Ivanov et al. Clinical Phytoscience (2015) 1:7
DOI 10.1186/s40816-015-0008-x

Clinical Phytoscience
a SpringerOpen Journal

ORIGINAL CONTRIBUTION Open Access

An open label, non-controlled, multicentre, interventional trial to investigate the safety and efficacy of Canephron® N in the management of uncomplicated urinary tract infections (uUTIs)

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Abstract

Background: Despite of increasing prevalence of bacterial resistance to antibiotics and possible adverse drug reactions (ADRs), uncomplicated lower urinary tract infections (uUTIs) are usually treated with antibiotics. Canephron® N, a herbal medicinal product, was now investigated for safety and efficacy in women suffering from uUTI.

Methods: In an open-label, non-randomized, multicentre clinical trial 125 Caucasian female patients suffering from uUTI with a sum score of at least 6 for the symptoms dysuria, frequency and urgency (each rated 0–4) were enrolled. Patients were treated with 3x2 tablets Canephron® N for seven days. Symptom assessment was performed by the patient on a daily basis and by the investigator at Day 0, Day 7, Day 37. Primary endpoint was the incidence of ADRs during the treatment. Secondary endpoints were clinical cure (none of the main symptoms scored as worse than mild ("1")) on Day 7, severity of uUTI symptoms on Day 7 and Day 37, patients requiring antibiotic treatment until Day 7, duration of uUTI symptoms and patients with early recurrence. Changes in safety data (dipstick analysis of urine; analysis of blood and urine in a central laboratory) were analysed descriptively. Further post hoc analyses were performed.

Results: None of 19 adverse events within the study period was considered as drug-related or serious. The responder rate was 71.2 % on Day 7 and 85.6 % on Day 37 (FAS, N = 125) with a significant improvement of all symptoms (all: $p < 0.001$). Only 2.4 % of patients required antibiotics during the treatment period and none of the patients met the definition of recurrence until Day 37. The mean changes of the main symptoms on Day 7 and Day 37 compared to Day 0 were: −1.9/-2.3 (dysuria); −1.8/-2.4 (frequency); −1.6/-1.9 (urgency). Symptomatic improvement was accompanied by decreased nitrite, leukocytes as well as erythrocyte levels from Day 0 to Day 7 and Day 37.

Conclusions: This trial emphasises the safe usage and efficacious opportunity of a non-antibiotic therapy approach with Canephron® N and fully justifies a large-scale controlled clinical trial.

Trial registration: Eudra CT nr. 2011-000838-11. ClinicalTrials.gov Identifier NCT01478620

Keywords: Canephron® N; Uncomplicated urinary tract infection (uUTI); Herbal drug; Clinical trial; Safety; Efficacy

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Background

After infections of the respiratory tract, urinary tract infections (UTIs) represent the second most frequent bacterial infections in women. Most of them (80 %) are uncomplicated UTIs (uUTIs) [1, 2]. Common uUTI symptoms are frequency, urgency, dysuria and suprapubic pain, while low back/flank complaints, loin pain and fever are often considered as symptoms of pyelonephritis. The acute uUTI is characterized by absence of structural and functional abnormalities of the urinary tract, whereas relevant kidney diseases and relevant comorbidities increase the risk for complicated UTI with a more serious outcome [3]. Most frequent uropathogens are *Escherichia coli (E. coli)* followed by *Staphylococci*, *Enterococci* and *Enterobacteriaceae* such as *Klebsiella* subspecies (spp.). Acute uUTIs are usually treated by general practitioners in primary health care. Studies of UTIs usually focus on bacteriology, drug resistance and bacteriological outcome of antibiotic treatment although newer guidelines for treatment of UTIs require eradication of both bacteriuria and symptoms [4, 5]. In order to prevent unnecessary consumption, antibiotics should be prescribed for symptomatic bacteriuria and not for symptoms only [6, 7].

It is known that many women with uUTI do not seek medical help immediately, but try to wait or treat themselves with home remedies [8]. The natural course of untreated uUTIs has previously been described in several studies with a resolution rate of up to 50 % after three days [1, 3, 6, 7]. In a well-designed trial, Ferry et al. observed a spontaneous 28 % cure rate of symptoms in the first week, followed by 37 % after 5–7 weeks [7]. In a pilot trial, Bleidorn et al. reported on the non-inferiority of ibuprofen versus ciprofloxacin based on symptomatic outcome on Day 4, however, one third of patients of the ibuprofen group relapsed within the first week [6]. Thus, there is only little evidence for the natural course of untreated uUTIs or for proven alternative treatment options. Facing increasing antibiotic resistances, efforts to either optimize appropriate anti-microbial use or to develop alternative treatment gain in importance.

Canephron® N, a herbal medicinal product containing centaury herb, lovage root and rosemary leaves was developed for irrigation therapy of acute and chronic infections of the urinary tract and for the prevention of kidney gravel [9]. The individual components have been characterized to exhibit spasmolytic, diuretic, anti-inflammatory and antimicrobial properties [9]. Centaury herb, containing xanthones, has antibacterial and anticholinergic effects [10, 11] and is traditionally used as "supportive for expulsion of kidney stones" as well as for diuresis [12–14]. Lovage root with its furanocoumarines is proven to have spasmolytic and diuretic effects [15, 16] and is proven for irrigation therapy of lower urinary tract inflammation [15]. Rosemary leaves, containing diterpenes, polyphenols and phenols with antioxidative, antibacterial, antiviral, anti-inflammatory, spasmolytic and anticonvulsant effects [17, 18], are supportive for renal excretion and diuresis [4, 14, 19, 20]. In a systematic review of 17 clinical studies with 3115 patients, Canephron® N was shown to be effective in the treatment and prophylaxis of UTI compared with standard therapy, both in adults and children accompanied by a reduced number of relapses [21]. Only one adverse event (AE) was reported (skin rash). It was concluded that Canephron® N has a positive impact on infectious and inflammatory processes within the urinary tract. However, because some of these studies were not well-designed, their internal and external validity remains unclear [21].

