STUDY ON HAEMOLYTIC ACTIVITIES OF 10 TYPES OF COAL MINE DUSTS AND THEIR EFFECT FACTORS

XING GUO-CHANG • Lui Tie-Min • Lui Zung-Qi • Zhou Chen

Institute for Occupational Medicine of Ministry of Coal Industry, Beijing, China

INTRODUCTION

Prevalence rate of C.W.P. differs not only in different countries, but also in different coal mines in one country.[1,2,3] It is so in China. Of course, the difference is due to many factors. Walton, et al[4] and Reisner, et al[5] suggested that an unknown factor or factors must play a major role in determining the pneumoconiotic potential of individual dusts.

The haemolytic test is a simple and reliable method for estimating cytotoxicity of silica mineral dusts, which was one of the first systems used for exploring mineral dust cytotoxicity[6] and later on for cytotoxicity of colliery dust.[7] Reisner, et al[5] found that cytotoxicity or coal dust was correlated with the pneumoconiosis risk obtained by epidemiological survey.

In the present study, a vitro haemolytic technique was used to estimate preliminary cytotoxicity of most colliery dusts in China, providing scientific basis for fibrogenic potential of different colliery dusts.

MATERIALS AND METHODS

Preparation of Erythrocyte Suspensions

Healthy male New Zealand rabbits weighing 3 Kg were used for this study (provided by Animals Centre of Academy of Medical Sciences of China). Blood was drawn from the heart, centrifuged at 2000 rpm for 20 min., diluted with sterile physiological saline to make a 2% erythrocyte suspension.

Preparation of Dust Suspensions

10 types of coals used in this study were Fat Coal, Anthracite, Cindery Coal, Meagre Coal, Candle Coal, Weak Caking-Coal, Gas Coal, Lignite, Non-Caking Coal and Lean Coal, provided by Academy of Coal Science of China. The elemental compositions were listed in Table I and Table II. These coal samples were crushed in agate mortar to the particle size distribution less than 5 μm in dia. accounting for over 95%. Standard quartz dust less than 5 μm in dia. accounts for over 99%, provided by Academy of Preventive Medical Science of China.

A series of dust samples were dried, sterilized by ultraviolet ray for 30 min., suspended in sterile physiological saline to make the certain concentration and shaken in a high-speed water bath shaker for complete suspension.

Experiment Groups

Coal groups:

10 types of coals were divided into 10 groups. 3 ml erythrocyte suspensions were added into 2 ml of 20 mg/ml to 10 types of coal dust suspensions respectively and then incubated in IDI Water Bath Oscillator (at 37 ± 0.5 temperature, 120 times per min, cyclo-oscillation) for 60 min. The suspensions were centrifuged at 2000 rpm for 60 min and the optical density of the suspensions was measured at 540 nm in a 721 Spectrophotometer.

Quartz control groups:

- Quartz control I: 0.16 mg/ml.
- Quartz control II: 1.25 mg/ml.

Different Concentrations of Coal Groups

A series of Anthracite, Candle Coal and Non-Caking Coal were used as different doses of coal-groups (5, 10, 20 and 40 mg/ml).

Completely Lysed Control and Erythrocyte Fragility Control

3 ml of 2% erythrocyte suspension was centrifuged at 2000 rpm for 20 min and added with 5 ml distilled water to make a complete lysis control. 2 ml of 2% erythrocyte suspension was mixed with 2 ml physiological saline to make an erythrocyte fragility control.

$$\% \text{ of haemolysis} = \frac{OD_{540} \text{ Test Sample} - OD_{540} \text{ Fragility Control}}{OD_{540} \text{ Fully Lysed Control}} \times 100$$

RESULTS

Comparison of Haemolytic Activities among 10 Types of Coal Dusts

Results of tests done repeatedly 17–20 times are shown in Figure 1 and Table III. It is clearly seen that haemolytic activities of 10 types of coal dusts were different. Degree of haemolysis by Lean Coal and Fat Coal was respectively lowest and highest (range 10–35%). Statistics showed that haemolytic activities of quartz control I and II were significantly higher than those of coal dust-groups and that haemolytic activities of different doses of coal dust-groups were significantly

Table I
Composition of 10 Types of Coals

Types of Coal	Ash (g %)	Volatility	% of Carbon	% of Hydrogen	% of Nitrogen
Anthracite	22.962	3.471	79.944	1.080	0.715
Lean Coal	16.390	16.067	73.293	3.776	1.111
Cindery Coal	24.796	18.623	64.671	3.746	1.012
Gas Coal	11.244	29.332	76.436	4.640	1.323
Candle Coal	11.369	35.763	70.424	4.593	0.723
Lignite	10.042	41.623	63.925	4.020	0.960
Weak Caking Coal	10.340	26.150	71.680	4.380	
Non-Caking Coal	5.940	28.550	63.430	3.430	
Fat Coal	35.780	22.290	57.380	3.700	1.040
Meagre Coal	25.640	14.800	67.200	4.300	1.560

