Supplementary Material

Hydrogen peroxide-induced oxidative stress activates NF-κB and Nrf2/keap1 signals and triggers autophagy in piglets

Table S1.

Oxidative stress parameters in serum after exposure to H_2O_2. The measured variables include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase, total antioxidant capability (T-AOC), malondialdeyhde (MDA), OH•, H_2O_2. These parameters in serum were measured at 2, 50, 98, and 170 h after administration of H_2O_2. The details of methodology are described under Materials and methods. Data are presented as mean ± SE. The values having different superscript letters were significantly different ($P < 0.05$; $n = 6$).

Item	control group	gas-5% H_2O_2	gas-10% H_2O_2	per-10% H_2O_2
2 h (0 d)				
SOD (U/mgprot)	126.42±4.43	126.18±3.20	131.60±5.55	
MDA （nmol/mgprot）	3.22±0.25	2.75±0.36	2.76±0.52	
GSH-Px （U/mgprot）	58.10±4.37	52.14±6.64	59.05±7.80	
catalase （U/mgprot）	4.99±0.55	5.35±1.04	4.95±0.74	
T-AOC （U/mgprot）	3.05±0.23	2.84±0.44	3.21±0.25	
OH· （U/mgprot）	1464.46±70.13	1491.62±74.19	1228.53±51.39	
H_2O_2 (mmol/gprot)	18.70±4.92	16.17±2.38	18.21±4.38	
50 h (2 d)				
SOD（U/mgprot）	122.94±4.40	127.88±4.86	128.23±10.18	
MDA （nmol/mgprot）	2.88±0.36	2.54±0.40	2.56±0.19	
GSH-Px （U/mgprot）	75.62±5.04b	87.43±6.18ab	95.71±5.49a	
catalase （U/mgprot）	5.89±0.95a	2.79±0.54b	1.72±0.27b	
T-AOC （U/mgprot）	3.73±0.59	2.98±0.49	2.50±0.28	
OH· （U/mgprot）	1337.18±55.59	1242.11±54.46	1174.01±72.73	
H_2O_2 (mmol/gprot)	23.70±3.13	20.13±2.15	20.09±0.52	
98 h (4 d)				
SOD（U/mgprot）	112.69±2.28	110.21±9.65	121.98±4.96	
MDA （nmol/mgprot）	2.62±0.20	2.36±0.39	3.05±0.32	
GSH-Px （U/mgprot）	98.10±1.90a	98.10±3.01a	85.52±3.49b	
catalase （U/mgprot）	4.02±1.92	6.74±1.97	5.96±1.39	
T-AOC （U/mgprot）	3.33±1.03	2.74±0.22	2.66±0.48	
OH· （U/mgprot）	1218.83±68.68	2219.10±596.00	1880.14±378.64	
H_2O_2 (mmol/gprot)	13.22±6.08	14.59±6.02	34.63±15.32	
170 h (7 d)				
SOD（U/mgprot）	116.17±11.27	118.66±7.70	118.12±5.50	105.68±2.76
MDA （nmol/mgprot）	2.40±0.38	2.66±0.26	2.76±0.40	1.97±0.15
GSH-Px （U/mgprot）	89.52±5.08a	96.35±5.15a	64.00±7.93b	83.57±1.31a

catalase （U/mgprot）	3.13±0.61ab	5.13±0.87a	3.90±0.98a	0.99±0.08b
T-AOC （U/mgprot）	2.20±0.30	1.89±0.18	1.65±0.37	1.65±0.15
OH· （U/mgprot）	865.17±82.86b	1209.28±92.10a	1235.71±99.23a	941.19±94.491b
H_2O_2 (mmol/gprot)	5.73±0.87	20.30±1.38	15.76±2.84	15.88±1.58

Table S2.

Oxidative stress parameters in the greater curvature (A) and jejunum (B) after exposure to H_2O_2. The measured variables include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase, total antioxidant capability (T-AOC), malondialdeyhde (MDA), OH•, H_2O_2. These parameters were measured at 7 d (170 h) after administration of H_2O_2. The details of methodology are described under Materials and methods. Data are presented as mean ± SE. The values having different superscript letters were significantly different ($P < 0.05$; $n = 6$).