The objective of this trial was to assess safety and therapeutic impact of a non-antibiotic therapy approach, treatment with Canephron® N, in the management of uUTIs.

Methods

The present open-label, non-controlled, multicentre, interventional clinical trial was conducted in nine Ukrainian sites between October 2011 and June 2012.

The study consisted of a seven-day treatment period of Canephron® N and a follow-up period until Day 37. Three visits were planned on Day 0 (Visit 1: Enrolment, start of treatment), Day 7 ± 1 day (Visit 2: End of treatment) and Day 37 ± 1 day (Visit 3: End of study). In case the patients experienced persisting or worsening of symptoms, antibiotic therapy was allowed, stopping Canephron® N application (Fig. 1).

The severity of uUTI symptoms was assessed by the patient on a daily basis in the diary as well as by the investigator at each study visit (Fig. 1) in the CRF. The following uUTI symptoms were rated on a 5-point scale (Table 1): dysuria, frequency, urgency, acute development of incontinence or worsening of incontinence, nocturia, pain or discomfort in lower abdomen or pelvic areas and increased body temperature (fever). For clinical outcome (clinical cure, improvement, failure) only the three main symptoms, dysuria, frequency, urgency, were assessed. The symptom score was developed on the basis of several guidelines for urological diseases [6, 22–24].

Exploratory variables were haematology and clinical blood chemistry (visit 1 and 2) as well as midstream urine analysis (specific gravity, pH, leucocytes, erythrocytes, nitrite, glucose, protein, ketone, urobilinogen, bilirubin, blood; Combur 10 Dipstick Test, Roche Germany) and standard microbiological culture of a central laboratory (visit 1, 2 and 3).

In total 125 patients were enrolled into the trial (full analysis set (FAS)). Of these, 105 patients were distributed to the per protocol set (PPS, Fig. 2).

The study was performed in accordance with the Declaration of Helsinki, International Conference on Harmonization and Good Clinical Practice (ICH-GCP)

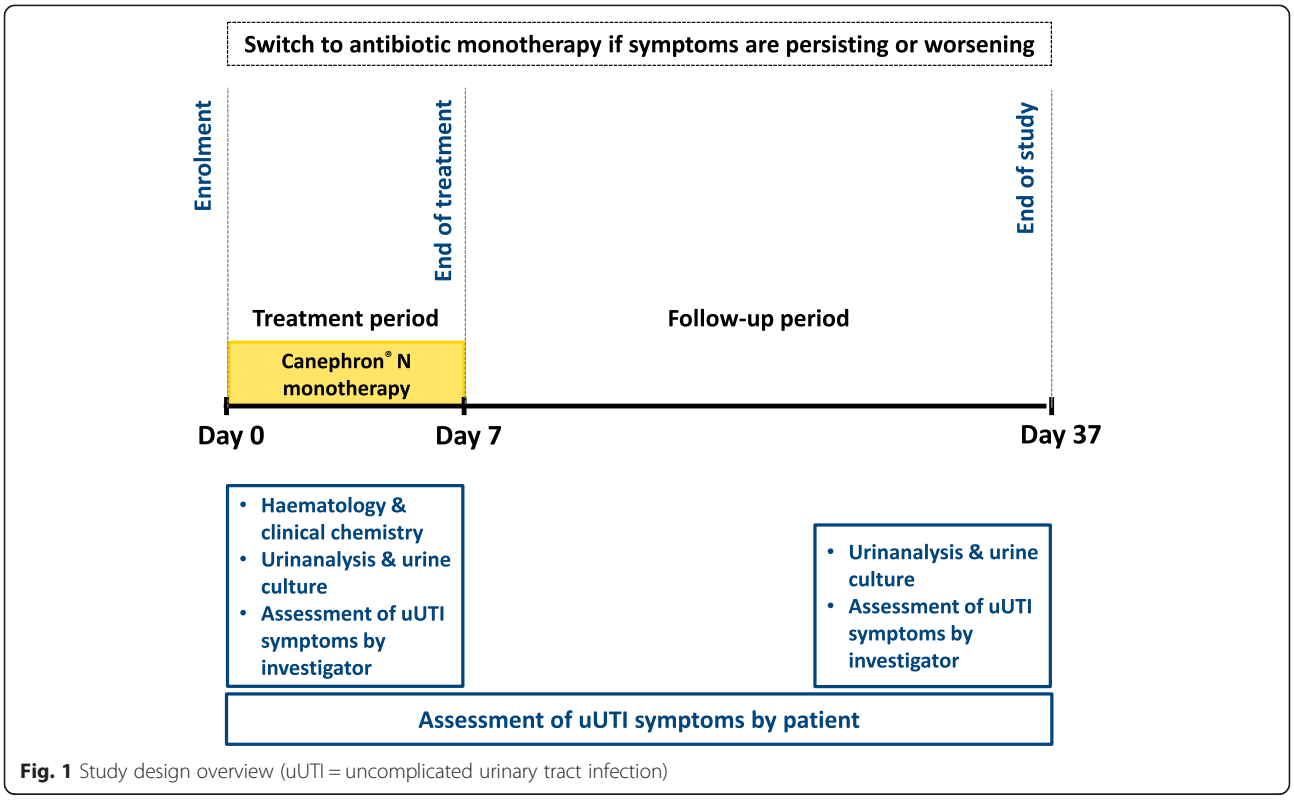

Fig. 1 Study design overview (uUTI = uncomplicated urinary tract infection)

regulations. A central laboratory was used in order to standardize the test results.

Main inclusion criteria, patient characteristics

Signed informed consent; female outpatients aging from 18–65 years; patients suffering from acute symptoms of uUTI (cystitis) at screening exhibiting a total sum score of at least 6 for the following symptoms: dysuria (pain during micturition), frequency (pollakisuria), urgency (Table 1); development of symptoms within a maximum of six days before screening; non-lactating female patients or patients with a negative pregnancy test at screening willing to use effective contraception methods.