Table II
Composition of Ashes of 10 Types of Coals

Types of Coals	% of SiO_2	% of Fe_2O_3	% of Al_2O_3	% of CaO	% of MgO	% of SO_3	% of TiO_2	% of K_2O	% of Na_2O	% of P_2O_5
Anthracite	52.67	5.31	30.89	4.13	0.99	1.08	1.13	1.48	0.80	0.47
Lean Coal	46.59	13.83	30.08	3.40	0.67	1.96	1.57	0.76	0.45	0.11
Cindery Coal	46.47	11.33	25.05	7.07	1.59	4.59	1.12	0.89	0.75	0.09
Gas Coal	59.42	5.42	26.75	2.367	0.67	1.61	1.16	1.26	0.33	0.08
Candle Coal	49.08	7.94	32.81	3.49	1.26	1.23	1.44	1.13	0.42	0.34
Lignite	50.42	12.25	22.18	6.68	1.16	3.58	1.06	1.52	0.552	0.18
Weak Caking Coal	52.16	21.47	17.15	2.38	1.09	2.54	1.92	1.06	0.12	
Fat Coal	48.67	4.90	35.04	3.78	1.92	2.90	1.48			
Meagre Coal	49.77	3.34	35.71	3.95	1.54	0.54	1.31			

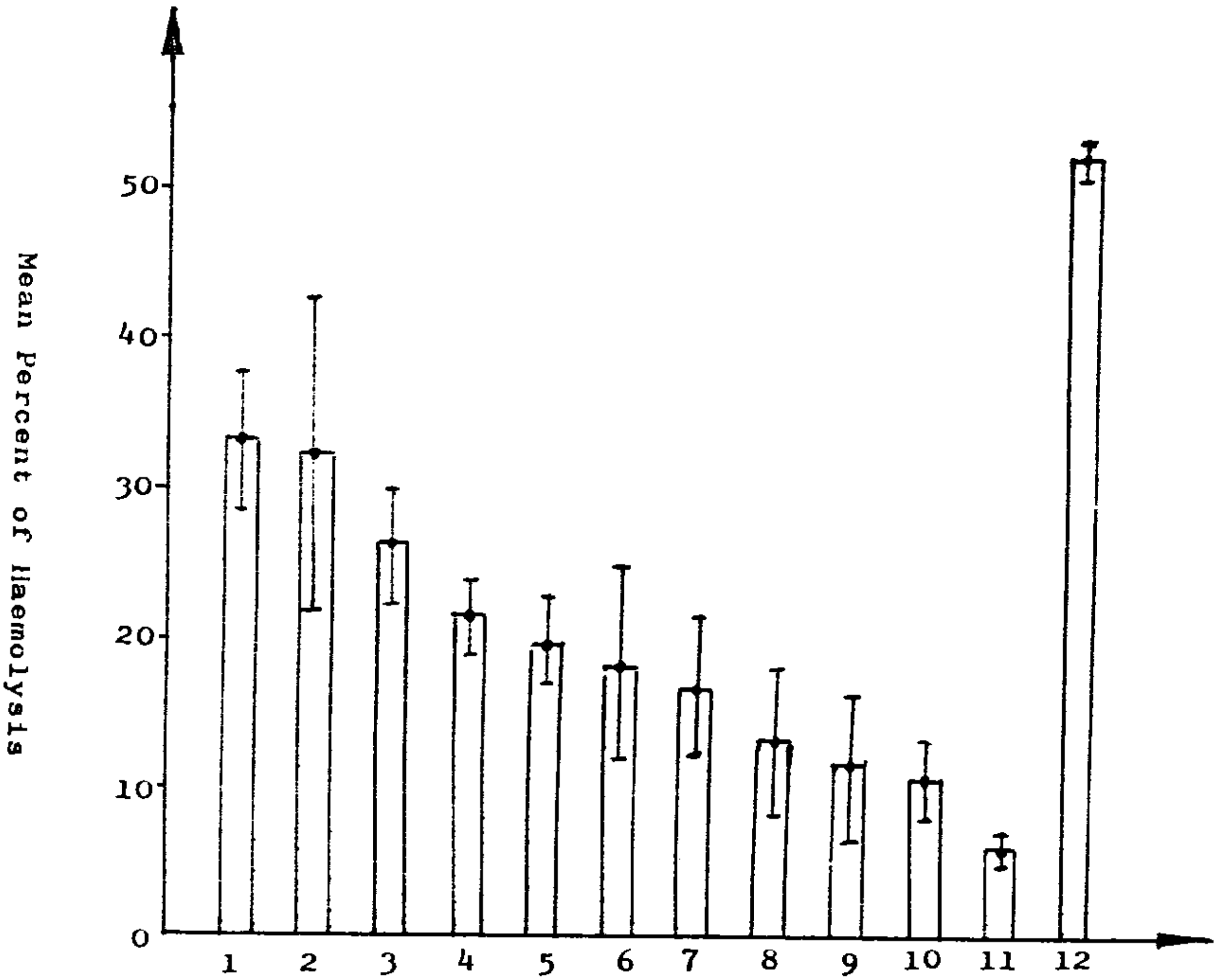

Figure 1. Mean percent of haemolysis of 10 types of coals—(1) Fat Coal, (2) Anthracite, (3) Cindery Coal, (4) Meagre Coal, (5) Candle Coal, (6) Weak Caking Coal, (7) Gas Coal, (8) Lignite, (9) Non-Caking Coal, (10) Lean Coal, (11) Quartz I, (12) Quartz II.

Table III
Endangerment Levels of Coal Dusts

Types of Coals	% Haemolysis	Levels
Fat Coal Anthracite Cindery Coal	33-26	Highest
Meagre Coal Candle Coal Weak Caking-Coal Gas Coal	21-16	High
Lignite Non-Caking Coal Lean Coal	13-10	Low

different ($P < 0.01$, analysis of Variance). Further F-Test showed that except for Anthracite and Candle Coal, Lignite and Lean Coal, haemolytic activities of remaining coal dusts showed statistical significance. Therefore, 10 types of coal dusts were divided into three levels by haemolytic activities, listed in Table III.

Comparison of Haemolytic Activities by the Different Doses of Coal Dusts

