

The Role of Cellular Proliferation and Apoptosis in Acute Lung Injury: An Experimental Study

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Abstract

Introduction: Acute Lung Injury (ALI) is a widespread damage to cells and structures of the alveolar capillary membrane that occurs within hours to days of a predisposing insult. A comprehensive understanding of the mechanisms of apoptosis in the initial injury and repair of lung epithelial and endothelial cells and other key cells involved in ALI is lacking. Further information is required regarding cytokine-signaling pathways, patterns of gene and protein expression. The present study attempts to correlate various parameters of lung damage and expression of certain proteins that regulate the cell cycle in an experimental model of ALI.

Material and methods: ALI was induced in five groups of mice via inhalation of paraquat. The animals of each group were sacrificed on days 1,2,3,4 and 6, respectively and their lungs were examined for the following parameters: focal thickening of alveolar membranes, capillary congestion, pulmonary oedema, intra-alveolar haemorrhage, interstitial neutrophil infiltration and intra-alveolar neutrophil infiltration scored from 0 to 3 according to the severity of the pathological changes. Specimens were also examined with immunoassay for the quantitative expression (percentile) of Ki-67, TUNEL staining (Todd-mediated dUTP nick end-labeling), p21, p16, p27, Cyclin B, Cyclin D1 and Cyclin E. A control group of animals that were not exposed to paraquat was also studied.

Results: All variables were significantly altered in all the study groups as compared with the control group suggesting the deleterious effect of the paraquat inhalation. Focal thickening of alveolar membranes and intra-alveolar haemorrhage were the more significant pathological changes. Multiple comparisons showed significantly higher mean differences in TUNEL staining and in the expression of ki-67, cyclins and cyclin-dependent kinase inhibitors (CDKIs) particularly after 2-3 days from the paraquat inhalation.

Conclusion: These findings implicate that lung tissue damage is characterized by enhanced cellular proliferation and apoptosis both being regulated by complex interactions of cyclins and CDKIs.

Keywords: Acute lung injury; Apoptosis; Paraquat

Introduction

Acute lung injury (ALI) may be either a consequence of direct causes such as pneumonia, aspiration, pulmonary contusion, fat or air embolism and inhalation injury, or indirect causes such as sepsis, burn, severe trauma, cardiopulmonary bypass, drug overdose and acute pancreatitis [1]. In ALI, widespread damage to cells and structures of the alveolar capillary membrane occurs within hours to days of a predisposing insult. A comprehensive understanding of the mechanisms of apoptosis in the initial injury and repair of lung epithelial and endothelial cells and other key cells involved in ALI is lacking. Further information is required regarding cytokine-signaling pathways, patterns of gene and protein expression, and functional responses in mesenchymal cells that lead to deregulated matrix remodeling and relentless fibrosis [2].

The present study attempts to correlate various parameters of lung damage and expression of certain proteins that regulate the cell cycle in an experimental model of ALI.

Materials and Methods

The experimental protocol was approved by the bioethics committee of the University of Athens. ALI was induced in five groups of mice via inhalation of paraquat [3]. Each group was consisted of 8 animals. Twenty microlitres of PQ solution was applied through the nares. The animals of each group were sacrificed on days 1,2,3,4 and 6, respectively and their lungs were examined for the following parameters: focal thickening of alveolar membranes, capillary congestion, pulmonary

oedema, intra-alveolar haemorrhage, interstitial neutrophil infiltration and intra-alveolar neutrophil infiltration scored from 0 to 3 according to the severity of the pathological changes. Specimens (i.e. lung tissue) were also examined with immunohistochemistry for the quantitative expression (percentile) of Ki-67, TUNEL staining (Todd-mediated dUTP nick end-labeling), p21, p16, p27, Cyclin B, Cyclin D1 and Cyclin E. A control group of eight animals that were not exposed to paraquat was also studied. Normality test of Kolmogorov-Smirnov and equal variances test of Levene indicated that the variables did not meet the parametric requirements and therefore comparisons were made with the non-parametric Kruskal-Wallis test. Furthermore, multiple comparisons between all pairs of variables were performed with the Bonferroni test.