Table 1 Rating scale for assessment of uncomplicated urinary tract infection (uUTI) symptoms by patient and investigator.

Score	Rating of uUTI symptoms
0	absent
1	mild (no influence on daily activities or sleep)
2	moderate (minor influence on daily activities or sleep)
3	severe (major influence on daily activities or sleep)
4	very severe (impossible to carry out daily activities or to sleep)

Assessed uUTI symptoms are dysuria, frequency, urgency, acute development of incontinence or worsening of incontinence, nocturia, pain or discomfort in lower abdomen or pelvic areas, increased body temperature. Assessed uUTI symptoms for inclusion and clinical outcome (clinical cure, improvement, clinical failure) are only the main symptoms, dysuria, frequency and urgency

Main exclusion criteria

Exhibiting any signs evoking complicated UTI, pyelonephritis (fever ≥ 38 °C, back pain, chills and shivers) and/or concomitant vulvovaginitis; conditions leading to complicated infections (renal diseases, urinary tract abnormalities, past urinary surgery, urine catheterization, etc.); chronic infection of the urinary tract requiring an intravenous pyelogram, ultrasound or cystoscopy; current signs of severe, progressive or uncontrolled life-threatening systemic disease, that is endocrine, pulmonary, cardiac, neurological, cerebral, renal, hepatic, haematological or gastrointestinal disease; other acute infection requiring antibiotic treatment; patients with proven UTI within four weeks before enrolment; antibiotic, immunosuppressive or immune-stimulant therapy within four weeks prior to study entry.

Further concomitant therapies were prohibited: Rosmarini folium, Levistici radix and Centauri herba supplements other than the study product, anti-inflammatorydrugs, spasmolytics, herbal drugs or supplements, Cranberry juice, kidney or bladder teas. Paracetamol as primary analgetic drug was allowed for relieving pain.

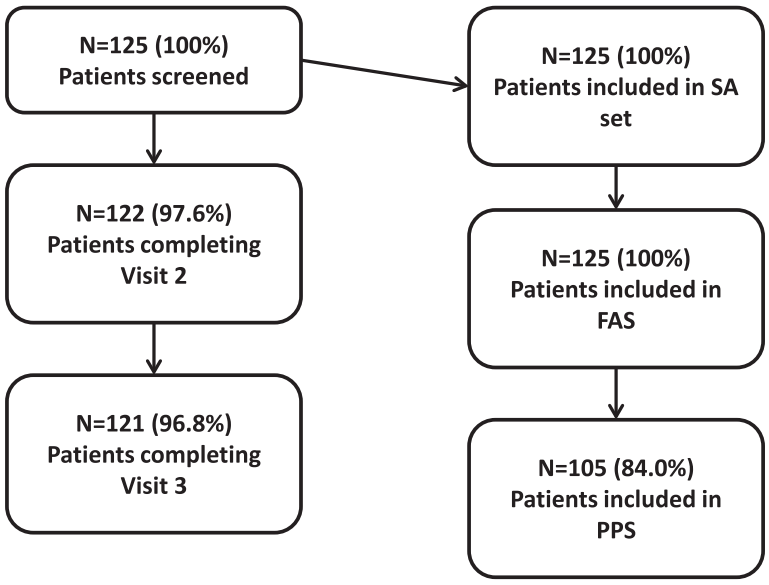

Fig. 2 Number of screened patients in different statistical analysis sets. This figure shows the number of patients screened, completing Visit 2, completing Visit 3 and included in the safety set (SA), full analysis set (FAS) and per protocol set (PPS)

Investigational medicinal product (IMP), dose and mode of administration

The IMP, Canephron® N coated tablets, was administrated orally for seven consecutive days, two tablets three times a day (as specified in the Ukrainian summary of product characteristics (SmPC)).

Definitions

Based on the symptom assessments a sum score (ranging from 0 to 12) was calculated considering only the main symptoms dysuria, frequency and urgency. A responder (clinical cure) was defined as a patient with none of these three symptoms assessed as "worse than mild" with a maximum sum score of 3. The number of days from start of treatment until the respective symptom was assessed as "not worse than mild" was defined as the duration of each symptom. For early recurrence the following criteria had to be fulfilled: patient considered to be a responder on Day 7, worsening of symptoms in the follow-up period (sum score ≥ 6) and a microbiological test result indicating ≥ 10^4 colony forming units (CFU/ml). For calculation of responder (clinical cure) rates and early recurrences, investigators' assessments were used whereas the duration of symptoms was analysed based on patient diaries' data.

For more detailed information, a post-hoc analysis was performed dividing the non-responders into two subcategories, namely patients whose symptoms improved (but were not cured) and patients with clinical failure of the treatment. In this classification responders were denominated as patients who were clinically cured. Patients were considered as improved if the sum score < 6 and if they do not fulfil the criterion for clinical cure. In case of clinical failure of the treatment, the sum score of the patient was ≥ 6 (Table 2). Due to the fact that the methods of the central laboratory for determining the bacterial count were sensitive enough to consistently pick up even lower bacterial counts as initially assumed during generating the study protocol, it was decided to change the criterion for significant bacteriuria from 10^4 CFU/ml to the more sensitive threshold of 10^3 CFU/ml.

Study endpoints

Primary endpoint was the incidence of adverse drug reactions (ADRs) during the seven-day treatment of uUTI symptoms with Canephron® N. An ADR was defined as any untoward medical occurrence which started during treatment with Canephron® N and which has a causal relationship to this treatment. AEs were assessed either by

Table 2 Categories of patients' outcome based on symptom assessment by investigator

Definition according to study protocol		Definition for post-hoc analysis	
Responder	None of the symptoms dysuria, frequency and urgency worse than mild (i.e. sum score ≤3)	Clinical cure	None of the symptoms dysuria, frequency and urgency worse than mild (i.e. sum score ≤3)
Non-Responder	At least one of the symptoms dysuria, frequency and urgency worse than mild	Improved	Sum score < 6 and criterion for clinical cure not fulfilled
		Clinical Failure	Sum score ≥ 6

non-directive questioning of the patient at each visit, via self-reporting by the patient or via physical examination or laboratory tests.

