Age-related physiological and biological changes, such as hyperlipidemia, hypertension, and diabetic mellitus, have received strong attention as a possible background for urinary bladder disorders. [25] In the present study, blood chemical analysis revealed significantly higher serum lipid levels in the O + AL group. In addition, the insulin value significantly increased in the O + AL group compared with the Y and O + CR groups. These findings were in line with previous reports suggesting that the insulin levels increase with aging because of insulin resistance, and that CR decreased the insulin levels owing to improvement of insulin sensitivity in rats [26] and humans. [27] Furthermore, serum testosterone level significantly decreased with aging, which is similar to observations in humans. [28] Interestingly, long-term CR maintained high serum testosterone level in the present study, which is consistent with a previous report that long-term CR can prevent reductions in steroidogenesis. [29]

There are some limitations in the present study. To evaluate bladder function *in vivo* more in detail cystometric investigations would be desirable, and to explore the background of aging-induced changes, gene- and protein-molecular examinations will be required. Such further studies may reveal a possible mechanism of age-related urinary bladder dysfunction. Furthermore, under the current experimental conditions, the influence of major co-morbidities could be avoided, implying that

changes occurring under "real life" conditions would not be detected, and that the alterations demonstrated in the study may have been quantitatively underestimated. It may be that potential effects of CR could be more conspicuous if tested in animal models of disease. **Previously preventive effects of CR on chronic disease, such as type 2 diabetes and cardiovascular disease, were revealed in human and animal trials.[13, 14] It is, however, difficult to translate these results of rodent studies to human health problems. Recently, Lorenzini suggested that there are two possible interpretations of CR, one is that excess fat is deleterious for health, and the second that leanness from a normal body weight might contribute to health. [30] In the present study, we were not able to determine whether the effects of CR could be explained by any of these interpretations.**

Conclusions

The present study demonstrates for the first time that CR has a preventive effect against age-related functional and morphological changes of the rat urinary bladder. Thus, age-related impairment of detrusor contractility seems to be related to decreased expression of M_3 receptors and fibrosis of the bladder wall. These findings may contribute to an increased understanding of the mechanisms of age-related detrusor underactivity.

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Figure legends

Figure 1. Contractile responses to high K^+ (A), carbachol (CCh, B), and adenosine triphosphate (ATP, C) and relaxant responses to isoproterenol (D) in young (Y), old fed with normal food (O + AL), and old with calorie restriction (O + CR) groups of rats. Values are expressed as means ± SEM. $^*P<0.05$ compared with the Y group according to the Kruskal-Wallis H test with a post hoc test.

Figure 2. Contractile responses to electrical field stimulation (EFS, A), and their cholinergic- (B) and purinergic- (C) components in young (Y), old fed with normal food (O + AL), and old with calorie restriction (O + CR) groups of rats. $^*P<0.05$ compared with the Y group according to Kruskal-Wallis H test with a post hoc test. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared between two groups according to the Kruskal-Wallis H test with a post hoc test.

Figure 3. cDNA expressions of muscarinic 1,2 and 3 (M_1, M_2 and M_3) receptors and $P2X_1$ receptor in young (Y), old fed with normal food (O + AL), and old with calorie restriction (O + CR) groups of rats.

$^{\#}P<0.05$, $^{\#\#}P<0.01$ compared between two groups according to the Kruskal-Wallis H

test with a post hoc test.

Figure 4. Representative mictroscopic images with Masson-trichrome staining of the bladder (A: low power field (upper) and high power field (lower)) and the collagen-deposition rate in the detrusor layer (B) and whole bladder layer (C) in young (Y), old fed with normal food (O + AL), and old with calorie restriction (O + CR) groups of rats.

$^{\#}P$ <0.05, $^{\#\#}P$ <0.01 compared between two groups according to the Kruskal-Wallis H test with a post hoc test.

Table 1. Primer list for real-time RT-PCR analysis.

