

Supplementary data to:

**THE THERAPEUTIC POTENTIAL OF LOSARTAN IN LUNG
METASTASIS OF COLORECTAL CANCER**

Milad Hashemzahi^{1,2*}, Niloufar Naghibzadeh^{2*}, Fereshteh Asgharzadeh^{1,2*}, Asma Mostafapour³, Seyed Mahdi Hassanian³, Gordon A. Ferns⁵, William C. Cho⁶, Amir Avan^{2,3,4,#}, Majid Khazaei^{2,3,#}

¹ Department of Medical Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

² Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

³ Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

⁴ Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

⁵ Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK

⁶ Department of Clinical Oncology, Queen Elizabeth Hospital, Kowloon, Hong Kong, China

* These authors contributed equally as first author.

Corresponding authors: Majid Khazaei, MD, Ph.D., and Amir Avan, Ph.D. Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; Tel: +98 513 8002298; E-mail: Khazaeim@mums.ac.ir; avana@mums.ac.ir;

<http://dx.doi.org/10.17179/excli2020-2093>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>).

Supplementary Table 1: Body weight, Lung weight, and Lung to body weight ratio in treatment groups

Group	Body weight (g)	Lung weight (g)	Lung to body weight ratio
Control, Mouse 1	15.53	0.18	0.01159047005795235
Control, Mouse 2	16.22	0.19	0.011713933415536375
Control, Mouse 3	16.33	0.17	0.01041028781383956
Control, Mouse 4	26.81	0.21	0.007832898172323759
Control, Mouse 5	25.22	0.25	0.009912767644726409
5-FU, Mouse 1	17.33	0.13	0.007501442585112522
5-FU, Mouse 2	19.0	0.17	0.008947368421052633
5-FU, Mouse 3	14.35	0.13	0.009059233449477353
Losartan, Mouse 1	29.3	0.14	0.00506168933881683
Losartan, Mouse 2	26.12	0.12	0.006846556584776481
Losartan, Mouse 3	28.6	0.16	0.005361930294906167
Losartan, Mouse 4	28.39	0.16	0.004750593824228029
Losartan+5-FU, Mouse 1	31.61	0.16	0.004778156996587031
Losartan+5-FU, Mouse 2	24.83	0.17	0.004594180704441041
Losartan+5-FU, Mouse 3	26.11	0.14	0.005594405594405594
Losartan+5-FU, Mouse 4	29.47	0.14	0.005635787249031349

5-FU, Fluorouracil; Conc, concentration; g, gram of tissue; Four experimental groups comprised: a group untreated with either 5-FU or losartan; a 5-FU group that received 5 mg/kg/every other day, administered intraperitoneally (ip); a losartan group received 90 mg/kg/day; ip; a combination group that received 5-FU (5 mg/kg/every other day; ip) plus (losartan 90 mg/kg/day; ip). [Raw data to Figure 1, main document]

Supplementary Table 2: Number of lung metastatic in treatment groups

Group	Number of lung metastatic
Control, Mouse 1	23.0
Control, Mouse 2	22.0
Control, Mouse 3	28.0
5-FU, Mouse 1	6.0
5-FU, Mouse 2	5.0
5-FU, Mouse 3	4.0
Losartan, Mouse 1	4
Losartan, Mouse 2	2
Losartan, Mouse 3	1
Losartan, Mouse 4	3
Losartan+5-FU, Mouse 1	0
Losartan+5-FU, Mouse 2	1

5-FU, Fluorouracil; Conc, concentration; g, gram of tissue; Four experimental groups comprised: a group untreated with either 5-FU or losartan; a 5-FU group that received 5 mg/kg/every other day, administered intraperitoneally (ip); a losartan group received 90 mg/kg/day; ip; a combination group that received 5-FU (5 mg/kg/every other day; ip) plus (losartan 90 mg/kg/day; ip). [Raw data to Figure 2, main document]

Supplementary Table 3: Percent of metastatic area and Number of metastatic nodule in treatment groups

