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Chlorine gas induced acute lung injury in isolated rabbit lung

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ABSTRACT: This study was designed to investigate the pathogenesis of chlorine gas (Cl_2) induced acute lung injury and oedema.

Isolated blood-perfused rabbit lungs were ventilated either with air (n=7) or air plus 500 parts per million (ppm) of Cl_2 (n=7) for 10 min.

Capillary pressure, measured by analysing the pressure/time transients of pulmonary arterial, venous and double (both arterial and venous) occlusions, was unchanged in both groups. In Cl_2-exposed lungs, the fluid filtration rate increased from -0.228±0.25 to 1.823±1.23 $mL \cdot min^{-1} \cdot 100\ g^{-1}$ ($p<0.001$) and the filtration coefficient increased from 0.091±0.01 to 0.259±0.07 $mL \cdot min^{-1} \cdot cmH_2O^{-1} \cdot 100\ g^{-1}$ ($p<0.001$). No changes were observed in the control lungs. The extravascular lung water/blood-free dry weight ratio was 8.6±1.6 in the Cl_2 group and 4.0±0.5 in the control group ($p<0.001$), confirming that the increase in lung weight was related to accumulation of extravascular fluid.

Although the alveolar flooding by oedema is explained, in part, by the Cl_2-induced epithelial injury, our results suggest that Cl_2 exposure induces acute lung injury and oedema due to an increased microvascular permeability.

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Most accidental exposures to chlorine gas (Cl_2) occur in the pulp and paper industry (where Cl_2, hydrochloric acid, or chlorine dioxide are used as bleaching agents) [1], in transportation mishaps [2] and in swimming pool preparations [3]. Exposure of the general population may occur through inappropriate mixing of household cleaning agents, such as hypochlorite (bleach) and acids (cleaners) [4]. Following acute Cl_2 inhalation, pulmonary oedema may manifest itself clinically by the presence of blood-tinged frothy fluid in the airways. The diagnosis may be confirmed by the chest radiograph, and sometimes by postmortem observations and microscopic examination of the lungs [2–5]. Massive pulmonary oedema may also be produced by inhalation of other choking agents, such as phosgene, diphosgene and chloropicrine [6].

The injurious effects of the inhalation of a toxic gas depend both on the duration of exposure and on the concentration of the gas [7, 8]. The median lethal concentration (LC_{50}) of Cl_2 in dogs, using 30 min exposures and 3 day observation periods, is 600 parts per million (ppm) [9]. The most complete studies of the cellular, tissue, and organic lesions created by Cl_2 inhalation are those of WINTERNITZ *et al.* [10], based on experimental work with dogs. Above LC_{50}, animals dying within 24 h of exposure (acute death) had tracheitis, bronchitis with peribronchial inflammation and focal pulmonary necrosis. Extreme congestion and oedema were also observed in the entire respiratory tract, including peribronchial tissues and the sheaths of the large blood vessels. BARROW and SMITH [7] identified pulmonary oedema in rabbits exposed to 100 and 200 ppm of Cl_2 for 30 min, by a frothy exudation in the trachea, and changes in lung function and histopathology.

Massive exposure to Cl_2 may be used as a model of acute lung injury and oedema induced by the inhalation of choking agents. Compared to phosgene, Cl_2 has the distinct advantage of a noticeable odour at nontoxic concentrations, enabling early detection of accidental leaks. However, despite its paramount role as a toxic inhalant, the pathogenesis of Cl_2-induced injury has received little attention in most reviews on irritant injury of the lung. More specifically, no experimental studies have quantified the changes in intravascular pressure and in vessel wall permeability of the pulmonary microcirculation induced by acute inhalation of high concentrations of Cl_2. Therefore, the current study was designed to establish an experimental model of pulmonary oedema following 10 min of Cl_2 inhalation, and to investigate the pathogenesis of this oedema. The isolated perfused rabbit lung preparation was used to: 1) isolate the effects of Cl_2 inhalation on the alveolar blood/gas barrier from its effects on the bronchial circulation; 2) estimate pulmonary fluid filtration rate, pulmonary capillary pressure, and vascular permeability; and, finally, 3) to measure the extravascular lung water to assess the amount of pulmonary oedema formation.