Secondary endpoints were the proportion of responder (clinical cure) on Day 7, severity of uUTI symptoms on Day 7 and Day 37, duration of symptoms, proportion of patients requiring antibiotic treatment until Day 7 and the proportion of patients with early recurrence.

Statistics

The primary endpoint of the study, the incidence of ADRs within the seven-day treatment with Canephron® N, was evaluated descriptively. Statistical hypothesis tests of the primary as well as secondary endpoints were not planned; the sample size was discussed with respect to the accuracy of the expected proportions of ADRs. Clopper–Pearson confidence limits for the incidence rate were calculated.

The assessment of symptom severity was based on a 5-point rating scale (Table 1). Data were analysed descriptively. Changes of the severity of uUTI symptoms from baseline were analysed by the Wilcoxon-rank test. Results of vital signs measurements, physical examination and safety laboratory tests were also analysed descriptively.

The safety set consisted of patients who received at least one dose of the study medication, whereas the FAS included all patients of the safety set who reported at least one efficacy endpoint. All patients of the FAS without relevant protocol violations participating in the study were distributed to the PPS.

The following calculations were performed during the post-hoc analysis: comparison of the patient and investigator sum score in a descriptive manner, followed by a Kaplan-Meier curve. In case of significant bacteriuria, the spectrum of uropathogens was analysed. In case of "unknown bacteriuria" or "no growth" no bacterial analysis was possible. For post-hoc analysis the FAS was used.

Ethical considerations

The clinical trial approval was granted by the competent authorities of the Ukraine and a favourable opinion of the Central Commission on Ethics Questions of the Ministry of Health of Ukraine, the relevant independent ethics committee, was given prior to the start of the clinical trial. Written informed consent was obtained from all patients prior to enrolment.

Results

In total, 125 Caucasian women with a mean age of 43.8 years diagnosed with uUTI were enrolled in this open-label, non-randomized, multicentre interventional study at nine Ukrainian sites. Eighty-three patients (66.4 %) had an acute uUTI, whereas 42 patients (33.6 %) suffered from an acute episode of a recurrent infection. The patients demonstrated excellent compliance (mean 101.8 %). Only 3 out of 122 patients who completed the treatment phase were out of the accepted compliance range between 80 and 120 % of the number of tablets to be taken. Twenty patients were excluded from PPS due to major protocol violations resulting in 105 patients within the PPS (Table 3). No patient dropped out due to switching to antibiotics before Day 3.

Table 3 Reasons for patients being excluded from the per protocol set (PPS)

Number of patients	Reason for exclusion from PPS
1	Non-compliance (<80 %)
2	Missing of relevant lab data
2[a]	Drop out before Day 3
7	Major deviation from visit window
8	Intake of forbidden concomitant therapy

[a]Dropped out due to lost to follow-up and withdrawal of informed consent

Canephron® N was safe and well tolerated. In total 19 AEs, but no serious adverse event (SAE), were reported. Eleven (8.8 %) of 125 patients experienced at least one AE, most frequently reporting headache (4.0 %), followed by abdominal distension (1.6 %). None of the AEs was considered as drug-related. Thus, the primary endpoint of the study, the incidence of ADRs within the seven-day treatment with Canephron® N was 0 % with a 95 % Clopper-Pearson Confidence Interval (CI) between 0.0 and 2.9 % in the safety set.

At the end of the seven-day treatment period, 71.2 % of patients of the FAS (Table 4) and 70.8% of the PPS met the definition of clinical cure. The clinical cure rates on Day 37

Table 4 Change of patient symptom status from Day 7 to Day 37 categorized by age

		Day 7		Day 37	
		N	%	N	%
Total (N = 125)	Clinical Cure	89	71.2	107	85.6
	Improvement	24	19.2	10	8.0
	Clinical Failure	12	9.6	8	6.4
≤45 years (N = 68)	Clinical Cure	55	80.9	64	94.1
	Improvement	9	13.2	2	2.9
	Clinical Failure	4	5.9	2	2.9
>45 years (N = 57)	Clinical Cure	34	59.7	43	75.4
	Improvement	15	26.3	8	14.0
	Clinical Failure	8	14.0	6	10.5

Number (N) and percentage (%) of patients whose symptom status was assessed as "clinical cure" (sum score* ≤ 3), "improvement" (3 < sum score < 6) or "clinical failure" (sum score ≥ 6) on Day 7 and Day 37, for all patients and categorized into women aged ≤ 45 years or > 45 years (full analysis set, N = 125)

*sum of the three main symptoms (dysuria, frequency and urgency) rated on a 5-point scale (0 – absence, 1 – mild, 2 – moderate, 3 – severe, 4 – very severe)

were 85.6 % (FAS) and 85.8 % (PPS) respectively. The clinical cure rate was higher in younger women (≤45 years; cure rate of 80.9 % on Day 7 and 94.1 % on Day 37 respectively) than in women over the age of 45 years (cure rate of 59.7 % on Day 7 and 75.4 % on Day 37 respectively) (FAS; Table 4). On Day 7, the average improvement (mean change from baseline, FAS) in the sum score of dysuria, frequency and urgency was −5.4 on Day 7 and −6.6 on Day 37 compared to a mean baseline score of 7.3 (Fig. 3). The mean changes of the individual symptoms on Day 7/37 (FAS) compared to Day 0 were: −1.9/-2.3 (dysuria); −1.8/-2.4 (frequency); −1.6/-1.9 (urgency); −0.4/-0.5 (incontinence); −0.8/-1.1 (nocturia) and −1.0/-1.2 (pain). All changes from baseline were statistically significant ($p < 0.001$). There were only minor differences between the symptom ratings by the investigators and by the patients (Fig. 3). The Kaplan-Meier plot illustrated in Fig. 4 depicts the number of responders (symptoms absent versus symptoms not worse than mild) within the study period demonstrating a fast relieving of