Group	Percent of metastatic area	Number of metastatic nodule
Control, Mouse 1	1.159047005795235	23.0
Control, Mouse 2	1.1713933415536375	22.0
Control, Mouse 3	1.041028781383956	21.0
Control, Mouse 4	0.7832898172323759	
Control, Mouse 5	0.9912767644726409	
5-FU, Mouse 1	0.7501442585112522	9.0
5-FU, Mouse 2	0.8947368421052633	8.0
5-FU, Mouse 3	0.9059233449477353	7.0
Losartan, Mouse 1	0.506168933881683	
Losartan, Mouse 2	0.6846556584776481	
Losartan, Mouse 3	0.5361930294906168	
Losartan, Mouse 4	0.4750593824228029	
Losartan+5-FU, Mouse 1	0.47781569965870313	
Losartan+5-FU, Mouse 2	0.4594180704441041	
Losartan+5-FU, Mouse 3	0.5594405594405595	
Losartan+5-FU, Mouse 4	0.5635787249031349	

5-FU, Fluorouracil; Conc, concentration; g, gram of tissue; Four experimental groups comprised: a group untreated with either 5-FU or losartan; a 5-FU group that received 5 mg/kg/every other day, administered intraperitoneally (ip); a losartan group received 90 mg/kg/day; ip; a combination group that received 5-FU (5 mg/kg/every other day; ip) plus (losartan 90 mg/kg/day; ip). [Raw data to Figure 3, main document]

Supplementary Table 4: Oxidative stress markers

Group	MDA	Thiol	SOD	CAT
Control, Mouse 1	7.13	1.15	2.01	3.25
Control, Mouse 2	7.58	1.16	1.89	3.19
Control, Mouse 3	7.94	1.27	1.62	3.71
5-FU, Mouse 1	8.36	0.88	1.01	2.25
5-FU, Mouse 2	9.26	0.76	1.12	2.51
5-FU, Mouse 3	9.22	0.74	1.08	2.35
Losartan, Mouse 1	10.38	0.61	0.29921	1.82
Losartan, Mouse 2	10.67	0.63	0.45	1.72
Losartan, Mouse 3	11.29	0.59	0.52	1.9
Losartan+5-FU, Mouse 1	12.03	0.41	0.41	1.15
Losartan+5-FU, Mouse 2	12.83	0.39	0.315696	1.21
Losartan+5-FU, Mouse 3	13	0.52	0.145053	1.19

Malon dialdehyde: MDA; Superoxide dismutase activity: SOD; Catalase activity: CAT; Total thiol group: Thio; 5-FU, Fluorouracil; Conc, concentration; g, gram of tissue; Four experimental groups comprised: a group untreated with either 5-FU or losartan; a 5-FU group that received 5 mg/kg/every other day, administered intraperitoneally (ip); a losartan group received 90 mg/kg/day; ip; a combination group that received 5-FU (5 mg/kg/every other day; ip) plus (losartan 90 mg/kg/day; ip). [(Raw data to Figure 4, main document)]

Supplementary Table 5: IL-6 levels in treatment groups

Group	OD	Conc. pg/mL	g tissue	Conc. pg/g tissue
Control, Mouse 1	0.68	114.40	0.04	2790.24
Control, Mouse 2	0.553	93.57	0.04	2599.22
Control, Mouse 3	0.545	92.26	0.03	3075.33
Control, Mouse 4	0.534	90.46	0.03	3015.20
Average	0.578	97.67		2870.00
5-FU, Mouse 1	0.626	105.54	0.07	1507.77
5-FU, Mouse 2	0.571	96.52	0.04	2413.10
5-FU, Mouse 3	0.566	95.70	0.04	2392.60
5-FU, Mouse 4	0.6	101.28	0.03	3376.00
Average	0.59075	99.76		2422.37
Losartan, Mouse 1	0.657	110.63	0.05	2212.56
Losartan, Mouse 2	0.558	94.39	0.08	1179.90
Losartan, Mouse 3	0.659	110.96	0.10	1109.56
Losartan, Mouse 4	0.621	104.72	0.06	1745.40
	0.62375	105.18		1561.86
Losartan+5-FU, Mouse 1	0.682	114.73	0.10	1147.28
Losartan+5-FU, Mouse 2	0.549	92.92	0.10	929.16
Losartan+5-FU, Mouse 3	0.5	84.88	0.10	848.80
Losartan+5-FU, Mouse 4	0.6	101.28	0.10	1012.80
Average	0.58275	98.45		984.51