Differences between the groups were considered statistically significant when *P* value was less than 0.05. Statistical analysis was performed using the statistical package SPSS version 12.0.1.

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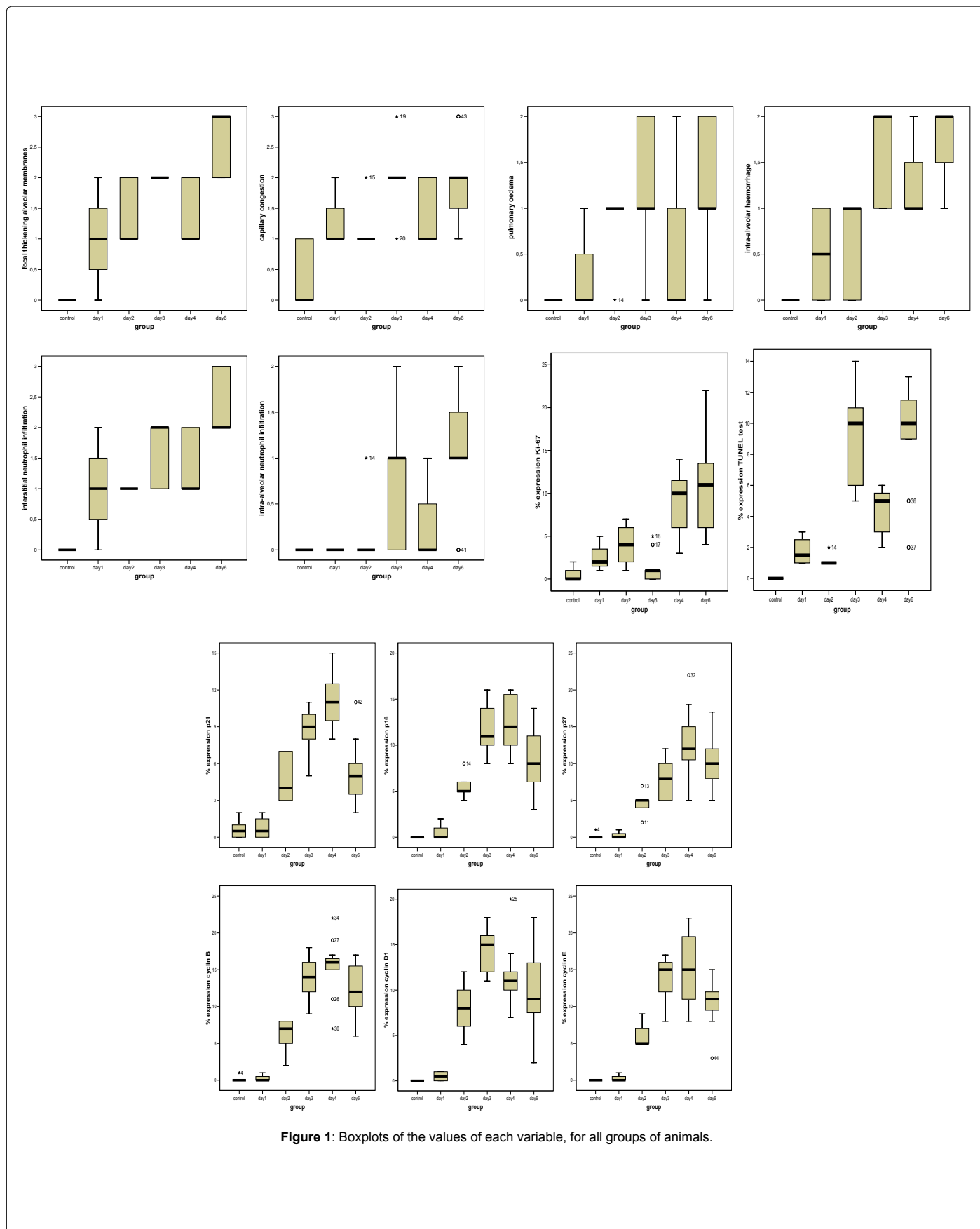


Figure 1: Boxplots of the values of each variable, for all groups of animals.