A

Item	control group	gas-5% H_2O_2	gas-10% H_2O_2
SOD（U/mgprot）	90.04±2.25b	103.27±2.05a	80.83±3.34b
MDA （nmol/mgprot）	0.37±0.02b	0.46±0.01a	0.47±0.02a
GSH-Px （U/mgprot）	26.87±3.77	28.74±3.50	25.31±4.31
catalase （U/mgprot）	1.01±0.07	1.12±0.05	0.95±0.07
T-AOC （U/mgprot）	0.96±0.14a	0.34±0.14b	0.55±0.08b
OH^- （U/mgprot）	177.38±13.27b	244.87±12.01ab	301.78±33.80a
H_2O_2 (mmol/gprot)	2.36±0.16	1.60±0.25	2.07±0.51

B

Item	control group	gas-5% H_2O_2	gas-10%H_2O_2	per-10%H_2O_2
SOD（U/mgprot）	51.31±3.18	49.46±1.52	53.17±2.18	50.10±0.51
MDA （nmol/mgprot）	0.33±0.02b	0.37±0.03ab	0.48±0.06a	0.30±0.06b
GSH-Px （U/mgprot）	14.35±1.68	14.04±1.04	10.53±0.76	12.09±2.09
catalase （U/mgprot）	0.71±0.08	0.67±0.05	0.68±0.08	0.65±0.06
T-AOC （U/mgprot）	0.65±0.11a	0.33±0.01b	0.34±0.05b	0.41±0.03b
OH^- （U/mgprot）	203.71±7.95	216.30±5.49	207.22±8.12	225.90±7.06
H_2O_2 (mmol/gprot)	3.77±0.43	2.99±0.12	3.39±0.67	2.62±0.64

Table S3.

The intestinal villus height and crypt depth after exposure to H_2O_2. The duodenal, jejunal, and ileal villus height and crypt depth were measured at 7 d (170 h) after administration of H_2O_2. The details of methodology are described under Materials and methods. Data are presented as mean ± SE. The values having different superscript letters were significantly different ($P < 0.05$; n = 6).

Item	control group	gas-5% H_2O_2	gas-10% H_2O_2	per-10% H_2O_2
duodenal villus height (um)	429.00±22.01	405.50±18.53	399.22±34.58	387.92±23.34
duodenal crypt depth (um)	152.02±11.88	123.00±11.18	141.53±16.30	134.45±10.95
duodenal V/C	2.90±0.28	2.87±0.23	2.89±0.21	2.80±0.32
jejunal villus height (um)	332.57±13.00b	365.90±120.02a	372.86±23.65a	342.14±20.45ab
jejunal crypt depth (um)	182.50±17.37	163.27±15.57	168.08±22.56	164.42±10.69
jejunal V/C	1.97±0.02	2.31±0.14	1.94±0.22	2.03±0.19
ileal villus height (um)	257.28±9.17b	307.94±10.09a	302.48±14.40a	281.40±14.48ab
ileal crypt depth (um)	104.54±11.23	125.07±9.07	96.70±11.29	129.86±18.96
ileal V/C	2.43±0.22	2.56±0.23	2.91±0.34	2.34±0.34

Table S4.

Gene expression of occludin and ZO-1 in the pylorus, great curvature, duodenum, jejunum, and ileum after exposure to H_2O_2 at 7 d (170 h). The details of RT-PCR are described under Materials and methods. Data are presented as mean ± SE. The values having different superscript letters were significantly different ($P < 0.05$; n = 6)

Item	control group	gas-5% H_2O_2	gas-10% H_2O_2	per-10% H_2O_2
great curvature				
Occludin	1.00±0.20a	0.51±0.06b	0.45±0.07b	
ZO-1	1.00±0.13	0.73±0.12	0.82±0.22	
pylorus				
Occludin	1.00±0.20	1.31±0.14	1.09±0.16	
ZO-1	1.00±0.12b	1.18±0.08ab	1.44±0.18a	
duodenum				
Occludin	1.00±0.15b	1.58±0.18a	1.03±0.11b	1.20±0.17b
ZO-1	1.00±0.09b	1.33±0.10a	0.57±0.10c	1.05±0.13ab
ileum				
Occludin	1.00±0.19a	0.30±0.07b	0.22±0.02b	0.40±0.10b
ZO-1	1.00±0.16a	0.54±0.05b	0.63±0.06b	0.80±0.10ab

Table S5.