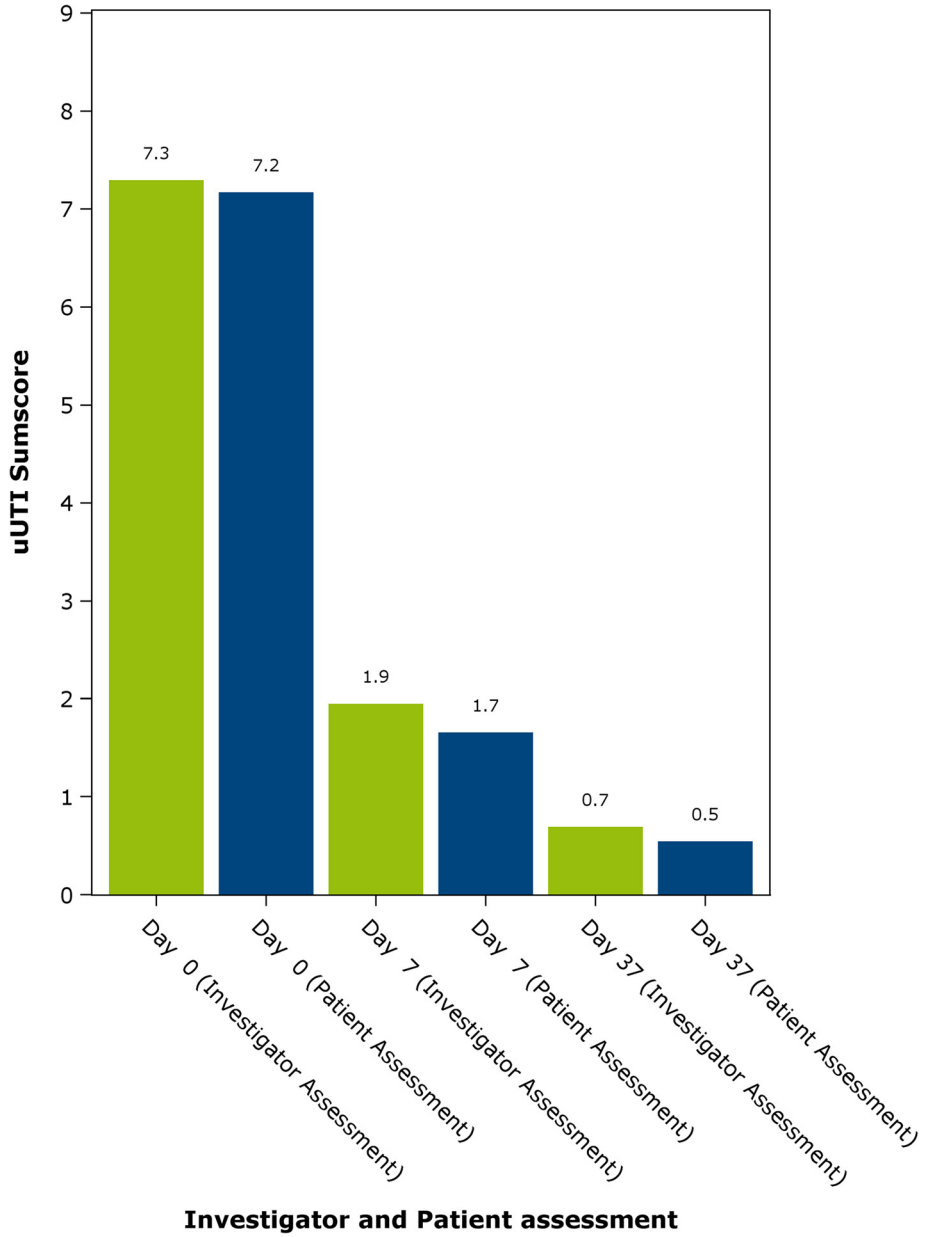

Fig. 3 Comparison between uUTI sumscore assessed by patients and investigators. The uUTI Sumscore includes the symptoms dysuria, frequency and urgency; uUTI Sumscore was assessed on Day 0, Day 7 and Day 37 (full analysis set, N = 125)