	Focal thickening of alveolar membranes	Capillary congestion	Pulmonary oedema	Intra-alveolar haemorrhage	Interstitial neutrophil infiltration	Intra-alveolar neutrophil infiltration	Ki-67 %	TUNEL test %	p21 %	p16 %	p27 %	Cyclin B %	Cyclin D1 %	Cyclin E %
Controls	0	0 (0-1)	0	0	0	0	0 (0-2)	0	0.5 (0-2)	0	0 (0-1)	0 (0-1)	0	0
Day 1	1 (0-2)	1 (1-2)	0 (0-1)	0.5 (0-1)	1 (0-2)	0	2 (1-5)	1.5 (1-3)	0.5 (0-2)	0 (0-2)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Day 2	1 (1-2)	1 (1-2)	1 (0-1)	1 (0-1)	1	0 (0-1)	4 (1-7)	1 (1-2)	4 (3-7)	5 (4-8)	5 (2-7)	7 (2-8)	8 (4-12)	5 (5-9)
Day 3	2	2 (1-3)	1 (0-2)	2 (1-2)	2 (1-2)	1 (0-2)	1 (0-5)	10 (5-14)	9 (5-11)	11 (8-16)	8 (5-12)	14 (9-18)	15 (11-18)	15 (8-17)
Day 4	1 (1-2)	1 (1-2)	0 (0-2)	1 (1-2)	1 (1-2)	0 (0-1)	10 (3-12)	5 (2-6)	11 (8-15)	12 (8-16)	12 (5-22)	16 (7-22)	11 (7-20)	15 (8-22)
Day 6	3 (2-3)	2 (1-3)	1 (0-2)	2 (1-2)	2 (2-3)	1 (0-2)	11 (4-22)	10 (2-13)	5 (2-11)	8 (8-14)	10 (5-17)	12 (6-17)	9 (2-18)	11 (3-15)

Table 1: Median and range of values for all variables.

	focal thickening of alveolar membranes	capillary congestion	pulmonary oedema	intra-alveolar haemorrhage	interstitial neutrophil infiltration	intra-alveolar neutrophil infiltration	% expression Ki-67	% expression TUNEL test	% expression p21	% expression p16	% expression p27	% expression cyclin B	% expression cyclin D1	% expression cyclin E
Chi-Square	33,523	21,803	18,236	28,149	31,234	19,196	32,011	36,001	36,042	32,825	33,272	31,454	30,130	32,874
P value	0.000	0.001	0.003	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2: Comparisons of the median values of all variables with the non-parametric Kruskal-Wallis test.