Materials and methods

Experimental preparation

New Zealand white rabbits (2.6–3.2 kg) were anaesthetized with sodium pentobarbital (30 mg·kg^{-1} body weight (BW)) administered through an ear vein. After placement of a tracheal cannula *via* a tracheostomy, the animals were mechanically ventilated. Five minutes after an injection of 300 international units (IU)·kg^{-1} heparin *via* the ear vein, the rabbits were exsanguinated through the carotid artery. The blood collected (~130 mL), was supplemented by a 5% albumin solution to obtain a haematocrit of 14–16%, and then used to fill the perfusion circuit. The albumin solution contained (in mM): $CaCl_2.2H_2O$ (1.80); $MgSO_4$ (0.81); KCl (5.36); NaCl (116.4); NaH_2PO_4 (1.02); phenol red sodium (0.029); and D-glucose (5.55). After exsanguination, a median sternotomy was performed and rigid plastic cannulae (6 mm internal diameter (ID)) were inserted into the pulmonary artery *via* the right ventricle and into the left atrium. The pulmonary circulation was washed free of blood at a slow rate (~20 mL·min^{-1}) with the 5% albumin solution until the lungs were removed and connected to the perfusion circuit. A ligature was placed around the ventricles to occlude their lumen. Following complete ligation of all connections to surrounding tissues, the heart and lungs were removed *en bloc* and weighed. The isolated lungs were then suspended in air from a force transducer (Grass, FT 03, Quincy, USA) by a string tied around the tracheal cannula for continuous weight measurement.

The perfusion circuit consisted of a 100 mL venous reservoir, a roller pump (MasterFlex, 7523-02; Cote-Parmer Inst. Co., Chicago, IL, USA), a heat exchanger, and a 10 mL bubble trap placed upstream from the arterial cannula. Taking care to avoid air emboli, the lung preparation was connected to the circuit with inflow and outflow cannulae. Blood flow was then slowly increased to 100 mL·min^{-1}·kg^{-1} BW, and maintained at this level during the experiment. After a brief inflation (intratracheal pressure ~25 cmH_2O) to reverse any atelectasis, the lungs were cyclically ventilated with a gas mixture containing 22% O_2/5% CO_2/73% N_2 at a rate of 34 cycles·min^{-1}, a tidal volume of ~3 mL·kg^{-1} BW and an end-expiratory pressure of 3 cmH_2O. The minute ventilation was 100 mL·min^{-1}·kg^{-1} BW. The lungs and trachea were covered with a plastic wrap to prevent dessication. The whole lung was perfused under zone 3 conditions, *i.e.* venous pressure (6 cmH_2O) was kept above airway pressure (3 cmH_2O) in all regions of the lung. Blood gases and haematocrit (Ht) were measured at the beginning and at 15 min intervals throughout the experiment. The pH was adjusted to 7.35–7.45, when necessary, by the addition of $NaHCO_3$.

Lung haemodynamic measurements

Pulmonary arterial (P_a) and venous (P_v) pressures were continuously measured with pressure transducers (Viggo-Spectramed, 5299 702; BOC Health Co., Bilthoven, the Netherlands) connected to the side-ports of the arterial and venous cannulae. The zero reference level for vascular pressures was set at the top of the lung. Tracheal pressure was measured (Microswitch, 142PC01D; Honeywell, PA, USA) *via* the tracheal cannula, and end-expiratory pressure was assumed to represent alveolar pressure. P_v was adjusted by changing the height of the venous reservoir.