Gene expression of LC3 and beclin1 in the pylorus, great curvature, duodenum, jejunum, and ileum after exposure to H_2O_2 at 7 d (170 h). The details of RT-PCR are described under Materials and methods. Data are presented as mean ± SE. The values having different superscript letters were significantly different ($P < 0.05$; n = 6)

Item	control group	gas-5% H_2O_2	gas-10% H_2O_2	per-10% H_2O_2
great curvature				
LC3	1.00±0.13	0.93±0.09	1.26±0.31	
Beclin1	1.00±0.16	0.65±0.09	1.34±0.29	
pylorus				
LC3	1.00±0.10	1.36±0.12	1.15±0.17	
Beclin1	1.00±0.12	0.83±0.11	0.73±0.06	
duodenum				
LC3	1.00±0.14b	2.05±0.48a	0.68±0.07b	1.26±0.39b
Beclin1	1.00±0.15	1.67±0.38	1.21±0.16	1.29±0.21
jejunum				
LC3	1.00±0.43b	2.47±0.72a	1.10±0.40b	2.70±0.73a
Beclin1	1.00±0.09	1.36±0.24	0.98±0.10	1.86±0.23
ileum				
LC3	1.00±0.12a	0.58±0.04b	0.92±0.13a	1.07±0.17a
Beclin1	1.00±0.20a	0.39±0.05b	0.66±0.13b	0.67±0.11b

Table S6.

Proinflammatory cytokines (IL-6, IL-8, IL-17 and TNF-α) gene expression in the pylorus, great curvature, duodenum, jejunum, and ileum were detected with RT-PCR after exposure to H_2O_2 at 7 d (170 h). The details of RT-PCR are described under Materials and methods. Data are presented as mean ± SE. The values having different superscript letters were significantly different ($P < 0.05$; n = 6).

Item	control group	gas-5% H2O2	gas-10% H2O2	per-10%H2O2
great curvature				
IL-6	1.00±0.20a	0.44±0.05b	0.71±0.09ab	
IL-8	1.00±0.06b	3.48±0.36a	3.55±0.58a	
IL-17	1.00±0.43	0.49±0.20	0.26±0.13	
TNF-α	1.00±0.20	1.01±0.06	1.14±0.09	
pylorus				
IL-6	1.00±0.04b	1.85±0.12a	1.65±0.12ab	
IL-8	1.00±0.15	1.11±0.17	1.47±0.45	
IL-17	1.00±0.12	0.82±0.09	0.86±0.18	
TNF-α	1.00±0.20	0.85±0.08	1.06±0.18	
duodenum				
IL-6	1.00±0.07	0.78±0.14	0.99±0.13	0.76±0.08
IL-8	1.00±0.09a	1.02±0.15a	0.88±0.14ab	0.62±0.11b
IL-17	1.00±0.11b	1.99±0.33a	1.52±0.22ab	1.51±0.42ab
TNF-α	1.00±0.13a	1.02±0.11a	0.99±0.08a	0.69±0.07b
ileum				
IL-6	1.00±0.11	0.93±0.12	0.71±0.16	1.11±0.21
IL-8	1.00±0.23a	0.21±0.09b	0.40±0.03b	0.43±0.09b
IL-17	1.00±0.14	0.85±0.17	1.27±0.23	0.88±0.11
TNF-α	1.00±0.16b	1.62±0.21ab	2.58±0.30ab	2.26±0.26a

Table S7

PCR primer sequences: the forward primers (F) and the reverse primers (R) used in this study are tabulated along with their accession No. and product length.

