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BENEFICIAL EFFECTS OF GOSHA-JINKI-GAN AND GREEN TEA EXTRACT IN RATS WITH CHEMICAL CYSTITIS

Hypothesis / aims of study

Interstitial cystitis (IC) is a chronic inflammatory disease of the bladder. Major characteristics are urinary frequency, urgency, and suprapubic pain. An intraperitoneal injection of cyclophosphamide (CYP) was shown to induce reproducible dose-dependent chemical cystitis in rats, therefore, it has been used as an experimental model of hemorrhagic cystitis [1]. Previous studies have implied that urinary cytokines or chemokines are involved in CYP-induced cystitis [2] and in patients with cystitis [3]. Many reports have described the efficacy and safety of phytotherapeutic natural products for urinary dysfunction. However, the use of these agents in the treatment of lower urinary tract symptoms due to IC has been limited by a lack of scientific knowledge underlying the possible mechanism of pharmacological action. Gosha-jinki-gan (GJG) is a traditional Japanese medicine used for urinary disorders, and is composed of 10 crude drugs in fixed proportions. GJG has been shown to decrease the frequency of urination in patients with urinary disturbance, while the mechanism of pharmacological action of GJG is not yet clear. The medicinal value of green tea is well known and reportedly contains the highest concentration of powerful antioxidants called polyphenols, also known as green tea catechins. Green tea polyphenolic compounds present in the green tea extract (GTE) have antioxidant, anticarcinogenic, antiinflammatory, and antimicrobial properties in human, animal, and *in vitro* studies. The current study aimed to characterize pharmacological effects of GJG and GTE, on urodynamic parameters, bladder receptors, and urinary cytokines in rats with CYP-induced cystitis.

Study design, materials and methods

Rats received an injection of CYP (150 mg/kg, i.p.) for acute cystitis and were used for the experiment three days later. Sham rats received a volume-matched injection of saline (5 mL/kg, i.p.), CYP+GJG and CYP+GTE-treated animals were orally administered GJG (1000 mg/kg/day) and GTE (400 mg/kg/day) prepared in 0.5% methylcellulose and distilled water, respectively, for three days before and after the CYP treatment (seven consecutive days). Vehicle was administered orally to sham and CYP-treated rats. Cystometry and measurements of muscarinic receptor binding and urinary cytokines were performed seven days after the first administration of GJG or GTE. The muscarinic and purinergic receptors in the bladder and submaxillary gland were measured by radioreceptor assays using [N-methyl-^{3}H] scopolamine chloride ([^{3}H]NMS) and αβ-methylene-ATP [2,8-^{3}H] tetrasodium salt ([^{3}H]αβ-MeATP), respectively. Urinary cytokines (interleukin-1β [IL-1β], IL-6 and L-17) were measured with ELISA kits.

Results

Micturition interval and micturition volume were significantly decreased and the frequency of micturition and basal pressure were significantly increased in CYP-treated rats compared with sham-operated rats. The GJG treatment significantly ameliorated changes in urodynamic parameters in CYP-treated rats. Similar treatment with GTE slightly attenuated changes in urodynamic parameters. The maximal number of binding sites for [^{3}H]NMS and [^{3}H]αβ-MeATP in the bladder was significantly lower in CYP-treated rats than in sham rats. Such a reduction in receptor density was significantly attenuated by the GJG treatment. GTE treatment also significantly attenuated the down-regulation of muscarinic receptors, but not P2X receptors in bladders of rats with CYP-induced cystitis. The elevation in urinary cytokine levels in CYP-treated rats was effectively attenuated by GJG treatment. The elevation in cytokine levels in CYP-treated rats was alleviated by GTE treatment.

Interpretation of results

The present study is the first to demonstrate that GJG and GTE may improve effectively bladder overactivity in rats with CYP-induced cystitis. Our previous studies showed that treatment with CYP caused a decrease in the number of muscarinic and purinergic receptors in the rat bladder. Therefore, urinary cytokines and pharmacologically relevant bladder receptors may serve as direct therapeutic targets or potential biomarkers for the development of targeted therapy designed to prevent chronic bladder inflammatory conditions such as IC. The down-regulation of muscarinic and purinergic receptors in the bladder and an elevation of cytokines in urine of CYP-treated rats were effectively attenuated by repeated treatment with these phytotherapeutic agents, suggesting a viable alternative in the pharmacological treatment of cystitis.

Concluding message

GJG may be a potential therapeutic agent for improving clinical symptoms of cystitis. GTE also appears to have some ameliorative effects on the altered parameters observed in CYP-treated rats.

Table 1. Urodynamic parameters in sham, CYP-treated, and CYP+GJG-treated rats

Variable	Sham			CYP			CYP+GJG		
Micturition interval (min)	12	±	2.4	3.5	±	0.8*	15	±	2.9††
Mean micturition volume (mL)	0.68	±	0.14	0.20	±	0.04*	0.94	±	0.14††
Frequency of micturition (number/hr)	6.0	±	0.9	17	±	2.9**	4.7	±	0.9†††
Maximum micturition pressure (mmHg)	41	±	2.7	43	±	6.9	35	±	4.3
Basal pressure (mmHg)	2.6	±	0.7	10	±	2.2**	2.0	±	0.5††
Threshold pressure (mmHg)	8.1	±	1.1	15	±	3.6	10	±	0.8
Residual urine (mL)	0.03	±	0.02	0.06	±	0.04	0.04	±	0.04

Each value represents the mean ± SE (n = 5-6). *P<0.05, **P<0.01, significantly different from sham rats. ††P<0.01, †††P<0.001, significantly different from CYP-treated rats.

Table 2. Dissociation constant (K_d) and maximal number of binding sites (B_{max}) for specific binding of [^{3}H]NMS and [^{3}H]αβ-MeATP in sham, CYP-treated, and CYP + GJG-treated rats

Variable	K_d			B_{max}		
[^{3}H]NMS binding	(pM)			(fmol/mg protein)		
Bladder						
Sham	265	±	9	224	±	15 (1.0)
CYP	234	±	15	147	±	8 (0.65)**
CYP + GJG	240	±	10	211	±	19 (0.94)†
Submaxillary gland						
Sham	190	±	12	143	±	7
CYP	182	±	4	149	±	8
CYP + GJG	167	±	7	132	±	7
[^{3}H]αβ-MeATP binding	(pM)			(pmol/mg protein)		
Bladder						
Sham	975	±	133	9.3	±	0.66 (1.0)
CYP	970	±	171	4.6	±	0.38 (0.49)***
CYP + GJG	910	±	108	6.5	±	0.28(0.69)**,†

Each value represents the mean ± SE (n=7–9). **P<0.01, ***P<0.001, significantly different from sham rats. †P<0.05, significantly different from CYP-treated rats.

References

1. 1 Cell Biol Toxicol 23: 303-312, 2007
2. 2 Urol 73: 421-426, 2009
3. 3 J Infect Chemother 4: 24-27, 1998

Disclosures

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