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	P value	
focal thickening alveolar membranes	control	day 1	-1.000	.297	.025	
		day 2	-1.400	.279	.000	
		day 3	-2.000	.243	.000	
		day 4	-1.455	.234	.000	
		day 6	-2.636	.234	.000	
		day 1	control	1.000	.297	.025
	day 1	day 2	-.400	.309	1.000	
		day 3	-1.000	.277	.012	
		day 4	-.455	.269	1.000	
		day 6	-1.636	.269	.000	
		day 2	control	1.400	.279	.000
		day 1	.400	.309	1.000	
	day 2	day 3	-.600	.257	.368	
		day 4	-.055	.248	1.000	
		day 6	-1.236	.248	.000	
		day 3	control	2.000	.243	.000
		day 1	1.000	.277	.012	
		day 2	.600	.257	.368	
	day 3	day 4	.545	.207	.178	
		day 6	-.636	.207	.057	
		day 4	control	1.455	.234	.000
		day 1	.455	.269	1.000	
		day 2	.055	.248	1.000	
		day 3	-.545	.207	.178	
day 4	day 6	-1.182	.196	.000		
	day 6	control	2.636	.234	.000	
	day 1	1.636	.269	.000		
	day 2	1.236	.248	.000		
	day 3	.636	.207	.057		
	day 4	1.182	.196	.000		
capillary congestion	control	day 1	-.917	.374	.279	
		day 2	-.867	.351	.267	
		day 3	-1.778	.305	.000	
		day 4	-1.121	.294	.007	
		day 6	-1.576	.294	.000	
		day 1	control	.917	.374	.279
pulmonary oedema	control	day 2	.050	.388	1.000	
		day 3	1.636	.269	.000	
		day 4	1.236	.248	.000	
		day 6	.636	.207	.057	
		day 1	1.182	.196	.000	
		day 2	-.917	.374	.279	
pulmonary oedema	control	day 1	-.250	.400	1.000	
		day 2	-.800	.375	.587	
		day 3	-1.111	.326	.023	
		day 4	-.455	.314	1.000	
		day 6	-1.273	.314	.003	
		day 1	control	.250	.400	1.000
	day 1	day 2	-.550	.416	1.000	
		day 3	-.861	.372	.389	
		day 4	-.205	.362	1.000	
		day 6	-1.023	.362	.109	
		day 2	control	.800	.375	.587
		day 1	.550	.416	1.000	
	day 2	day 3	-.311	.346	1.000	
		day 4	.345	.334	1.000	
		day 6	-.473	.334	1.000	
		day 3	control	1.111	.326	.023
		day 1	.861	.372	.389	
		day 2	.311	.346	1.000	
	day 3	day 4	.657	.278	.350	
		day 6	1.576	.294	.000	
		day 1	.659	.338	.874	
		day 2	.709	.312	.429	
		day 3	-.202	.260	1.000	
		day 4	.455	.247	1.000	
day 4	day 6	.202	.260	1.000		
	day 1	1.121	.294	.007		
	day 2	.911	.323	.111		
	day 3	.657	.260	.236		
	day 4	.657	.260	.236		
	day 6	-.455	.247	1.000		
day 6	control	1.778	.305	.000		
	day 1	.861	.348	.265		
	day 2	.911	.323	.111		
	day 3	-.911	.323	.111		
	day 4	-.255	.312	1.000		
	day 6	-.709	.312	.429		
day 6	control	1.778	.305	.000		
	day 1	.861	.348	.265		
	day 2	.911	.323	.111		
	day 3	-.911	.323	.111		
	day 4	-.255	.312	1.000		
	day 6	-.709	.312	.429		

		day 6	-.162	.278	1.000
	day 4	control	.455	.314	1.000
		day 1	.205	.362	1.000
		day 2	-.345	.334	1.000
		day 3	-.657	.278	.350
		day 6	-.818	.264	.053
	day 6	control	1.273	.314	.003
		day 1	1.023	.362	.109
		day 2	.473	.334	1.000
		day 3	.162	.278	1.000
		day 4	.818	.264	.053
intra-alveolar haemorrhage	control	day 1	-.500	.303	1.000
		day 2	-.600	.284	.612
		day 3	-1.556	.247	.000
		day 4	-1.273	.238	.000
		day 6	-1.727	.238	.000
	day 1	control	.500	.303	1.000
		day 2	-.100	.314	1.000
		day 3	-1.056	.282	.008
		day 4	-.773	.274	.110
		day 6	-1.227	.274	.001
	day 2	control	.600	.284	.612
		day 1	.100	.314	1.000
		day 3	-.956	.261	.011
		day 4	-.673	.253	.167
		day 6	-1.127	.253	.001
	day 3	control	1.556	.247	.000
		day 1	1.056	.282	.008
		day 2	.956	.261	.011
		day 4	.283	.211	1.000
		day 6	-.172	.211	1.000
	day 4	control	1.273	.238	.000
		day 1	.773	.274	.110
		day 2	.673	.253	.167
		day 3	-.283	.211	1.000
		day 6	-.455	.200	.426
	day 6	control	1.727	.238	.000
		day 1	1.227	.274	.001
		day 2	1.127	.253	.001
		day 3	.172	.211	1.000
		day 4	.455	.200	.426
interstitial neutrophil infiltration	control	day 1	-1.000(*)	.311	.039
		day 2	-1.000	.292	.022
		day 3	-1.556	.254	.000
		day 4	-1.364	.245	.000
		day 6	-2.364	.245	.000
	day 1	control	1.000	.311	.039
		day 2	.000	.324	1.000
		day 3	-.556	.290	.938
		day 4	-.364	.282	1.000
		day 6	-1.364	.282	.000
	day 2	control	1.000	.292	.022
		day 1	.000	.324	1.000
		day 3	-.556	.269	.683
		day 4	-.364	.260	1.000
		day 6	-1.364	.260	.000
	day 3	control	1.556	.254	.000