Muscarinic receptors	
M_1	5'- GTCAACACCGAGCTCAAGA- CAG-3'
	5'- CGTGGTATAGAGGTTCATGGAGAAG-3'
M_2	5'-TCCCGGGCAAGCAAGAGTAG-3'
	5'-CCATCACCACCAGGCATATTGTTA-3'
M_3	5'- AGGACTCGAGTGGGACAGCTAC-3'
	5'- ATATGGTTCAGT- CAATCCACAGTTC-3'
Purinoceptor	
$P2X_1$	5'- TCCGTCTGATCC- AGTTGGTG-3'
	5'- GATGAGGTCACTTGAGGTCTGG-3'
Gapdh	5'- ATCAACGGGAAACCCATCAC-3'
	5'-GACATACTCAGCACCAGCATCAC-3'

Table 2. Parameters of frequency volume measurements for 24 h in young (Y), old fed with normal food (O + AL), and old with calorie restriction (O + CR) groups of rats

	Y (N = 8)	O + AL (N = 8)	O + CR (N = 8)
Voiding frequency (times)	14.88 ± 1.11	14.38 ± 2.08	18.13 ± 1.53
P value	0.427 (vs O + AL)	0.113 (vs O + CR)	0.100 (vs Y)
Voided volume in a day (ml)	10.01 ± 1.26	9.69 ± 2.87	9.19± 0.77
P value	0.208 (vs O + AL)	0.294 (vs O + CR)	0.674 (vs Y)
Voided volume per micturition (ml/time)	0.70 ± 0.10	0.62 ± 0.08	0.51 ± 0.02
P value	0.753 (vs O + AL)	0.208 (vs O + CR)	0.208 (vs Y)
Mean uroflow rate (ml/s)	0.21 ± 0.02	0.18 ± 0.02	0.17 ± 0.003
P value	0.298 (vs O + AL)	0.401 (vs O + CR)	0.121 (vs Y)
Water intake (ml)	15.12 ± 4.02	12.88 ± 5.56 $^{\#}$	25.68 ± 1.35
P value	0.397 (vs O + AL)	0.012 (vs O + CR)	0.058 (vs Y)
Food intake (g)	14.20 ± 1.30	12.66 ± 2.24 $^{\#}$	19.52 ± 1.06 **
P value	0.529 (vs O + AL)	0.046 (vs O + CR)	0.006 (vs Y)

The values are expressed as mean ± SEM.

N = number of animals

$^{**}P<0.01$: significant difference from Y (Kruskal-Wallis H test with a post hoc test)

$^{\#}P<0.05$: significant difference from O + CR (Kruskal-Wallis H test with a post hoc test)

Table 3. The body and bladder weights (A), cardiac parameters (B) and blood chemical analysis (C) in young (Y), old fed with normal food (O + AL), and old with calorie restriction (O + CR) groups of rats

A: body and bladder weights

	Y (N = 16)	O + AL (N = 15)	O + CR (N = 16)
Body weight (g)	369.19 ± 7.75	407.8 ± 11.39 **, ###	255.94 ± 3.13 ***
Bladder weight (mg)	93.38 ± 2.59	108.75±3.98 **, ###	82.37 ± 1.58 **
Bladder / body weight (mg/g)	0.25 ± 0.01	0.27 ± 0.02 ##	0.32 ± 0.01 ***

B: cardiac parameters

	Y (N = 8)	O + AL (N = 8)	O + CR (N = 8)
Heart rate (bpm)	403.96 ± 17.41	352.13 ± 24.13	368.63 ± 10.39
Systolic blood pressure (mmHg)	119.54 ± 3.90	122.08 ± 7.22	119.79 ± 3.01
Diastolic blood pressure (mmHg)	96.88 ± 2.81	94.92 ± 6.16	97.96 ± 3.87
Mean blood pressure (mmHg)	85.67 ± 2.82	80.08 ± 6.83	87.25 ± 4.55

