The role of Urinary Cations in the etiology of interstitial cystitis: A Multisite Study

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Objectives

To determine if patients with interstitial cystitis (IC) had elevated levels of toxic urinary cations compared to controls. To identify and quantify these cationic metabolites in patients with IC vs control subjects and determine their cytotoxicity to cultured urothelial cells.

Methods

Isolation of cationic fraction (CF) was achieved by solid phase extraction using an Oasis® MCX cartridge on urine specimens of IC patients and controls. C18 reverse phase high performance liquid chromatography (RP-HPLC) was used to profile cationic metabolites, which were quantified by area under the peaks and normalized to creatinine. Major CF peaks were identified by RP-HPLC and liquid chromatography-tandem mass spectrometry. HTB-4 urothelial cells were used to determine the cytotoxicity of CF and of individual metabolites.

Results

The RP-HPLC analysis was performed on CF metabolites isolated from urine samples of 70 IC patients and 34 controls. The mean (SEM) for control vs patient was 3.84 (0.20) vs 6.71 (0.37) mAU*min/µg creatinine, respectively (p =0.0001).
The CF cytotoxicity normalized to creatinine for control vs patient in mean (SEM) percent was -7.79 (3.32)% vs 20.03 (2.75)% (p < 0.0005). The major toxic cations were 1-methyladenosine, 1-methylguanine, N2,N2-dimethyl guanosine and L-tryptophan.

Conclusions

These multisite data confirm a single site report on the elevation of toxic cations in the urine of IC patients compared to controls. The cytotoxicity of cationic metabolites was significantly higher in IC patients compared to controls. These toxic cations likely are the primary cause of IC, because they injure bladder mucus and initiate epithelial leak.

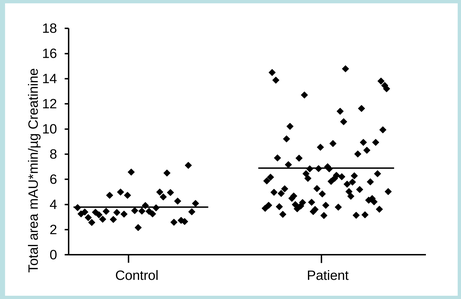

Figure 1. The scatter plot of sum of area under the peak for RT 5min-20min of HPLC of MCX CF per µg of creatinine of control vs patients with IC. Horizontal bars represent the mean. The mean (SEM) was 3.84 (0.20) vs 6.71 (0.37) mAU*min/µg creatinine, respectively (p=0.0001).

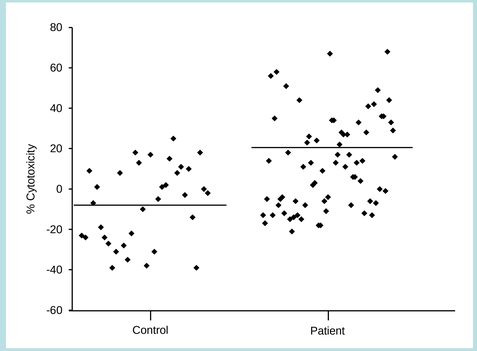

Figure 2. A scatter plot of cytotoxicity of MCX cationic fraction of control (n =34) vs patient (n=70). Horizontal bars represent the mean. The mean (SEM)% cytotoxicity in control vs patient was -7.79(3.32)% vs 20.03(2.75)% respectively (p<0.0005). Means were compared with Student's t-test. Negative number represents cell growth.

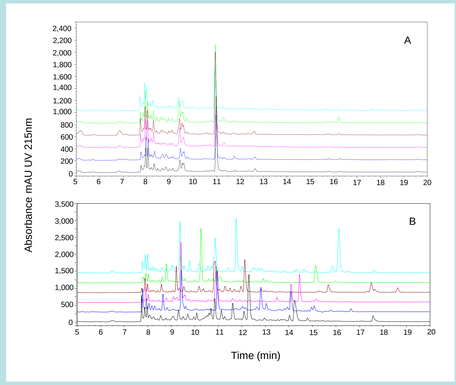

Figure 3. A. C_{18} RP-HPLC profiles of 6 representative controls showing reproducible pattern with single high intensity peak at RT 11 minutes, identified as L-tryptophan. **B.** C_{18} RP-HPLC profiles from 6 representative IC patients showing multiple high intensity peaks indicating high cationic metabolite content compare to controls.

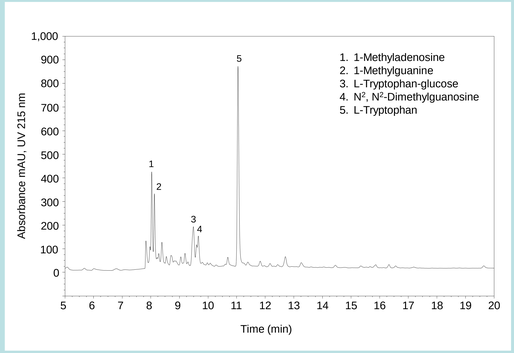

Figure 4. C_{18} reverse phase high performance liquid chromatography profile of MCX cationic fraction of representative control sample with major identified metabolites with RT were: 1-Methyladenosine, RT 8.10min;1-Methylguanine, RT 8.20min; L-tryptophan-glucose RT 9.42min; N^2,N^2-dimethylguanosine, RT 9.60 min; L-tryptophan, RT 11.02min.