		day 1	.556	.290	.938
		day 2	.556	.269	.683
		day 4	.192	.217	1.000
		day 6	-.808	.217	.009
	day 4	control	1.364	.245	.000
		day 1	.364	.282	1.000
		day 2	.364	.260	1.000
		day 3	-.192	.217	1.000
		day 6	-1.000	.206	.000
	day 6	control	2.364	.245	.000
		day 1	1.364	.282	.000
		day 2	1.364	.260	.000
		day 3	.808	.217	.009
		day 4	1.000	.206	.000
			.000	.345	1.000
intra-alveolar neutrophil infiltration	control	day 1			
		day 2	-.200	.324	1.000
		day 3	-.778	.282	.131
		day 4	-.273	.271	1.000
		day 6	-1.091	.271	.004
	day 1	control	.000	.345	1.000
		day 2	-.200	.359	1.000
		day 3	-.778	.321	.303
		day 4	-.273	.312	1.000
		day 6	-1.091	.312	.018
	day 2	control	.200	.324	1.000
		day 1	.200	.359	1.000
		day 3	-.578	.298	.899
		day 4	-.073	.289	1.000
		day 6	-.891	.289	.055
	day 3	control	.778	.282	.131
		day 1	.778	.321	.303
		day 2	.578	.298	.899
		day 4	.505	.240	.630
		day 6	-.313	.240	1.000
	day 4	control	.273	.271	1.000
		day 1	.273	.312	1.000
		day 2	.073	.289	1.000
		day 3	-.505	.240	.630
		day 6	-.818	.228	.014
	day 6	control	1.091	.271	.004
		day 1	1.091	.312	.018
		day 2	.891	.289	.055
		day 3	.313	.240	1.000
		day 4	.818	.228	.014
% expression Ki-67	control	day 1	-2.000	2.292	1.000
		day 2	-3.500	2.150	1.000
		day 3	-.944	1.871	1.000
		day 4	-8.409	1.802	.001
		day 6	-10.136	1.802	.000
	day 1	control	2.000	2.292	1.000
		day 2	-1.500	2.382	1.000
		day 3	1.056	2.133	1.000
		day 4	-6.409	2.073	.054
		day 6	-8.136	2.073	.005
	day 2	control	3.500	2.150	1.000
		day 1	1.500	2.382	1.000
		day 3	2.556	1.980	1.000
		day 4	-4.909	1.915	.213
		day 6	-6.636	1.915	.019

	day 3	control	.944	1.871	1.000
		day 1	-1.056	2.133	1.000
		day 2	-2.556	1.980	1.000
		day 4	-7.465	1.596	.000
		day 6	-9.192	1.596	.000
	day 4	control	8.409	1.802	.001
		day 1	6.409	2.073	.054
		day 2	4.909	1.915	.213
		day 3	7.465	1.596	.000
		day 6	-1.727	1.514	1.000
	day 6	control	10.136	1.802	.000
		day 1	8.136	2.073	.005
		day 2	6.636	1.915	.019
		day 3	9.192	1.596	.000
		day 4	1.727	1.514	1.000
% expression TUNEL test	control	day 1	-1.750	1.480	1.000
		day 2	-1.200	1.389	1.000
		day 3	-9.222	1.209	.000
		day 4	-4.364	1.164	.008
		day 6	-9.455	1.164	.000
	day 1	control	1.750	1.480	1.000
		day 2	.550	1.538	1.000
		day 3	-7.472	1.378	.000
		day 4	-2.614	1.339	.870
		day 6	-7.705	1.339	.000
	day 2	control	1.200	1.389	1.000
		day 1	-.550	1.538	1.000
		day 3	-8.022	1.279	.000
		day 4	-3.164	1.237	.217
		day 6	-8.255	1.237	.000
	day 3	control	9.222	1.209	.000
		day 1	7.472	1.378	.000
		day 2	8.022	1.279	.000
		day 4	4.859	1.031	.000
		day 6	-.232	1.031	1.000
	day 4	control	4.364	1.164	.008
		day 1	2.614	1.339	.870
		day 2	3.164	1.237	.217
		day 3	-4.859	1.031	.000
		day 6	-5.091	.978	.000
	day 6	control	9.455	1.164	.000
		day 1	7.705	1.339	.000
		day 2	8.255	1.237	.000
		day 3	.232	1.031	1.000
		day 4	5.091	.978	.000
% expression p21	control	day 1	-.083	1.319	1.000
		day 2	-4.133	1.237	.027
		day 3	-8.222	1.077	.000
		day 4	-10.515	1.037	.000
		day 6	-4.515	1.037	.001
	day 1	control	.083	1.319	1.000
		day 2	-4.050	1.371	.078
		day 3	-8.139	1.228	.000
		day 4	-10.432	1.193	.000
		day 6	-4.432	1.193	.009
	day 2	control	4.133	1.237	.027
		day 1	4.050	1.371	.078
		day 3	-4.089	1.140	.014
		day 4	-6.382	1.102	.000
		day 6	-.382	1.102	1.000