C: blood chemical analysis

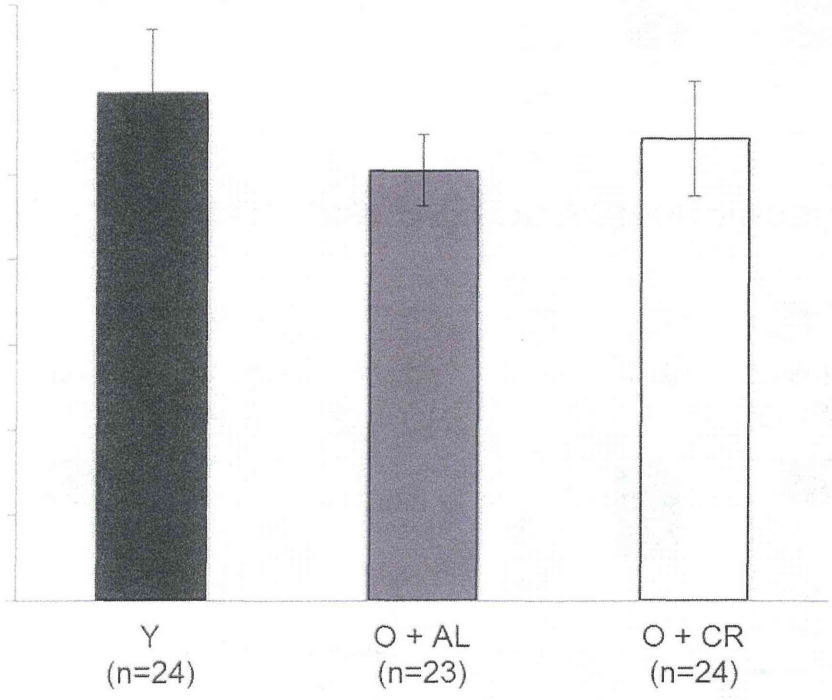
	Y (N = 8)	O + AL (N = 8)	O + CR (N = 8)
Albumin (g/dl)	3.73 ± 0.02	3.6 ± 0.11	3.61 ± 0.09
BUN (mg/dl)	22.1 ± 0.34	17.33 ± 0.57 ***	16.7 ± 0.85 ***
Creatinine (mg/dl)	0.29 ± 0.01	0.33 ± 0.01 ###	0.22 ± 0.01 **
HDL-Chol (mg/dl)	23.75 ± 0.84	30.75 ± 2.15 *	27.88 ± 2.70
LDL-Chol (mg/dl)	8.0 ± 0.24	19.75 ± 1.47 ***, ###	5.63 ± 0.32 **
Total-Chol (mg/dl)	69.63 ± 1.31	152.25 ± 13.55 ***, ###	62.38 ± 3.18
Triglyceride (mg/dl)	86.25 ± 8.86	114.75 ± 18.32 ###	32.13 ± 3.22 **
free fat acid (μEQ/l)	344.75 ± 68.28	311.13 ± 30.39 ##	153.75 ± 47.56 **
Glucose (mg/dl)	158.5 ± 11.58	150.43 ± 7.34	147.38 ± 10.91
HbA1c (%)	5.64 ± 0.13	4.67 ± 0.10 *** (n=7)	4.93 ± 0.06 ***
insulin (ng/ml)	1.31 ± 0.27	2.46 ± 0.29 *, #	1.41 ± 0.23
testosterone (ng/ml)	3.42 ± 1.02	0.28 ± 0.11 **, ##	2.05 ± 0.57
E2 (pg/ml)	25.25 ± 0.97	31.13 ± 2.12 *, ##	38.5 ± 3.96 ***
ADH (pg/ml)	110.95 ± 27.65	162.24 ± 41.78 (n=7)	126.71 ± 19.08

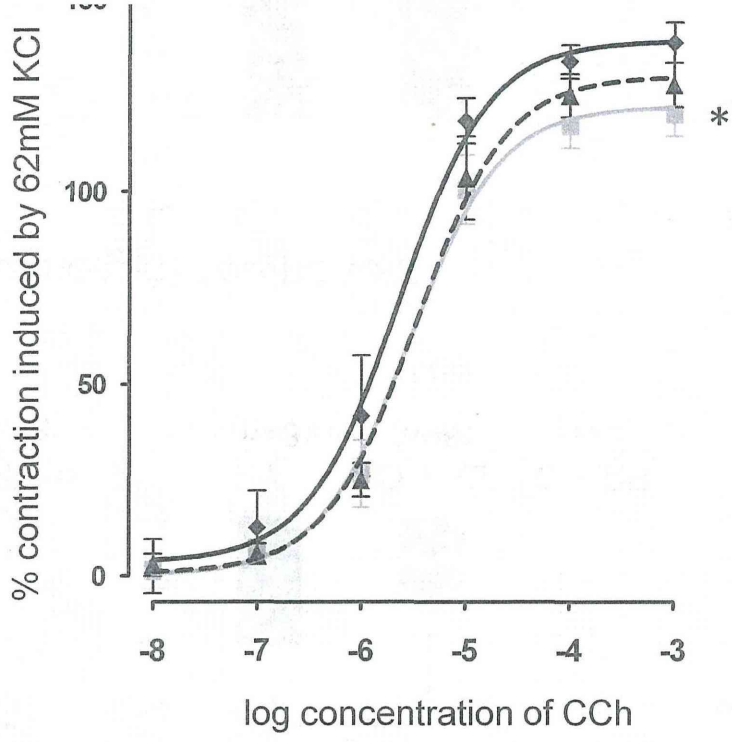
The values are expressed as mean ± SEM. N = number of animals