Anthracite, Candle Coal and Non-Caking Coal were selected as representatives of three levels of coals as defined above for dose-response test. The results are shown in Figure 2. Haemolytic activities of Anthracite, Candle Coal and Non-Caking Coals were respectively highest, high and low. But at the dust dose less than 10 mg/ml, their haemolytic activities were not significantly different. Starting from the dose of 10 mg/ml, their haemolytic activities increased with increasing dust doses.

Analysis of Effect Factors on Cytotoxicities of Coal Dust

Experimental data were analysed by multiple regression technique using computer to investigate the relationship among % Carbon content (X_1), % Ash content (X_2), % SiO_2 content (X_3) and % AlO_3 content (X_4) in dusts and haemolytic activities of dusts (Y). The analysis results were as follows.

Relationship between Y and X_1, $r = 0.89$
Relationship between Y and X_2, $r = 0.98$
Relationship between Y and X_3, $r = 0.92$
Relationship between Y and X_4, $r = 0.94$

Where r is Correlation coefficient.

Maximum amount comtributing variance were put into the equation. The result was:

$$Y = -2.382418 + 1.651106X_2 + 2373641X_3$$

Statistics showed that % of Ash content and SiO_2 in dusts made the largest contribution to variance.

DISCUSSION

Many scholars suggested a lot of hypothesis to explain the difference of C.W.P incidence rate in different coal mines with same dust concentration and similar workers' exposure time to dust. Some[5] thought that it was related to geological age and coal rank. Some[2] have identified rank and volatility of coal as factors associated with pneumoconiosis. Others[10] thought that it was related to non-coal mineral component and