Capillary pressure measurements ($P_{c,ao}$, $P_{c,vo}$, $P_{c,do}$) were performed by analysing the pressure/time transients of arterial, venous and double (both arterial and venous) occlusions, respectively, obtained by clamping (with solenoid valves) the arterial or/and the venous cannulae. Prior to each occlusion, ventilation was turned-off during expiration. To estimate $P_{c,ao}$, a monoexponential curve was fitted to the P_a/time curve following the arterial occlusion between 0.2 s and when P_a had fallen to 10% of the preocclusion arterio-venous pressure difference. $P_{c,ao}$ was obtained by extrapolation of the above exponential fit back to the time of occlusion. $P_{c,vo}$ was obtained by the extrapolation of a linear fit of the venous pressure trace 0.2 s after occlusion back to the time of occlusion. $P_{c,do}$ was measured as the common level reached by P_a and P_v after a double occlusion. These occlusion pressures were interpreted using a model of the pulmonary circulation, in which most of the compliance is in the capillary bed and most of the resistance in the small muscular arteries and veins [11]. $P_{c,ao}$ and $P_{c,vo}$ are the pressures prevailing in the arterial microvessels downstream from the major site of arterial resistance and in the venous microvessels upstream from the major site of venous resistance in the pulmonary circulation, respectively [12]. $P_{c,do}$ is the prevailing pressure in the capillaries [13]. Pressure drops across the pulmonary circulation were computed as the pressure differences $P_a - P_v = \Delta P_{tot}$ (total pressure gradient), $P_a - P_{c,ao} = \Delta P_a$ (arterial pressure gradient), $P_{c,ao} - P_{c,do} = \Delta P_{c,a}$ (capillary arterial pressure gradient), $P_{c,do} - P_{c,vo} = \Delta P_{c,v}$ (capillary venous pressure gradient) and $P_{c,vo} - P_v = \Delta P_v$ (venous pressure gradient).

Measurement of filtration coefficient and extravascular lung water

In order to measure the capillary filtration coefficient (K_f), P_v was raised by 5–7 cmH_2O and maintained at this level until the vascular volume changes were complete (at least 10–15 min after the increase in P_v). The increase in P_v results in an initial rapid weight gain, due mainly to vascular volume changes, followed by a slower weight gain, attributed mostly to transcapillary fluid filtration [14]. K_f can be measured on the slow phase of the weight *versus* time curve, using either the zero-time extrapolation [15] or the slope [16]. We chose the latter because its results more closely approximate those obtained using the haemoconcentration of the perfusate following a step increase in P_v in the isolated lung [16]. We verified that the K_f values obtained by the slope ($K_{f,s}$) and by the zero-time extrapolation ($K_{f,z}$) were well correlated ($K_{f,s} = 0.38 \times K_{f,z} + 0.03$; r=0.97; p<0.001; n=14). In the present study, the slope of the weight *versus* time curve between 12 and 15 min after the step P_v increase was used to measure K_f. This slope ($(\Delta w/\Delta t)_{12–15}$) was divided by the difference in $P_{c,do}$ measured before and during the K_f manoeuvre:

$$Kf = ((\Delta w/\Delta t)_{12-15})/\Delta P_{c,do}$$

Kf was expressed in $mL \cdot min^{-1} \cdot cmH_2O^{-1}$ and normalized to 100 g of the initial lung weight, assuming a filtrate density of 1 $g \cdot mL^{-1}$. Because the lungs exposed to Cl_2 were not isogravimetric, the rate of weight gain just before the increase in P_v was subtracted from the rate of weight gain during the hydrostatic challenge.

To measure lung water, lung lobes were weighed and homogenized with equal weights of distilled water in a Waring blender (Waring, New Hartford, USA). Two samples of the homogenate, two samples of the perfusate, and one sample of the supernatant of the centrifuged homogenate were weighed, dried in an oven and weighed again. The blood content of the lung was determined by measuring the haemoglobin concentration (spectrophotometer, OSM3; Radiometer, Copenhagen, Denamark) of the perfusate and of the supernatant. The lung water was expressed as the extravascular lung water/blood-free dry weight (EVLW/BFDW).

Histological examination

Three additional lungs were examined histologically; one was ventilated with air and two others exposed to Cl_2 (500 ppm for 10 min). At the completion of each experiment, the bronchoalveolar spaces were filled (tracheal pressure ~10 cmH_2O) with 4% formaldehyde, and then submerged in 4% formaldehyde for ≥24 h. After fixation, random sections were taken from upper and lower lobes. Samples were then routinely processed, embedded in paraffin, sectioned at 3 µm, and stained with haematoxylin and eosin for light microscopy analysis.