*P<0.05, **P<0.01, ***P<0.001: significant difference from Y (Kruskal-Wallis H test with a post hoc test)

#P<0.05, ##P<0.01, ###P<0.001: significant difference from O + CR (Kruskal-Wallis H test with a post hoc test)

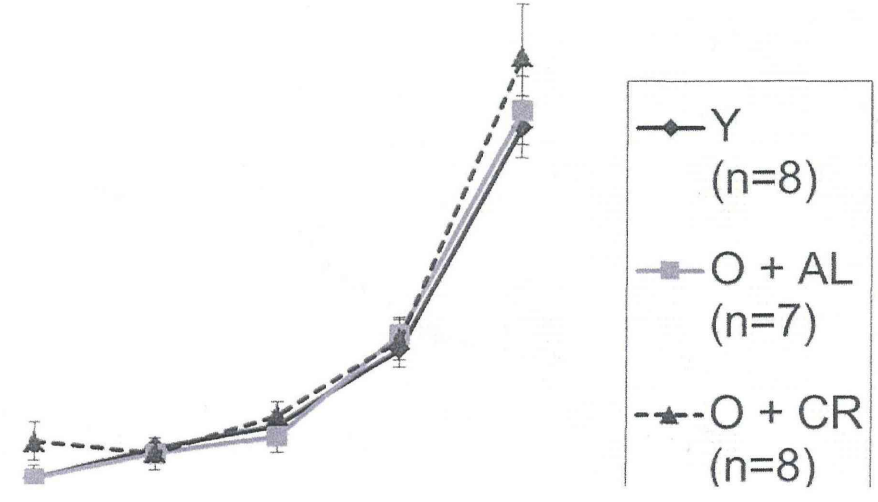
blood urea nitrogen: BUN, high density lipoprotein cholesterol: HDL- Chol, low density lipoprotein cholesterol: LDL- Chol, estradiol: E2 and antidiuretic hormone: ADH



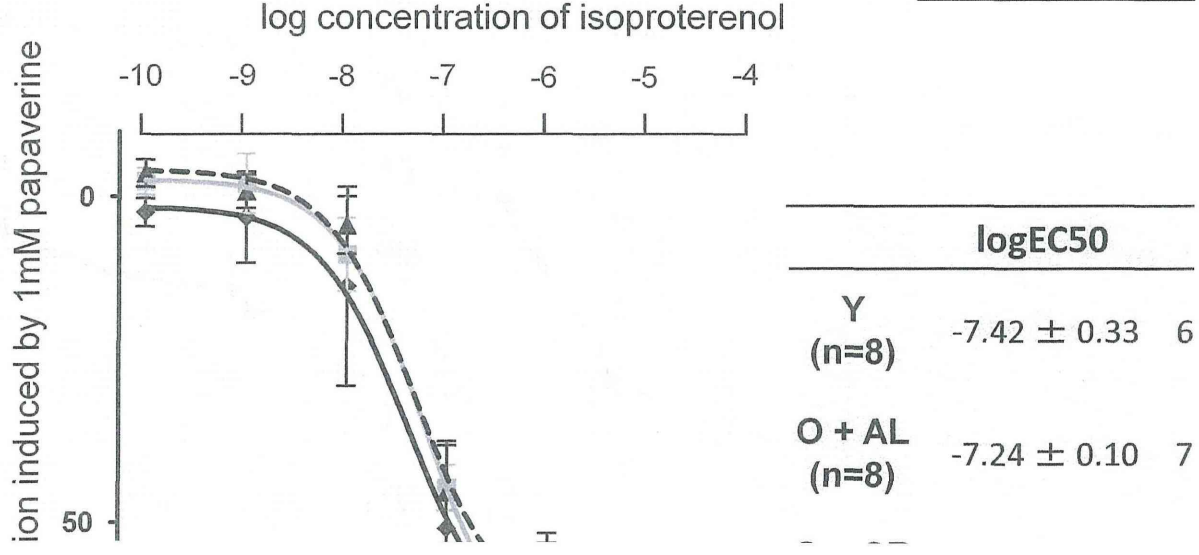


	logEC50	
Y (n=8)	-5.64 ± 0.14	13
O + AL (n=8)	-5.54 ± 0.11	122
O + CR (n=8)	-5.48 ± 0.10	13