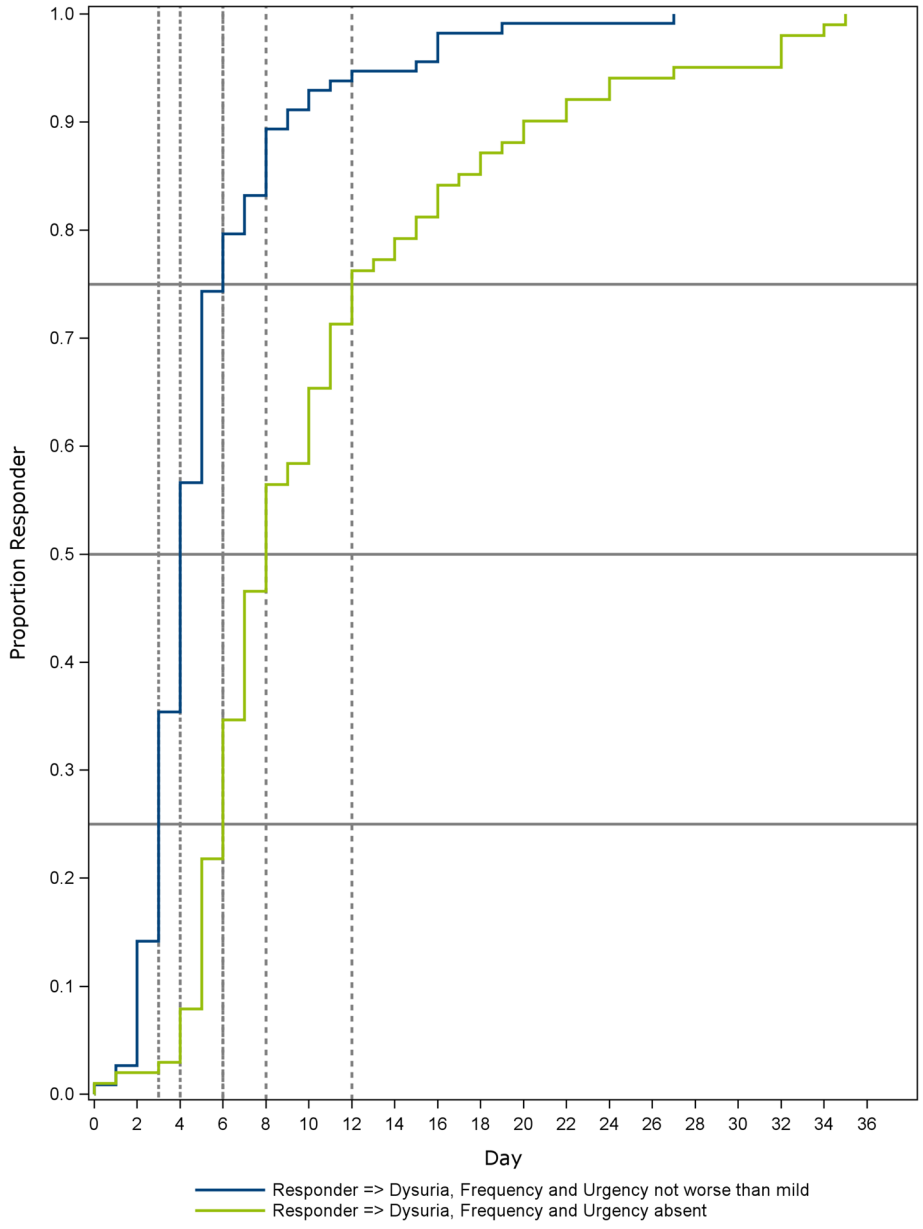

Fig. 4 Kaplan-Meier curve visualizing the proportion of responders at each study day. Responder defined as symptoms dysuria, frequency and urgency not worse than mild (blue line) or absent (green line) (full analysis set, N = 125)

symptoms in patients exhibiting none of the symptoms dysuria, frequency, urgency worse than mild within the first days of treatment (FAS).

The duration of symptoms was on average 5.1 days (frequency), 3.5 days (dysuria), 2.9 days (urgency), 2.4 days (nocturia), 1.7 days (pain) and 0.9 days (incontinence). The number of non-responders, i.e. the number of improved patients and patients with clinical failure (Table 2), decreased within the seven-day treatment period and up to Day 37 in favour of the proportion of clinical cured patients (Table 4; FAS).

An increased body temperature was not observed in any patient on Day 7 or Day 37. Three of 125 patients required antibiotics within the seven-day treatment period. After Day 7 none of the patients met the definition of recurrence. No clinically relevant trend was observed in the laboratory tests (haematology and clinical chemistry), vital signs and physical examinations. None of the patients finished the trial taking antibiotics.

There was a clear reduction in the number of patients exhibiting positive results in particular of erythrocytes (RBC), leucocytes (WBC) and nitrite in urinalysis. WBC

negativity improved from 17.6 % (Day 0) to 70.4 % (Day 7) and 77.6 % (Day 37). On Day 37, 93.6 % of the patients were nitrite-negative and 80.0 % RBC-negative (dipstick tests, Table 5, FAS). Of 65 patients suffering from significant bacteriuria on Day 0, only 40 still exhibited $\geq 10^3$ CFU/ml up to Day 37. However, of 50 patients without significant bacteriuria, 14 developed a significant bacteriuria up to Day 37. Of 10 patients with unknown bacteriuria on Day 0, 7 patients showed a significant bacteriuria on Day 37 (Fig. 5). Thus, the total number of patients with significant bacteriuria and the bacterial spectrum remained almost unchanged on Day 0, Day 7, and Day 37 with *E. coli* as the most frequent uropathogen followed by *Enterococci* (Table 6). The clinical cure rate of patients exhibiting $<10^3$ CFU/ml on Day 0 was 88.0 % on Day 7 and 98.0 % on Day 37, whereas the corresponding cure rates in patients exhibiting $\geq 10^3$ CFU/ml on Day 0 were 62.9 % on Day 7 and 77.4 % on Day 37, respectively, and thus significantly lower ($p < 0.005$). In the group $<10^3$ CFU/ml the number of patients with clinical failure was 1 (2.0 %) on Day 7 and 0 (0.0 %) on Day 37, whereas the number of patients with clinical failure in the group $\geq 10^3$ CFU/ml was 7 (11.3 %) on Day 7 and 4 (6.5 %) on Day 37 (Table 7).

Discussion

During one year, 11 % of women suffer from at least one UTI episode and more than half of all women have one or more infections during their lifetime [25]. Each year, in the United States, acute cystitis is responsible for 3.6 million office visits by women 18–75 years old, accounting for direct costs of $ 1.6 billion [25]. Most of acute lower UTIs (acute bacterial cystitis) are uncomplicated. They were not associated with signs or symptoms of upper UTIs (fever, chills, flank pain) or with other characteristics suggesting a higher risk of upper or complicated infection (e.g. diabetes mellitus, pregnancy, immunosuppression, previous pyelonephritis, structural abnormalities of the urinary tract). After initial infection many women have sporadic or multiple recurrences. *E. coli* causes 70 to 90 % of episodes of acute uUTI, followed by *Staphylococcus saprophyticus*, mainly in young women. Standard treatment is antibiotic therapy with associated rates of adverse reactions ranging from 7–40 % for trimethoprim, 0–40 % for nitrofurantoin, 7–21 % for norfloxacin and 13 % for ciprofloxacin [25]. Duration of antibiotic treatment in this indication is usually short-term: in a placebo-controlled clinical trial a significant symptomatic and bacteriological short-term benefit was observed in the therapy of uUTI with three-day treatment with nitrofurantoin, compared to placebo. After a follow-up period of two weeks, no statistically significant differences were found [21]. In addition, antibiotic use may harm patients suffering from asymptomatic bacteriuria. In the study of Cai et al. [26] it was concluded to be beneficial not to treat younger women suffering from asymptomatic bacteriuria with antibiotics due to a possible protective effect of colonizing bacteria. Solely symptomatic treatment of acute uUTI also seems to be an option. Bleidorn et al. reported similar results with ibuprofen versus ciprofloxacin based on symptomatic outcome in a pilot trial; however, one third of patients in the ibuprofen group relapsed within the first week [5].