Chlorine exposure conditions

Cl_2 was obtained from a gas cylinder containing a mixture of 0.96% Cl_2 in N_2 (Air Liquide, St. Quentin en Yveune, France). This Cl_2/N_2 mixture was diluted with compressed air and CO_2 to obtain the desired Cl_2 concentrations. The exposure dose of Cl_2 was 500 ppm for 10 min. The minute ventilation was 100 $mL \cdot min^{-1} \cdot kg^{-1}$ BW and the delivery rate of the Cl_2/N_2 mixture was ~5.2 $mL \cdot min^{-1} \cdot kg^{-1}$ BW. The Cl_2 concentration was continuously measured by the electrical potential of a chlorine-chlorine salt electrode. The Cl_2 in the expired gas was neutralized in a NaOH solution. The isolated lung and its ventilation and perfusion circuits were covered by a laboratory exhaust hood to contain any accidental leakage of Cl_2.

Experimental protocol

After weight and haemodynamic stabilization (~30 min), a first series of occlusions was performed to obtain $P_{c,ao}$, $P_{c,do}$ and $P_{c,vo}$. The initial Kf (Kf,i) was then measured. P_v was lowered to its original value and, 10–15 min later, another series of occlusions was made to obtain baseline haemodynamic values. The lungs were then randomly assigned either to the control group (n=7) or to a group exposed to Cl_2 at a concentration of 500 ppm for 10 min (n=7).

Series of occlusions were performed at the end of Cl_2 inhalation (time 0 min) and 30 min later (time 30 min). Afterwards, the final Kf (Kf,f) was measured. Perfusion lasted approximately 2.5 h. At the end of the study, the heart and lungs were weighed before and after dissection of the heart and main stem bronchi. This allowed calculation of the weight of the lungs before and after perfusion. The lung water was measured subsequently.

Statistical analysis

All data are expressed as mean±SD. Differences between control and Cl_2-exposed groups were determined by analysis of variance (ANOVA). Paired t-tests were used in each group to compare initial to final values. A difference at the 5% level was considered statistically significant.

Results

In preliminary dose-response experiments, we found that a very high dose of Cl_2 (1,000 ppm for 10 min) consistently caused devastating lung injuries. In these experiments, the lungs had such a high rate of weight gain that they were unable to sustain the venous pressure increase necessary to measure Kf at the end of the experiment. The Cl_2 concentration was therefore lowered to 500 ppm.

No change was observed in blood gases (pH, arterial oxygen tension (P_{a,O_2}), arterial carbon dioxide tension (P_{a,CO_2}) and haematocrit (Ht)) of the isolated lung perfusates in the control group or Cl_2-exposed group (pH = 7.39±0.03 *vs* 7.39±0.02; P_{a,O_2} = 17.3±2.3 *vs* 19.1±1.3 kPa (129.6±17.6 *vs* 143.4±9.4 mmHg); P_{a,CO_2} = 4.7±0.5 *vs* 5.1±0.2 kPa (35.5±3.9 *vs* 38.1±1.7 mmHg) and Ht = 14.4±1.2 *vs* 14.6±1.5%, respectively).

Table 1. – Effect of Cl_2 exposure on pulmonary haemodynamics

	Group (n=7)	Baseline	Observation period 0 min	30 min
P_a cmH_2O	Control	14.6±3.8	15.0±4.0*	16.6±4.9*
	Chlorine	16.6±2.0	17.6±2.4	19.7±5.0
$P_{c,ao}$ cmH_2O	Control	11.8±2.1	11.9±2.3	13.1±3.0*#
	Chlorine	12.5±1.6	13.5±2.4	13.9±2.6
$P_{c,do}$ cmH_2O	Control	10.8±0.9	10.9±1.0	11.3±1.1
	Chlorine	11.6±1.3	12.1±1.5	12.4±1.3
$P_{c,vo}$ cmH_2O	Control	9.0±1.2	9.1±0.8	9.2±0.6
	Chlorine	9.4±1.6	8.9±1.5	9.3±1.2
P_v cmH_2O	Control	6.1±0.2	6.1±0.2	6.1±0.1
	Chlorine	6.0±0.2	6.2±0.4	6.1±0.2

Data are presented as mean±SD. P_a: arterial pressure; $P_{c,ao}$: capillary pressure measured using arterial occlusion pressure analysis; $P_{c,do}$: capillary pressure estimated by double occlusion; $P_{c,vo}$: capillary pressure measured using venous occlusion pressure analysis; P_v: venous pressure; 0 min: beginning of the observation period following Cl_2 exposure; 30 min: end of observation period. *: $p<0.05$, compared to value at baseline; #: $p<0.05$, compared to value at 0 min.