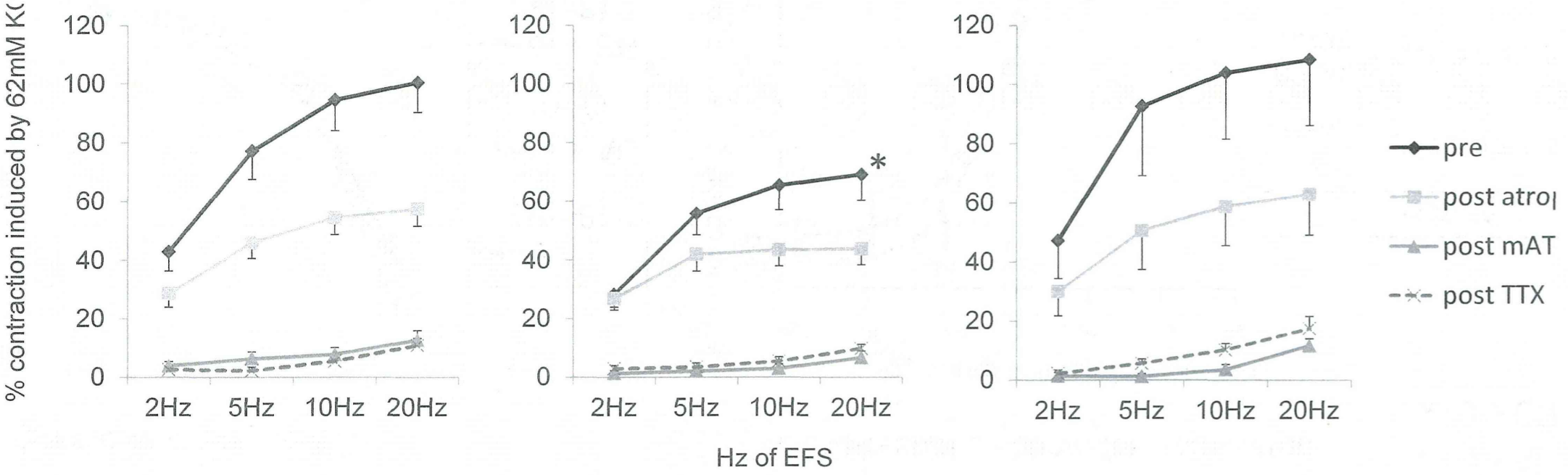
ntractile response to ATP



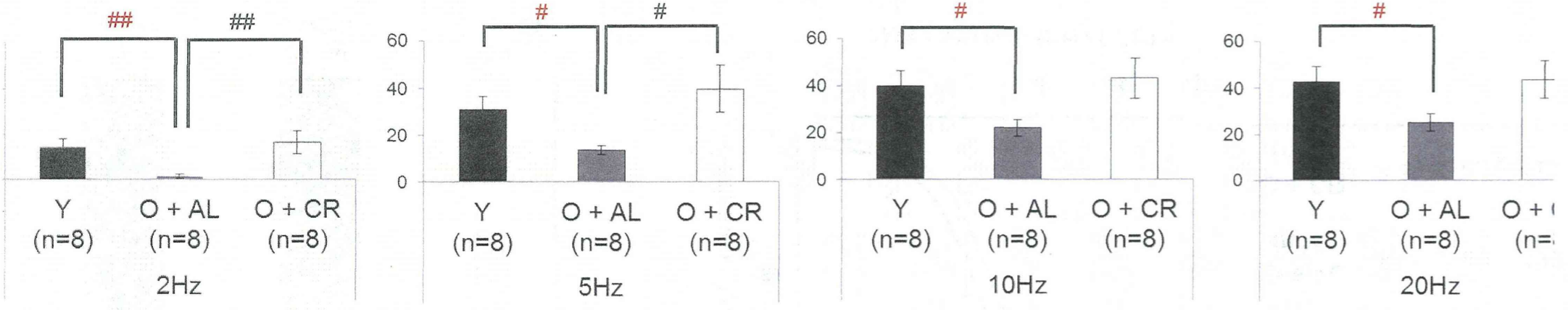
D Dose-response curve for isoproterenol



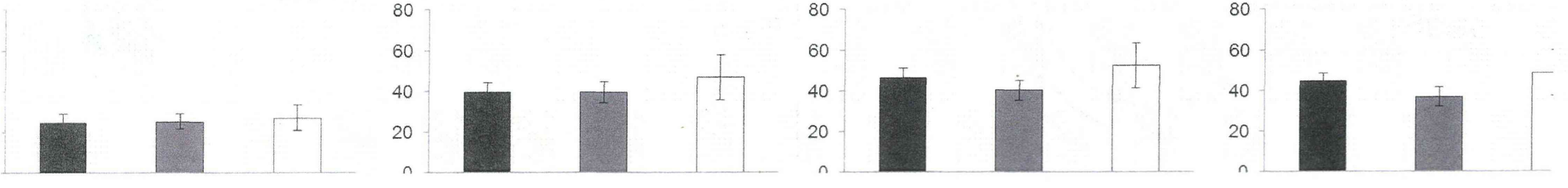
	logEC50	
Y (n=8)	-7.42 ± 0.33	6
O + AL (n=8)	-7.24 ± 0.10	7