Gene	Accession No.	Nucleotide sequence of primers (5′–3′)	Size (bp)
β-Actin	DQ845171.1	F: CTGCGGCATCCACGAAACT	147
		R: AGGGCCGTGATCTCCTTCTG	
MnSOD	NM_214127	F: GGACAAATCTGAGCCCTAACG	159
		R: CCTTGTTGAAACCGAGCC	
CuZnSOD	NM_001190422	F: CAGGTCCTCACTTCAATCC	255
		R: CCAAACGACTTCCASCAT	
GPx1	NM_214201	F: TGGGGAGATCCTGAATTG	183
		R: GATAAACTTGGGGTCGGT	
GPx4	NM_214407.1	F: GATTCTGGCCTTCCCTTGC	172
		R: TCCCCTTGGGCTGGACTTT	
catalase	NM_214301.2	F: CGAAGGCGAAGGTGTTTG	374
		R: AGTGTGCGATCCATATCC	
ZO-1	XM_003353439.2	F: CCTGCTTCTCCAAAAACTCTT	252
		R: TTCTATGGAGCTCAACACCC	
occludin	NM_001163647.2	F: ACGAGCTGGAGGAAGACTGGATC	238
		R: CCCTTAACTTGCTTCAGTCTATTG	
LC3	XM_002728433.2	F: CCGAACCTTCGAACAGAGAG	206
		R: AGGCTTGGTTAGCATTGAGC	
beclin1	NM_001034117.1	F: AGGAGCTGCCGTTGTACTGT	189
		R: CACTGCCTCCTGTGTCTTCA	
IL-6	NM_001252429.1	CAAAGCCACCACCCCTAAC	66
		TCGTTCTGTGACTGCAGCTT	
IL-8	XM_003361958.1	TTCTTCTTTATCCCCAAACTGG	63
		CCACATGTCCTCAAGGTAGGA	
IL-17	NM_001005729.1	CTCTCGTGAAGGCGGGAATC	137
		GTAATCTGAGGGCCGTCTGG	
TNFα	NM_214022.1	TTCCTCACTCACACCATCAGCC	224
		TGCCCAGATTCAGCAAAGTCC	

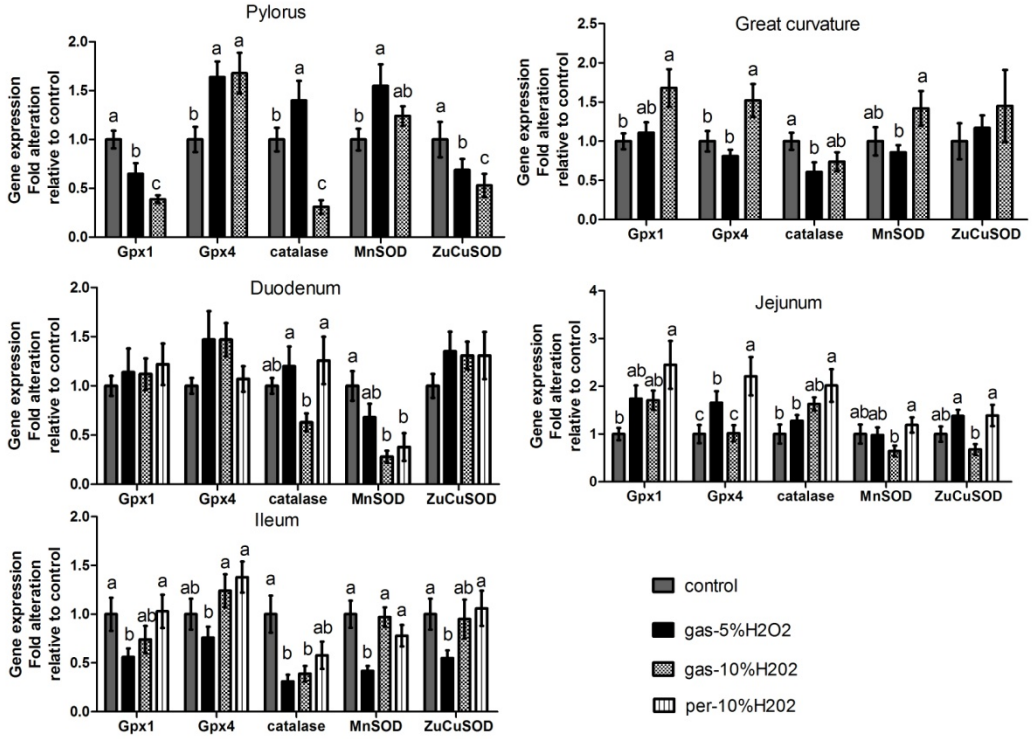

Figure S1. Gene expression of antioxidant enzymes (Gpx1, Gpx4, catalase, MnSOD, and ZnCuSOD) in the pylorus, great curvature, duodenum, jejunum, and ileum after exposure to H_2O_2 at 7 d. The details of RT-PCR are described under Materials and methods. Data are presented as mean ± SE. The values having different superscript letters were significantly different ($P < 0.05$; n = 6).