Therefore, alternatives to antibiotic treatment are warranted. Cranberry, long advocated for prevention of acute cystitis, contains proanthocyanidines inhibiting the attachment of pathogens to uroepithelium [27]. 200–500 mg cranberry juice or concentrate tablets reduce the risk of symptomatic recurrences by 12–20 %, but are not applicable for treatment of uUTIs [28].

With regard to the current state of uUTI treatment, the study was designed to evaluate the safety profile of

Table 5 Changes in Urine Dipstick status from Day 0 to Day7 and Day 37

Urine Dipstick		Day 0		Day 7		Day 37	
		N	%	N	%	N	%
Nitrite	Missing	0	0.0	3	2.4	4	3.2
	Negative	98	78.4	115	92.0	117	93.6
	Positive	27	21.6	7	5.6	4	3.2
RBC	Missing	0	0.0	3	2.4	4	3.2
	Negative	70	56.0	92	73.6	100	80.0
	Positive	55	44.0	30	24.0	21	16.8
WBC	Missing	0	0.0	3	2.4	5	4.0
	Negative	22	17.6	88	70.4	97	77.6
	Positive	103	82.4	34	27.2	23	18.4

Number (N) and percentage (%) of patients with missing, negative or positive dipstick urine analysis results regarding nitrite, red blood cells (RBC) and white blood cells (WBC) on Day 0, Day 7 and Day 37 (full analysis set, N = 125)

Table 6 Changes in patients bacteriuria spectrum

	Day 0		Day 7		Day 37	
	N	%	N	%	N	%
Patients	125	100	122	100	121	100
Patients with significant bacteriuria	65	52.0	65	53.3	61	50.4
Total isolates	87	100	83	100	79	100
E.coli	35	40.2	36	43.4	31	39.2
Klebsiella spp.	4	4.6	4	4.8	4	5.1
Proteus spp.	3	3.4	2	2.4	5	6.3
Other *Enterobacteriaceae*	1	1.1	3	3.6	2	2.5
Enterococci	34	39.1	28	33.7	32	40.5
Staphylococci	4	4.6	4	4.8	3	3.8
Streptococci	1	1.1	2	2.4	0	0.0
Other Gram positives	5	5.7	4	4.8	2	2.5

Number (N) and percentage (%) of patients with significant bacteriuria ($\geq 10^3$ colony forming units (CFU)/ml) on Day 0, Day 7 and Day 37 and the respective spectrum of uropathogens

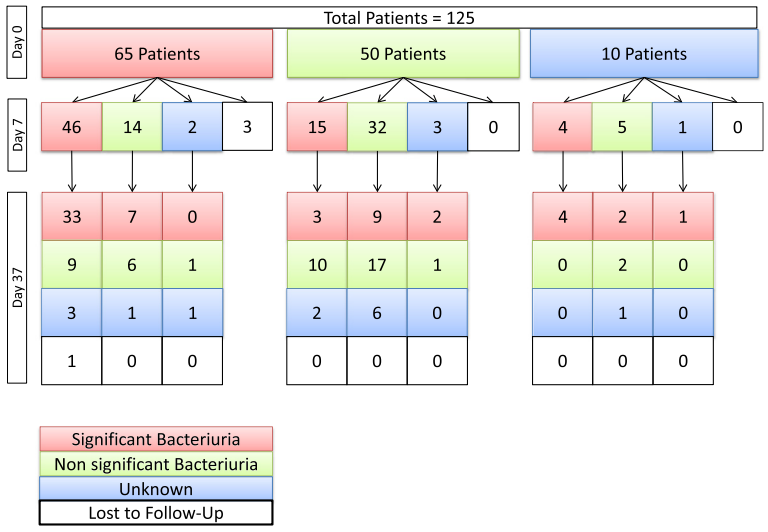

Fig. 5 Changes in bacteriuria status of patients throughout the clincical trial. Number of patients with significant, non significant and unknown bacteriuria on Day 0, Day 7 and Day 37 respectively and the changes in bacteriuria status throughout the clinical trial (full analysis set, N = 125). In case of "unknown bacteriuria" or "no growth" no bacterial analysis was possible

Canephron® N as well as the impact of a non-antibiotic therapy in the treatment of uUTI. The threshold for significant bacteriuria varies between 10^3 and 10^5 CFU/ml depending on uropathogens, gender and type of urine sample [7], therefore the selected thresholds of 10^4 CFU/ml and 10^3 CFU/ml for this study were in an acceptable range. The inclusion as well as exclusion criteria clearly fitted the intended indication which was demonstrated by only 2.4 % of patients receiving antibiotics.

Table 7 Changes of patient symptom status from Day 7 to Day 37 categorized by status of significant bacteriuria

		Day 7		Day 37	
		N	%	N	%
Total (N = 125[a])	Clinical cure	89	73.0	107	88.4
	Improvement	24	19.7	10	8.2
	Clinical failure	9	7.3	4	3.3
< 10^3/ml (N = 50)	Clinical cure	44	88.0[b]	49	98.0[b]
	Improvement	5	10.0	1	2.0
	Clinical failure	1	2.0	0	0.0
≥ 10^3/ml (N = 62)	Clinical cure	39	62.9[b]	48	77.4[b]
	Improvement	16	25.8	9	14.5
	Clinical failure	7	11.3	4	6.5
Unknown (N = 10)	Clinical cure	6	60.0	10	100.0
	Improvement	3	30.0	0	0.0
	Clinical failure	1	10.0	0	0.0

Number (N) and percentage (%) of patients whose symptom status was assessed as "Clinical cure", "Improvement" or "Clinical failure" on Day 7 and Day 37, for all patients and categorized into subgroups with (≥ 10^3 colony forming units (CFU)/ml) or without (< 10^3 CFU/ml) significant bacteriuria (full analysis set, N = 125)