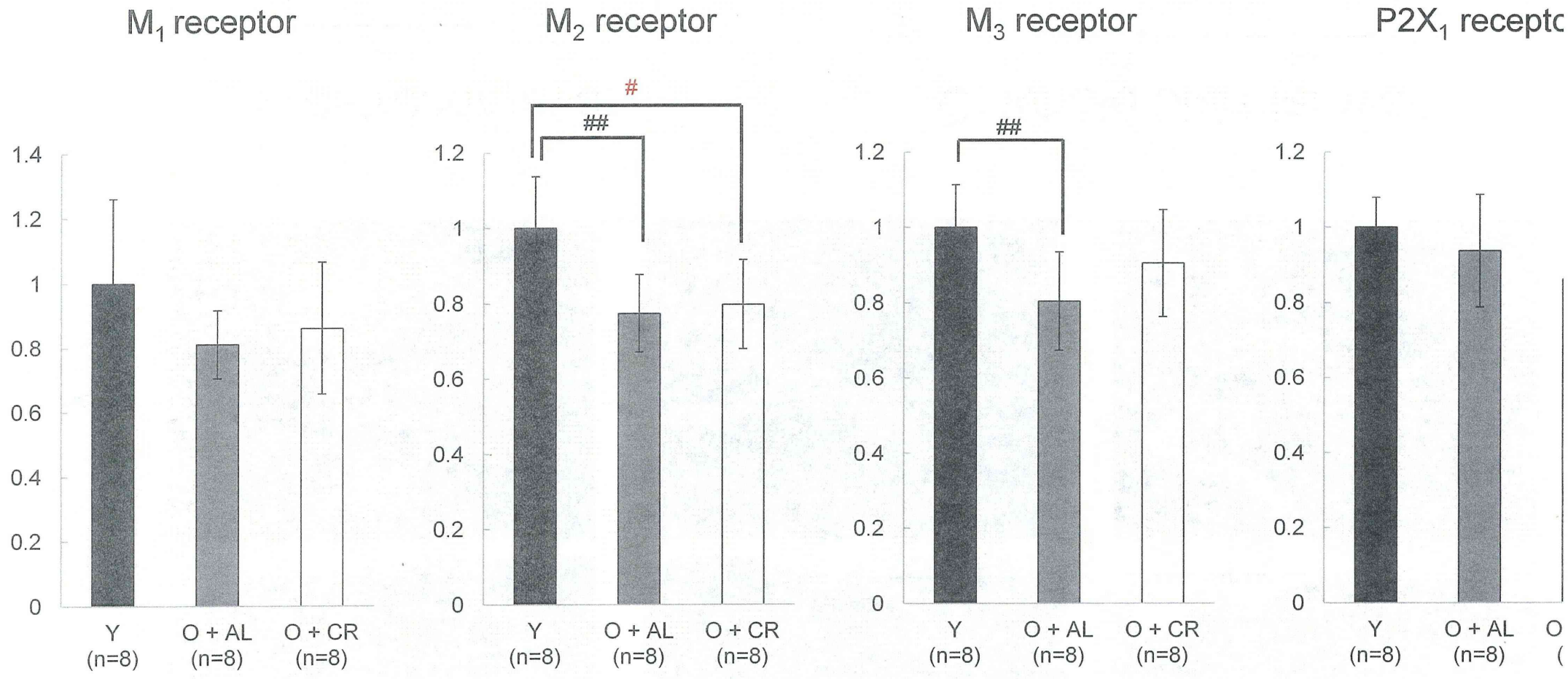


B Atropine sensitive (cholinergic) component



C Purinoceptor desensitization sensitive (purinergic) component




M1 receptor
M2 receptor
M3 receptor
P2X1 receptc