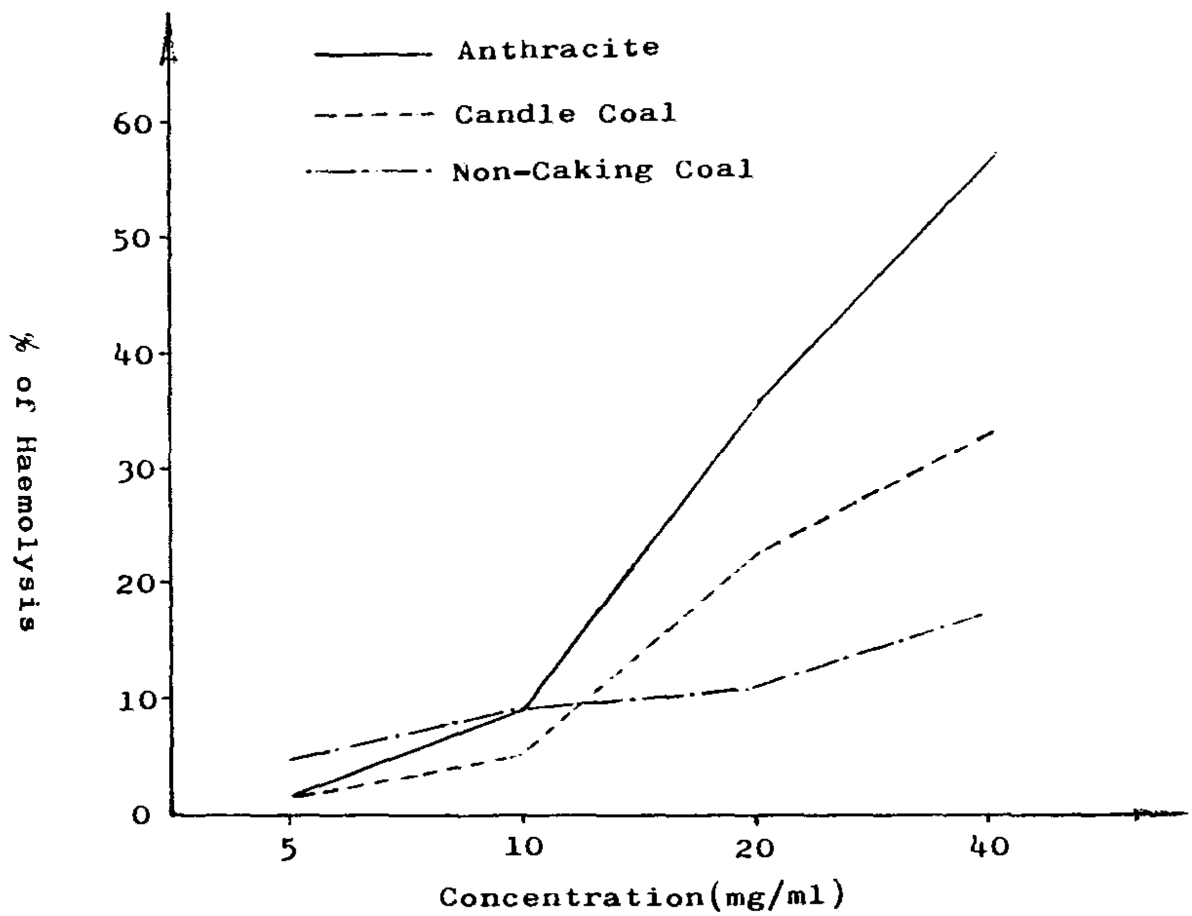

Figure 2. Relation between concentration and percent of haemolysis.

Table IV
Statistical Analysis of Mean Percent of 10 Types of Coals

Types of Coals	Number of Replicates	% of Haemolysis		Variance
		Mean	Standard Error	
Fat Coal	20	33.04	3.95	15.60
Anthracite	17	32.30	12.41	154.01
Cindery Coal	18	26.17	3.23	10.43
Meagre Coal	19	21.10	2.22	4.93
Candle Coal	18	19.43	2.43	5.91
Weak Caking Coal	20	18.48	6.92	47.89
Gas Coal	17	16.39	3.04	9.24
Lignite	19	13.14	4.21	17.72
Non-Caking Coal	19	11.60	4.46	19.89
Lean Coal	18	10.63	2.74	2.51

ash content. Others[7] thought rank and chemical composition of coal were important. It must be pointed out that unanimity of opinion has not been reached on the role played by SiO_2 in the development of pneumoconiosis. Some scholars[11,12] think that quartz, even if a small amount of it exists in dust, plays a role in developing pneumoconiosis, but others[13] don't agree with this opinion. Our experiment showed that from large to small sequence of haemolytic activities of coal dusts were Fat Coal, Anthracite, Cindery Coal, Meagre Coal, Candle Coal, Weak Caking Coal, Gas Coal, Lignite, Non-Caking Coal, and Lean Coal.

The mean haemolytic activities of 10 types of coals were listed in Table IV. It was clearly seen that haemolytic activity of coal was associated with coal type which is related to the geological age. Although times and conditions of coal formation and country rock component have an effect on coal quality, i.e. ash content and component, coal rank and carbon and ash content in 10 types of coals are in above order of haemolytic activity but volatility increased. Therefore, we think that SiO_2 in coal dusts plays an important role in cytotoxicity, which effect of coals themselves is related to period of coal formation. But other factors such as coal rank, volatility carbon content, ash content and SiO_2 content etc. are related to period or coal formation and are affected by condition of coal formation and country rock component.

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