	day 3	control	8.222	1.077	.000
		day 1	8.139	1.228	.000
		day 2	4.089	1.140	.014
		day 4	-2.293	.919	.252
		day 6	3.707	.919	.004
	day 4	control	10.515	1.037	.000
		day 1	10.432	1.193	.000
		day 2	6.382	1.102	.000
		day 3	2.293	.919	.252
		day 6	6.000	.871	.000
	day 6	control	4.515	1.037	.001
		day 1	4.432	1.193	.009
		day 2	.382	1.102	1.000
		day 3	-3.707	.919	.004
		day 4	-6.000	.871	.000
% expression p16	control	day 1	-.500	1.691	1.000
		day 2	-5.600	1.586	.016
		day 3	-11.778	1.381	.000
		day 4	-12.273	1.329	.000
		day 6	-8.364	1.329	.000
	day 1	control	.500	1.691	1.000
		day 2	-5.100	1.757	.090
		day 3	-11.278	1.574	.000
		day 4	-11.773	1.529	.000
		day 6	-7.864	1.529	.000
	day 2	control	5.600	1.586	.016
		day 1	5.100	1.757	.090
		day 3	-6.178	1.461	.002
		day 4	-6.673	1.413	.000
		day 6	-2.764	1.413	.862
	day 3	control	11.778	1.381	.000
		day 1	11.278	1.574	.000
		day 2	6.178	1.461	.002
		day 4	-.495	1.177	1.000
		day 6	3.414	1.177	.091
	day 4	control	12.273	1.329	.000
		day 1	11.773	1.529	.000
		day 2	6.673	1.413	.000
		day 3	.495	1.177	1.000
		day 6	3.909	1.117	.017
	day 6	control	8.364	1.329	.000
		day 1	7.864	1.529	.000
		day 2	2.764	1.413	.862
		day 3	-3.414	1.177	.091
		day 4	-3.909	1.117	.017
% expression p27	control	day 1	-.083	1.998	1.000
		day 2	-4.433	1.874	.344
		day 3	-7.500	1.631	.001
		day 4	-12.652	1.571	.000
		day 6	-10.288	1.571	.000
	day 1	control	.083	1.998	1.000
		day 2	-4.350	2.076	.638
		day 3	-7.417	1.860	.004
		day 4	-12.568	1.807	.000
		day 6	-10.205	1.807	.000
	day 2	control	4.433	1.874	.344
		day 1	4.350	2.076	.638
		day 3	-3.067	1.726	1.000
		day 4	-8.218	1.669	.000
		day 6	-5.855	1.669	.017

