end, during which time they exist as a quiescent reservoir after invading and multiplying in the urothelium⁽²⁾. Quiescence and infrequent mitosis confer considerable antibiotic and immune resistance.

To date similar studies have not been achieved in humans, but there are good reasons to explore this avenue. It has been shown that the culture of a concentrated suspension of urinary uroepithelial cells has proved extremely effective in isolating uropathogens from patients, in contrast to controls⁽³⁾. Urinary inflammatory exudates in patients with OAB has been reported and re-confirmed as commonplace⁽¹⁾.

This study tested the hypothesis that uropathogenic bacteria colonise urothelial cells in patients with OAB symptoms and not in controls. The experimental method used a novel assay that detected intracellular bacteria, after all extracellular contaminating microbes had been eliminated. This assay method was then developed further so as to make it suitable for adoption in ordinary clinical practice.

Study design, materials and methods

We studied 23 women, mean age 56 (sd=17); 16 had OAB symptoms and provided a CSU; 7 asymptomatic female controls, average age 29 (sd=12), provided a CSU. An aliquot of urine was examined immediately by microscopy and the urinary white cells evaluated. An aliquot of urine was sent for routine culture with a threshold of 10^5 cfu/ml. An intracellular invasion assay on the shed epithelial cells was performed: The cell sediment was extracted by centrifuge at 800g for 5 minutes. The deposit was incubated in cell culture media (Eagles Minimal Essential Medium) for 12 hours to encourage confluence of bladder epithelial cells and bacterial growth. The cells were then incubated with gentamicin 200µg/ml, lineozelid 200µg/ml and amoxicillin 200µg/ml for two hours to kill all extracellular bacteria. During this process the preparation was washed three times and reconstituted. The death of extracellular bacteria was confirmed through culture. After two hours, a sterile extracellular space was confirmed, and the cells were lysed using Triton X 0.1% and the viable intracellular bacteria were enumerated by culture. Species were identified by 16s-rRNA typing.

In a follow-on experiment the method was repeated in another sample of 26 patients and 8 controls which used chromogenic agar, providing immediate species identification, which was then confirmed by sub-culture and identification using API® strips (Analytical Profile Index)

The statistical analysis of the data was conducted using one-way analyses of variance (ANOVA). The statistical program SPSS was used for analysing data and calculating (ANOVA) and 95% confidence intervals. Where the data were the average of means the standard errors of the means were used to construct distribution bars. In all other cases the distribution bars were the 95% CI.

Results

26/49 (53%) of the OAB samples showed evidence of intracellular bacterial colonisation of the bladder epithelium and only 2/15 (13%) of the control specimens showed the same. The average colony counts post cell lysis were 1×10^6 cfu/ml¹ in patients. In the two positive control volunteers, these counts were $\leq 10^1$ cfu/ml¹ so that the difference was 5 orders of magnitude, ie $\approx 10^5$ (F=5.668, df=1, P=0.027). The figure illustrates the time course of the assay. The dominant bacteria in this sample of patients was *Enterococcus faecalis*, *Streptococcus agalactiae*, *Streptococcus aginosus*, *Escherichia coli* and *Proteus mirabilis*.

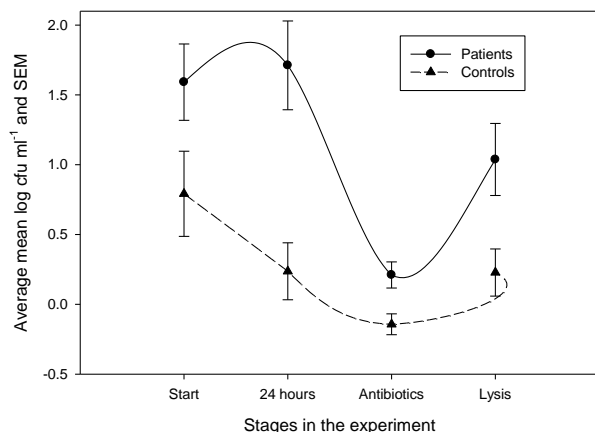
Interpretation of results

The extracellular bacterial population fell to zero, on colony counts, during the incubation with three antibiotics. After the final wash Triton-x lysed the cells. This event was followed by an immediate marked increase in the colony counts. The only explanation for this was efflux of the bacteria from intracellular sources. Every stage of the assay ran parallel controls of the methods so that error or contamination were excluded. The data from the human controls supported the proposition that intracellular colonisation is a pathological process.

Concluding message

This is the first time that evidence for intracellular bacterial invasion of bladder epithelium in patients with OAB has been shown. Intracellular colonisation is a known mechanism by which bacteria evade the host immunity and antibiotic treatment so that long-term persistence is a plausible outcome. A very important discovery was that *E.Coli* proved neither the only culprit nor the most common microbe implicated in this pathology.

The stages in the invasion assay showing colony counts



References

1. J.Urol., 17-3-2010, 183; 1843, 1847.
2. Antimicrob.Agents Chemother., 2010, 54; 1855, 1863.
3. Science., 4-7-2003, 301; 105, 107.

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| What were the subjects in the study? | HUMAN |
| Was this study approved by an ethics committee? | Yes |
| Specify Name of Ethics Committee | the Whittington and Moorefield's Research Ethical Committee, London. |
| Was the Declaration of Helsinki followed? | Yes |
| Was informed consent obtained from the patients? | Yes |