[a]lost for follow up: 3 patients on Day 7 and 4 patients on Day 37

[b]difference between patients with and without significant bacteriuria is statistically significant (chi^2 test; $p < 0.005$) on Day 7 and on Day 37

In this study a resolution rate of 71.2 % after seven-day treatment and 85.6 % after 37 days (FAS, N = 125) was achieved under Canephron® N treatment, which was much more effective than reported spontaneous resolution [6–8, 21 – 26]. This hints at the efficacy of Canephron® N treatment. A three-day treatment with nitrofurantoin, exhibited no significant symptomatic difference to untreated patients after 14 days [21]. In contrast the symptomatic as well as anti-inflammatory effect is extended up to four weeks after the end of treatment with Canephron® N on Day 7 (Fig. 4, Tables 4 and 5).

In a recent meta-analysis of 464 studies of uUTI in women, frequency and dysuria had a positive correlation with bacteriuria [29], supporting the observation of symptomatic as well as inflammatory resolution under treatment with Canephron® N in this study. On the other hand, asymptomatic bacteriuria does not necessarily need antibiotic therapy even in patients suffering from recurrent cystitis [3, 26].

During UTI, microorganism adhering to the bladder urothelium cause inflammatory reactions, the bladder gets hyperactive and patients suffer from increased micturition frequency and pain. Canephron® N possesses relevant anti-adhesive and anti-inflammatory activity, possibly modulating the overactive bladder function, easing bladder functions and preventing adhesion of *E. coli*, as demonstrated in several *in vitro* and *in vivo* experiments [30, 31]. The bacterial spectrum in patients suffering from significant bacteriuria did not change significantly during the treatment as well as observation period in this study (Table 6), indicating

no direct anti-microbial effect of the drug, possibly supporting the concept of an immunological adaption process between uroepithelium and adhering microorganism. The fast symptomatic resolution of uUTI under Canephron® N treatment supports the assumption that the multiple pharmacodynamic targets of Canephron® N are suitable for the treatment of uUTI.

Conclusions

Canephron® N was safe and well tolerated, no ADRs or SAEs were reported. The responder rate was 71.2 % on Day 7 and 85.6 % on Day 37 (FAS, N = 125). A significant improvement was observed for the sum score as well as for all single symptoms on Day 7 and Day 37. Only 2.4 % of patients required antibiotics during the seven-day treatment period and none of the patients suffered from a recurrence until Day 37 after assessed as clinical cure on Day 7. The resolution of symptoms continued four weeks after end of treatment in contrast to many anti-microbial therapies. As this study was designed to show the safety of Canephron® N treatment, it is not possible to give a definite statement about efficacy, but the fast symptomatic resolution of uUTI under Canephron® N treatment hints at the efficacy of Canephron® N that needs to be further investigated.

This trial emphasises the safe usage and potential opportunity of a non-antibiotic therapy approach with Canephron® N and fully justifies a large-scale controlled clinical trial to finally establish the significance of Canephron® N in the management of acute phases of uUTIs.

Abbreviations

AEs: adverse events; ADRs: adverse drug reactions; ASS: acetyl salicylic acid; BfArM: Bundesamt für Arzneimittel und Medizinprodukte (German Federal Institute for Drugs and Medical Devices); CFU: colony forming units; CI: confidence interval; *E. coli*: *Escherichia coli*; FAS: full analysis set; ICH-GCP: International Conference on Harmonization - Good Clinical Practice; IMP: Investigational medicinal product; NSAIDs: non-steroidal anti-inflammatory drugs; PPS: per protocol set; RBC: red blood cells; SA: safety set; SAEs: serious adverse events; SmPC: summary of product characteristics; spp.: subspecies; uUTI: uncomplicated urinary tract infection; UTI: urinary tract infections; WBC: white blood cells.

Competing interests

Dimitry Ivanov, Tatyana Kostinenko, Liliya Martynyuk, and Nikolay Kolesnik were investigators of this study sponsored by Bionorica SE, Neumarkt, Germany, and Dimitry Ivanov also Bionorica (speaker's bureau).
Dimitri Abramov-Sommariva, Kathrin Moritz, and Herbert Eskötter were emplyees of Bionorica.
Kurt G. Naber: Basilea (investigator, consultant), Bayer (speaker's bueau), Bionorica (investigator, consultant, scientific publication, speaker's bureau), Boehringer Ingelheim (consultant), Cubist/MSD (consultant), Daiichi Sankyo (speaker's bureau, scientific publication), Galenus (consultant), Leo Pharma (consultant, speaker's bureau), Melinta (consultant), MerLion (investigator, consultant, scientific publications), OM Pharma/Vifor (investigator, consultant, speaker's bureau, scientific publications), Paratek (consultant), Rempex Pharm (consultant), Rosen Pharma (investigator, consultant, scientific publications, speaker's bureau), Shionogi (consultant), Zambon (investigator, consultant, speaker's bureau).

Authors' contributions

KN, DAS, HE and KM contributed to the design of the study, analysis and review of study-results. DI, NK, TK and LM were clinically involved in execution of the study and data collection. All authors reviewed and approved the final manuscript.

Acknowledgements

We gratefully thank all participating clinical sites for their commitment and successful conduct of this clinical trial:.
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We acknowledge the extensive performance of the Contract Research Organisation Harrison Clinical Research now SynteractHCR throughout the project.
We thank Jörg Michael Schierholz, Starnberg, Germany, for his medical and scientific support as well as the valuable discussions regarding the preparation of the manuscript.
We greatly acknowledge the support extended throughout the study by Christine Neubauer, Martina Höller, Mirjam Rittmeyer, Patrick Dubovy and Hubert Steindl, Bionorica SE, Neumarkt i. d. OPf., Germany, in making this manuscript a success.

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Received: 22 January 2015 Accepted: 6 July 2015
Published online: 22 September 2015

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