5-FU, Fluorouracil; Conc, concentration; g, gram of tissue; OD, optical density; IL-6 measurements in lung tissue were performed using Zellbio ELISA kits [Raw data to Figure 5A, main document]

Supplementary Table 6: VEGF levels in treatment groups

Group	OD	Conc. pg/mL	g tissue	Conc. pg/g tissue
Control, Mouse 1	0.379	84.42	0.04	2059.02
Control, Mouse 2	0.381	84.98	0.04	2360.51
Control, Mouse 3	0.437	100.62	0.03	3354.07
Control, Mouse 4	0.346	75.20	0.03	2506.70
Average	0.38575	86.31		2570.07
5-FU, Mouse 1	0.464	108.16	0.07	1545.21
5-FU, Mouse 2	0.396	89.17	0.04	2229.22
5-FU, Mouse 3	0.438	100.90	0.04	2522.53
5-FU, Mouse 4	0.391	87.77	0.03	2925.73
Average	0.42225	96.50		2305.67
Losartan, Mouse 1	0.447	103.42	0.05	2068.31
Losartan, Mouse 2	0.469	109.56	0.08	1369.51
Losartan, Mouse 3	0.502	118.78	0.10	1187.80
Losartan, Mouse 4	0.447	103.42	0.06	1723.59
Average	0.46625	108.79		1587.30
Losartan+5-FU, Mouse 1	0.372	82.46	0.10	824.64
Losartan+5-FU, Mouse 2	0.525	125.20	0.10	1252.05
Losartan+5-FU, Mouse 3	0.502	118.78	0.10	1187.80
Losartan+5-FU, Mouse 4	0.449	103.97	0.10	1039.74
Average	0.462	107.61		1076.06

5-FU, Fluorouracil; Conc, concentration; g, gram of tissue; OD, optical density; Four experimental groups comprised: a group untreated with either 5-FU or losartan; a 5-FU group that received 5 mg/kg/every other day, administered intraperitoneally (ip); a losartan group received 90 mg/kg/day; ip; a combination group that received 5-FU (5 mg/kg/every other day; ip) plus (losartan 90 mg/kg/day; ip). VEGF/VEGFR-1 measurements in lung tissue were performed using Zellbio ELISA kits according to the manufacturer's protocol. [Raw data to Figure 5B, main document]

Supplementary Table 7: VEGFR level in treatment groups

Group	OD	Conc. pg/mL	g tissue	Conc. pg/g tissue
Control, Mouse 1	0.469	34.47	0.04	840.7
Control, Mouse 2	0.448	30.49	0.04	847.1
Control, Mouse 3	0.496	39.57	0.03	1319.1
Control, Mouse 4	0.474	35.41	0.03	1180.4
Average	0.47175	34.99		1046.8
5-FU, Mouse 1	0.422	25.58	0.07	365.4
5-FU, Mouse 2	0.615	62.09	0.04	1552.1
5-FU, Mouse 3	0.48	36.55	0.04	913.7
5-FU, Mouse 4	0.475	35.60	0.03	1186.7
Average	0.498	39.95		1004.5
Losartan, Mouse 1	0.621	63.22	0.05	1264.4
Losartan, Mouse 2	0.6	59.25	0.08	740.6
Losartan, Mouse 3	0.578	55.09	0.10	550.9
Losartan, Mouse 4	0.52	44.11	0.06	735.2
	0.57975	55.42		822.8
Losartan+5-FU, Mouse 1	0.836	103.89	0.10	1038.9
Losartan+5-FU, Mouse 2	0.626	64.17	0.10	641.7
Losartan+5-FU, Mouse 3	0.469	34.47	0.10	344.7
Losartan+5-FU, Mouse 4	0.472	35.03	0.10	350.3
Average	0.60075	59.39		593.9

5-FU, Fluorouracil; Conc, concentration; g, gram of tissue; OD, optical density; Four experimental groups comprised: a group untreated with either 5-FU or losartan; a 5-FU group that received 5mg/kg/every other day, administered intraperitoneally (ip); a Losartan group received 90 mg/kg/day; ip; a combination group that received 5-FU (5mg/kg/every other day; ip) plus (Losartan 90 mg/kg/day; ip). VEGF / VEGFR-1 measurements in lung tissue were performed using Zellbio ELISA kits according to the manufacturer's protocol. [Raw data to Figure 5C, main document]