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##
##
1.4
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Y (n=8)
O + AL (n=8)
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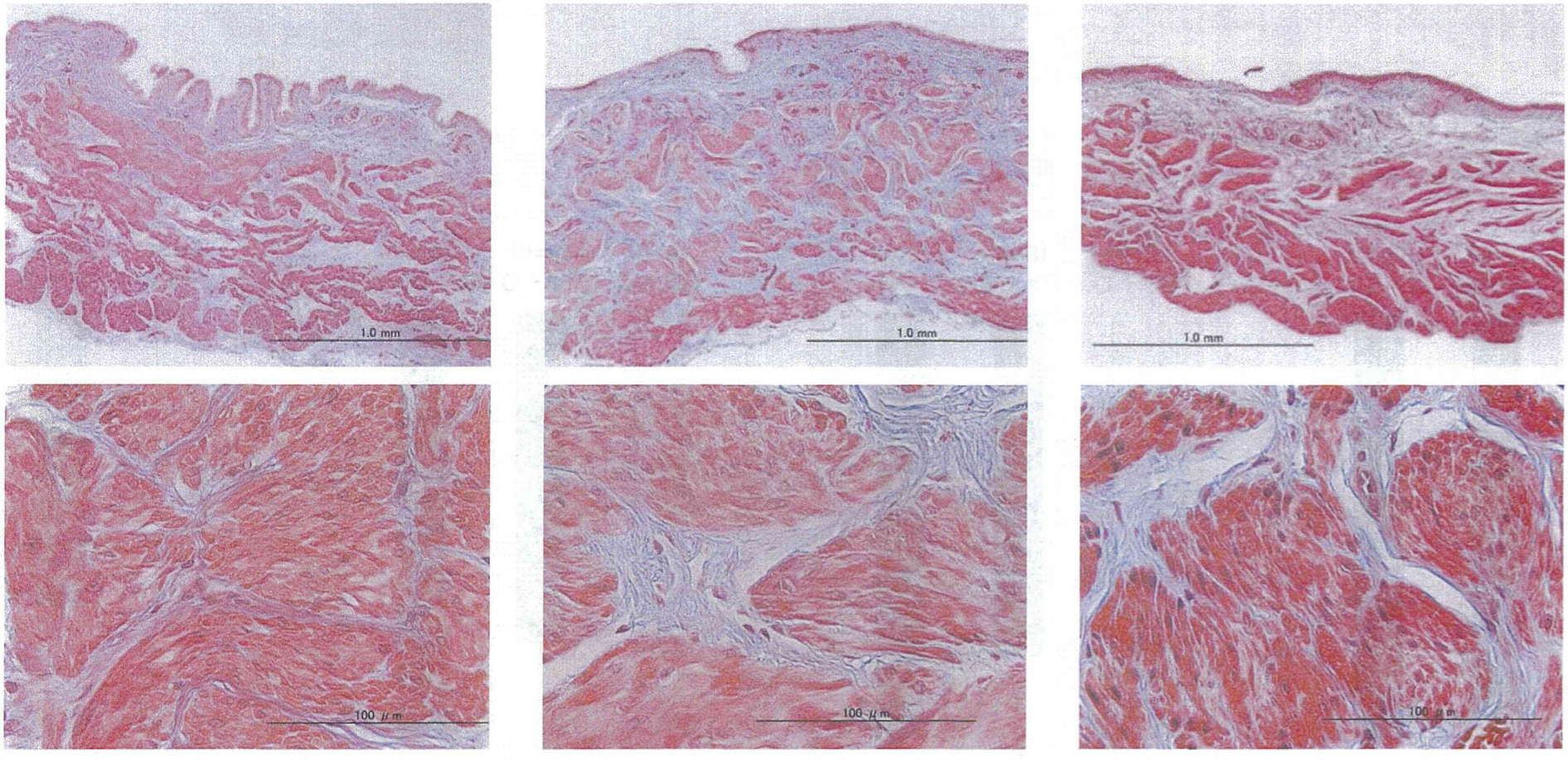