	day 3	control	7.500	1.631	.001
		day 1	7.417	1.860	.004
		day 2	3.067	1.726	1.000
		day 4	-5.152	1.391	.010
		day 6	-2.788	1.391	.778
	day 4	control	12.652	1.571	.000
		day 1	12.568	1.807	.000
		day 2	8.218	1.669	.000
		day 3	5.152	1.391	.010
		day 6	2.364	1.320	1.000
	day 6	control	10.288	1.571	.000
		day 1	10.205	1.807	.000
		day 2	5.855	1.669	.017
		day 3	2.788	1.391	.778
		day 4	-2.364	1.320	1.000
Dependent Variable	(I) group				
% expression cyclin B	control				
		day 2	-5.833	1.858	.048
	day 1				
		day 2	-5.750	2.058	.119
		day 3	-13.528	1.843	.000
		day 4	-15.114	1.791	.000
		day 6	-12.205	1.791	.000
	day 2	control	5.833	1.858	.048
		day 1	5.750	2.058	.119
		day 3	-7.778	1.711	.001
		day 4	-9.364	1.655	.000
		day 6	-6.455	1.655	.005
	day 3	control	13.611	1.617	.000
		day 1	13.528	1.843	.000
		day 2	7.778	1.711	.001
		day 4	-1.586	1.379	1.000
		day 6	1.323	1.379	1.000
	day 4	control	15.197	1.557	.000
		day 1	15.114	1.791	.000
		day 2	9.364	1.655	.000
		day 3	1.586	1.379	1.000
	day 6	day 6	2.909	1.308	.478
		control	12.288	1.557	.000
		day 1	12.205	1.791	.000
		day 2	6.455	1.655	.005
		day 3	-1.323	1.379	1.000
		day 4	-2.909	1.308	.478
% expression cyclin D1	control	day 1	-.500	2.124	1.000
		day 2	-8.000	1.992	.004
		day 3	-14.222	1.734	.000
		day 4	-11.455	1.670	.000
		day 6	-9.818	1.670	.000
	day 1	control	.500	2.124	1.000
		day 2	-7.500	2.207	.023
		day 3	-13.722	1.977	.000
		day 4	-10.955	1.921	.000
		day 6	-9.318	1.921	.000
	day 2	control	8.000	1.992	.004
		day 1	7.500	2.207	.023
		day 3	-6.222	1.835	.024

		day 4	-3.455	1.774	.879
		day 6	-1.818	1.774	1.000
	day 3	control	14.222	1.734	.000
		day 1	13.722	1.977	.000
		day 2	6.222	1.835	.024
		day 4	2.768	1.479	1.000
		day 6	4.404	1.479	.074
	day 4	control	11.455	1.670	.000
		day 1	10.955	1.921	.000
		day 2	3.455	1.774	.879
		day 3	-2.768	1.479	1.000
		day 6	1.636	1.403	1.000
	day 6	control	9.818	1.670	.000
		day 1	9.318	1.921	.000
		day 2	1.818	1.774	1.000
		day 3	-4.404	1.479	.074
		day 4	-1.636	1.403	1.000
% expression cyclin E	control	day 1	-.250	2.111	1.000
		day 2	-6.200	1.980	.049
		day 3	-13.889	1.723	.000
		day 4	-15.364	1.660	.000
		day 6	-10.455	1.660	.000
	day 1	control	.250	2.111	1.000
		day 2	-5.950	2.194	.147
		day 3	-13.639	1.965	.000
		day 4	-15.114	1.909	.000
		day 6	-10.205	1.909	.000
	day 2	control	6.200	1.980	.049
		day 1	5.950	2.194	.147
		day 3	-7.689	1.824	.002
		day 4	-9.164	1.764	.000
		day 6	-4.255	1.764	.308
	day 3	control	13.889	1.723	.000
		day 1	13.639	1.965	.000
		day 2	7.689	1.824	.002
		day 4	-1.475	1.470	1.000
		day 6	3.434	1.470	.368
	day 4	control	15.364	1.660	.000
		day 1	15.114	1.909	.000
		day 2	9.164	1.764	.000
		day 3	1.475	1.470	1.000
		day 6	4.909	1.394	.016
	day 6	control	10.455	1.660	.000
		day 1	10.205	1.909	.000
		day 2	4.255	1.764	.308
		day 3	-3.434	1.470	.368
		day 4	-4.909	1.394	.016

Table 3: Multiple comparisons between all pairs of variables with the Bonferroni test.

Results

Median and range of values for all variables are presented in Table 1 whereas Figure 1 contains the boxplots of each variable for all groups of animals. As shown in Tables 2 and 3, all variables were significantly altered in all the study groups as compared with the control group suggesting the deleterious effect of the paraquat inhalation. Focal thickening of alveolar membranes and intra-alveolar haemorrhage were the more significant pathological changes.

