Canephron® N reduced nociception in experimental cystitis and inhibited the pain-related targets NK1 receptor and ASIC1a

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Introduction

Canephron® N (Can N) is a herbal medicinal product that is used to treat urinary tract infections, such as uncomplicated cystitis. It contains rosemary leaves, lovage root and centaury herb. Can N prevents the adhesion of bacteria to bladder epithelial cells and exerts anti-inflammatory, anti-oxidative and spasmolytic effects.

Cystitis is one of the most frequent infections in humans mainly caused by Escherichia *coli* (E. *coli*) that enter the urinary bladder, adhere to the uroepithelium and invade epithelial bladder cells. As a consequence inflammatory processes are triggered to boost the clearance of E. *coli*. For the patients the most bothersome symptoms of cystitis are impaired bladder function (urge and frequency) and pain.

Here we investigated whether Can N exerts beneficial effects on pain in experimental cystitis. The aims of this study were to elucidate effects of Can N on nociception *in vivo* and to uncover possible molecular targets *in vitro*.

Methods

Nociceptive threshold and score were determined in rats. Experimental cystitis was induced by intraperitoneal injection of cyclophosphamide and pain sensation was measured in the abdominal area using von Frey filaments of increasing strength (1 to 60 g).

Nociceptive threshold is the minimal von Frey filament strength to elicit a pain response and is determined to detect allodynia (reduced threshold). The nociceptive score quantifies the response to a given von Frey filament strength. Nociceptive score was plotted against the von Frey filament strength, and the area under the curve (AUC) over the range of 10 to 60 g was determined as a measure of hyperalgesia (exaggerated pain response to a stimulus of given strength).

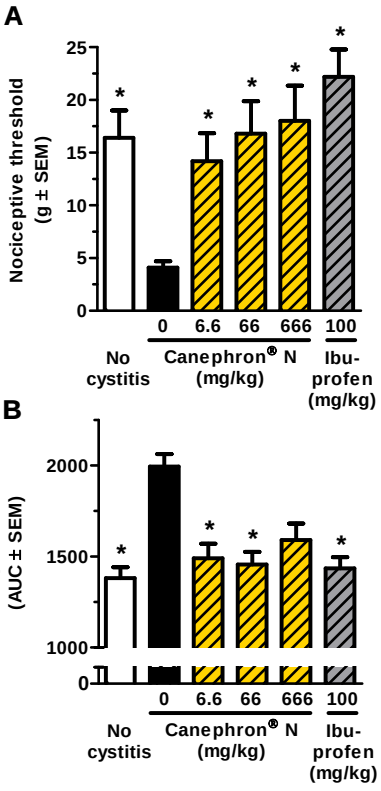
Can N was given orally twice a day for three days before the induction of cystitis at a dose of 6.6, 66, or 666 mg/kg (equivalent to 0.2x, 2x, or 20x the human equivalent dose (HED)). Ibuprofen (100 mg/kg, approximately 1x HED) was used as positive control.

Neurokinin receptor (NK1R) activity was measured in U2OS cells expressing NK1R. Stimulation with substance P (SP, 1 nM) induced an increase of the intracellular Ca^{2+} concentration and this increase was measured by fluorescence using the calcium indicator fura-2 AM. Can N was added 2.5 minutes before stimulation with SP in increasing concentrations (0.00001 - 400 µg/ml). The NK1 receptor inhibitor L-733,060 (50 nM) was used as positive control and completely blocked NK1R signaling.

Acid-sensing ion channel (ASIC1a) currents were measured in CHO-K1 cells expressing ASIC1a using standard patch-clamp techniques (conventional whole-cell configuration). ASIC1a was activated by applying a stimulation bath solution of pH 6.5 for 4 seconds. Can N was added in increasing concentrations (0 - 200 µg/ml) with the stimulation bath solution. The sodium channel blocker amiloride (100 µM) was used as positive control and inhibited ASIC1a current by approximately 90%.

Results

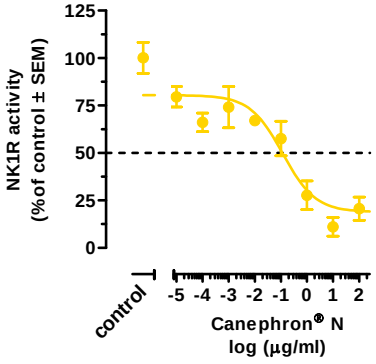
Figure 1: Canephron® N normalized pain sensation in a rat model of experimental cystitis



A: Induction of experimental cystitis by cyclophosphamide markedly reduced the nociceptive threshold, i.e. a stimulus that is normally not painful elicits a pain response (allodynia). Treatment with Can N prevented allodynia in a dose-dependent manner, even at the lowest dose tested (6.6 mg/kg) which is equivalent to 0.2x HED.

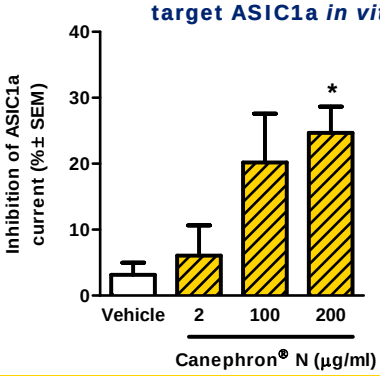
B: Induction of experimental cystitis also increased the nociceptive score and resulted in hyperalgesia. Treatment with Can N prevented hyperalgesia even at the lowest dose tested (6.6 mg/kg) which is equivalent to 0.2x HED.

Figure 2: Canephron® N inhibited the pain-related target NK1R *in vitro*



Stimulation of NK1R (expressed in U2OS cells) by substance P (1 nM) induced an increase in the intracellular concentration of Ca^{2+}. Pretreatment with Can N reduced the Ca^{2+}-concentration in a concentration-dependent manner with a low IC_{50} of approximately 0.15 µg/ml.

Figure 3: Canephron® N inhibited the pain-related target ASIC1a *in vitro*



Stimulation of ASIC1a expressed in CHO-K1 cells by a bath solution of pH 6.5 resulted in an inward current that was inhibited by application of Can N in a concentration-dependent manner with approximately 25% inhibition at 200 µg/ml.

Summary and conclusions

Canephron® N prevented allodynia and hyperalgesia in experimental cystitis *in vivo* at clinically relevant doses. It also inhibited ASIC1a as well as NK1R-mediated signaling *in vitro*. Because ASIC1a is involved in nociception and activation of NK1R leads to neurogenic inflammation and sensitization of afferent neurons, inhibition of these targets by Can N is likely to contribute to the anti-nociceptive effects of Can N. We conclude that Can N represents a suitable option to treat uncomplicated urinary tract infections by targeting inflammatory pain reactions as one of the most bothersome symptoms of cystitis.

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