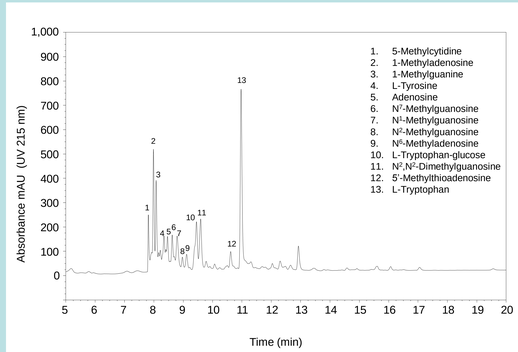

Figure 5. C_{18} reverse phase high performance liquid chromatography profile of MCX cationic fraction of representative sample from patient with IC. The 13 identified metabolites with RT were: 5-Methylcytidine, RT 7.90min; 1-Methyladenosine, RT 8.08min; 1-Methylguanine, RT 8.22min; L-Tyrosine, RT 8.46; Adenosine, RT 8.63min; N^7-Methylguanosine, RT 8.86 min; N^1-Methylguanosine, RT 9.18 min, N^2-Methylguanosine, RT 9.20 min, N^6-Methyladenosine, 9.30 min; L-tryptophan-glucose, RT 9.45 min; N^2, N^2-Dimethylguanosine, RT 9.59 min; 5'-Methylthioadenosine RT 10.60 min; L-tryptophan, RT 11. 00 min.

Table 1 Cationic metabolites identified by LC-MS and LC-MS/MS

	Metabolite	RT (min)	Molecular ion peak	Daughter ion
			m/z, $[M+H]^+$	m/z
1	5-Methylcytidine	7.89	258	126
2	1-Methyladenosine	8.08	282	150
3	1-Methylguanine	8.22	166	149
4	L-Tyrosine	8.47	182	165, 136
5	Adenosine	8.63	268	136
6	Tyramine	8.79	138	121
7	N^1,N^2,N^7 trimethylguanosine	8.97	326	194
8	5-Hydroxytryptophan	9.12	221	204
9	N^1-Methylguanosine	9.18	298	166
10	N^2-Methylguanosine	9.21	298	166
11	N^6-Methyladenosine	9.30	282	150
12	N^4-acetylcytidine	9.40	286	154
13	L-Tryptophan-glucose	9.45	367	247, 229
14	L-Phenylalanine	9.52	166	120
15	N^2,N^2-dimethylguanosine	9.59	312	180
16	Kynurenine	9.91	209	192
17	5'-Methylthioadenosine	10.62	298	136
18	L-Tryptophan	10.97	205	188

The RT (min), parent ion mass to charge ratio (m/z) and daughter ion m/z of all cationic metabolites identified in IC patients by LC-MS and LC-MS/MS using ESI in positive ion mode.

Table 2 Quantitative comparison of major cationic metabolites in control subjects vs patients with IC

Metabolite	Mean (SEM) ng/µg Creatinine		p value*
	Control (n = 34)	Patient (n = 70)	
1-MEA	7.12 (0.22)	7.88 (0.15)	< 0.01
1-MEG	5.76 (0.25)	6.47 (0.17)	< 0.05
L-Trp-glc	114.19 (8.58)	147.00 (14.50)	< 0.05
N^2,N^2-DMEG	4.53 (0.20)	6.14 (0.23)	< 0.0001
L-Trp	7.64 (0.55)	9.32 (0.34)	< 0.01

*Student's t-test. 1-MEA, 1-methyladenosine; 1-MEG, 1-methylguanine; L-Trp-glc, tryptophan-glucose glycoconjugate; N2,N2 -DMEG, N2,N2-dimethylguanosine; L-Trp, L-tryptophan.

Table 3 Cytotoxicity of cationic metabolite standard

	Metabolite	Cytotoxicity
1	5-Methylcytidine	34*
2	1-Methyladenosine	50*
3	1-Methylguanine	30*
4	L-Tyrosine	-9**
5	Adenosine	16*
6	Tyramine	-12**
7	5-Hydroxytryptophan	47*
8	N^2-Methylguanosine	9*
9	N^4-acetylcytidine	20*
10	L-Tryptophan-glucose	5*
11	L-Phenylalanine	-4**
12	N^2,N^2 Dimethylguanosine	19*
13	5'-Methylthioadenosine	15*
14	L-Tryptophan	22*

Cytotoxicity of the cationic metabolite standard 50µg/well (N=15) using HTB-4 urothelial cells 20,000/well.
*p < 0.001 compared to control well of cells using Student's test with a Bonferroni correction.
**Negative numbers represent cell growth greater than control well (p < 0.001).