Multiple comparisons showed significantly higher mean differences

in TUNEL staining and in the expression of ki-67, cyclins and CDKIs particularly after 2-3 days from the paraquat inhalation.

Discussion

ALI is a complex multifactorial process with wide heterogeneity of disease manifestations and outcomes. To optimize the understanding of the genetic basis for ALI, a major focus has been on candidate genes that are prominently involved in the primary pathophysiology of the disease, which includes widespread endothelial and epithelial disruption leading to increased permeability, protein-rich pulmonary oedema, neutrophilic inflammation, and surfactant dysfunction [4,5]. A common feature, regardless of the aetiological basis for disease, is an imbalance between pro- and anti-inflammatory cytokines, oxidants and antioxidants, procoagulants and anticoagulants, neutrophil recruitment/activation and clearance, proteases and protease inhibitors [1]. The pathogenesis of ALI is similar to that of sepsis, as both involve uncontrolled host defense responses that lead to inflammation, endothelial damage, enhanced coagulation, diminished fibrinolysis, and fibroproliferation [6]. Acute lung injury produced by paraquat causes progressive pulmonary insufficiency. Because of selective accumulation in the lungs, it causes severe lung injury manifested by edema, hemorrhage, interstitial inflammation, and progressive fibrosis [1,3]. Paraquat-induced lung injury is described on day 5 after PQ exposure in mice as pulmonary oedema, inflammatory cell recruitment, hemorrhage and light fibrosis [3]. Excessive productions of oxygen free radicals as well as lipid peroxidation are contributing factors for PQ-induced acute lung injury [7]. The cell cycle is regulated by various proteins among which cyclins play a key role. Cyclins are a family of proteins that control the progression of cells through the cell cycle by activating cyclin-dependent kinase (CDK) enzymes. On the other hand, cyclin-dependent kinase inhibitors (CDKIs) are proteins that bind to and inhibit the activity of CDKs. CDKIs are capable of suppressing growth and there is strong evidence suggesting that at least some CDKIs may be tumor suppressor proteins [8]. In the present study, the expression of cyclins B, D1 and E was examined as well as the expression of CDKIs p21, p16 and p27. The Ki-67 protein is a cellular marker for proliferation [9]. It is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). The TdT mediated dUTP nick end-labeling (TUNEL) method has been employed widely to demonstrate apoptotic cells in routinely prepared paraffin sections.

In the present study, an experimental model of paraquat-induced ALI was applied. Focal thickening of alveolar membranes and intra-alveolar haemorrhage were the more significant pathological changes. Two to three days after the lung damage, cellular proliferation was significantly increased as reflected by the expression of Ki-67. The expression of cyclins (B, D1, E) and CDKIs (p21, p16, p27) was also found at high levels. Furthermore, TUNEL test suggested increased apoptosis. These findings implicate that lung tissue damage is characterized by enhanced cellular proliferation and apoptosis both being regulated by complex interactions of cyclins and CDKIs. The outcome of the above process is remodeling of the lung architecture. Apoptosis is a form of regulated cell death in which activation of specific intracellular serine rich proteases (caspases) leads to cleavage and cell death. The literature confirms the presence of apoptosis in the alveolar and bronchial epithelial cells as an early feature in ALI [10]. Alveolar cell apoptosis likely contributes to ALI pathogenesis in response to various environmental stimuli, by induction of endothelial and epithelial barrier dysfunction [11]. While apoptosis may induce barrier dysfunction early in ALI, increasing evidence suggests that

apoptosis plays a beneficial role during ALI resolution. In fact, apoptosis may limit the duration of pulmonary inflammation by shortening neutrophil lifespan [12]. The beneficial influence of apoptosis in ALI can be further explained by the proregenerative role of clearance of apoptotic cells. This beneficial effect is mediated via the production of growth factors, including vascular endothelial growth factor (VEGF), from macrophages engulfing apoptotic cells [13].

Further studies need to elucidate the complex interactions of cyclins and CDKIs in ALI pathogenesis.

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