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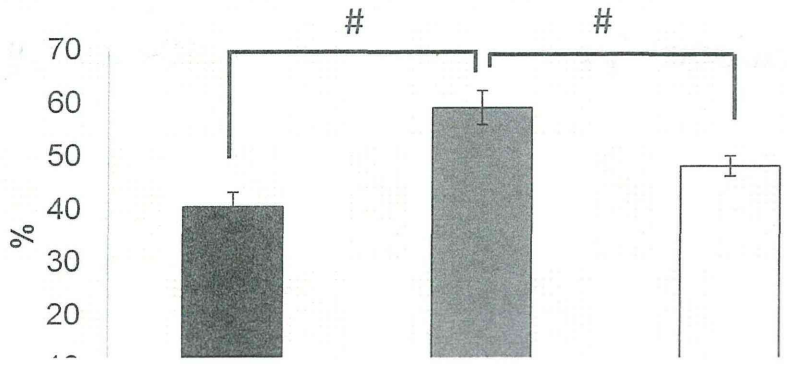

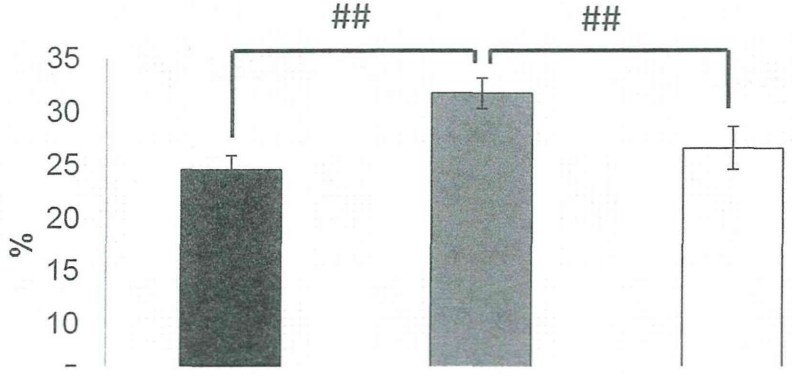

ABBREVIATIONS

ADH = antidiuretic hormone

ATP = adenosine triphosphate

BUN = blood urea nitrogen

CCh = carbachol

CR = caloric restriction

EFS = electrical field stimulation

E2 = estradiol

FV = frequency volume

HDL- Chol = high density lipoprotein cholesterol

LDL - Chol = low density lipoprotein cholesterol

M_1 = muscarinic 1

M_2 = muscarinic 2

M_3 = muscarinic 3

mATP = α,β-Methylene-ATP

N = number of animals

n = number of detrusor strips

O+AL = old ad libitum fed with normal food

O+CR = old with calorie restriction

RT-PCR= reverse transcription polymerase chain reaction

SEM = standard error of the mean

TTX = tetrodotoxin

Y = young

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Original Article

OXFORD

Original Article

Combination of docetaxel, ifosfamide and cisplatin (DIP) as a potential salvage chemotherapy for metastatic urothelial carcinoma

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Abstract

Objective: The aim of this study was to evaluate the efficacy and toxicity of the combination of docetaxel, ifosfamide and cisplatin as salvage chemotherapy after failure of standard cisplatin-based regimens for metastatic urothelial carcinoma.

Methods: We prospectively administered docetaxel, ifosfamide and cisplatin chemotherapy to patients with metastatic urothelial carcinoma refractory to standard cisplatin-based regimens from 2003 to 2013. Patients who had received only adjuvant and/or neoadjuvant chemotherapy were excluded. Eligible patients received every 28 days docetaxel 60 mg/m^2 on Day 1, ifosfamide 1.0 g/m^2 on Days 2–6 and cisplatin 20 mg/m^2 on Days 2–6. The primary endpoints were progression-free survival and overall survival, calculated from the start of docetaxel, ifosfamide and cisplatin chemotherapy. Secondary endpoints included objective response and related toxicity.

Results: Twenty-six cases received a median of 3.0 cycles of docetaxel, ifosfamide and cisplatin chemotherapy (interquartile range: 2–5), resulting in a median progression-free survival of 3 months (interquartile range: 2–9.5 months) and median overall survival of 8.5 months (interquartile range: 6.5–18.75 months), respectively. Of 26 patients, seven (27%) achieved major treatment responses, with one complete response (4%) and six partial responses (23%). Most of Grade 3/4 toxicities were hematologic events, including leukopenia (77%), anemia (54%) and thrombocytopenia (46%). No death from toxicity was observed.

Conclusions: Our results indicate that docetaxel, ifosfamide and cisplatin chemotherapy is a tolerable and moderately active regimen for metastatic urothelial carcinoma after failure of standard cisplatin-based regimens.

Key words: bladder cancer, urothelial carcinoma, metastatic urothelial carcinoma, salvage chemotherapy