Table 2. – Effect of Cl_2 exposure on total and segmental vascular drops

	Group (n=7)	Baseline	Observation period 0 min	30 min
ΔP_{tot} cmH_2O	Control	8.5±3.8	8.8±3.9	10.5±4.8*#
	Chlorine	10.6±1.9	11.4±2.1*	13.7±5.1
ΔP_a cmH_2O	Control	2.8±1.8	3.0±1.8	3.5±2.5
	Chlorine	3.9±2.2	3.9±2.2	5.6±3.2
$\Delta P_{c,a}$ cmH_2O	Control	1.0±1.9	1.1±1.9	1.8±2.7
	Chlorine	1.2±1.1	1.7±1.6	1.8±1.8
$\Delta P_{c,v}$ cmH_2O	Control	1.8±0.7	1.8±0.7	2.0±1.1
	Chlorine	2.1±1.0	3.2±1.0*	3.1±1.7
ΔP_v cmH_2O	Control	2.9±1.2	3.0±0.8	3.2±0.6
	Chlorine	3.4±1.7	2.8±1.3	3.2±1.2

Total (ΔP_{tot}) and segmental (ΔP_a, $\Delta P_{c,a}$, $\Delta P_{c,v}$, ΔP_v) vascular pressure drops measured in control lungs (control) and in lungs injured with Cl_2 (chlorine). Data are presented as mean±SD. ΔP_a: arterial pressure gradient; $\Delta P_{c,a}$: capillary arterial pressure gradient; $\Delta P_{c,v}$: capillary venous pressure gradient; ΔP_v: venous pressure gradient; 0 min: beginning of the observation period following Cl_2 exposure; 30 min: end of the observation period. *: $p<0.05$, compared to value at baseline; #: $p<0.05$, compared to value at 0 min.

Pulmonary haemodynamics

There were no significant differences in any haemodynamic variable between the control and the Cl_2-exposed groups during the baseline period (tables 1 and 2). Within each group, we observed a small increase in P_a and ΔP_{tot} which reached significance ($p<0.05$) in the control group. Following Cl_2 inhalation, there was a transient increase in the pressure drop across the venous microvessels ($\Delta P_{c,v}$) at time 0 of the observation period.

Lung transvascular filtration and lung water

Control lungs remained isogravimetric throughout the entire experiment. Cl_2-exposed lungs displayed a slight and transient decrease in weight (-0.228±0.25 to -0.435±0.53 $g \cdot min^{-1} \cdot 100\ g^{-1}$ wet lung weight) coinciding with Cl_2 inhalation, immediately followed by a continuous increase ($p<0.01$) in lung weight ($\Delta w/\Delta t$ = 1.823±1.23 $g \cdot min^{-1} \cdot 100\ g^{-1}$) during the observation period. Extravascular lung water content doubled ($p<0.001$) in the Cl_2-exposed group compared to the control group (fig. 1).

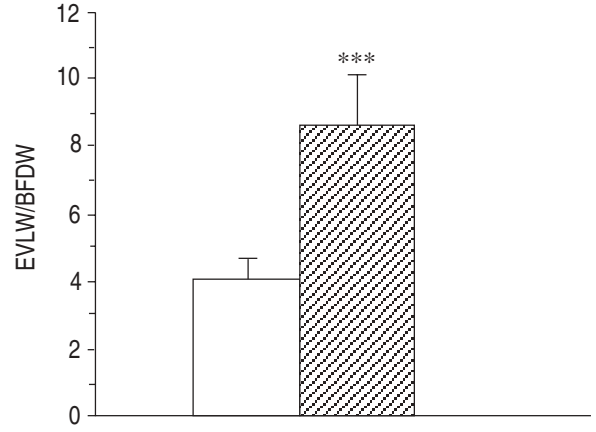

Fig. 1. – Effect of Cl_2 exposure on lung water. EVLW/BFDW: extravascular lung water/blood-free dry weight ratio. ***: $p<0.001$, compared to the control group. ▭: control group (n=7); ▬: chlorine-exposed group (n=7).

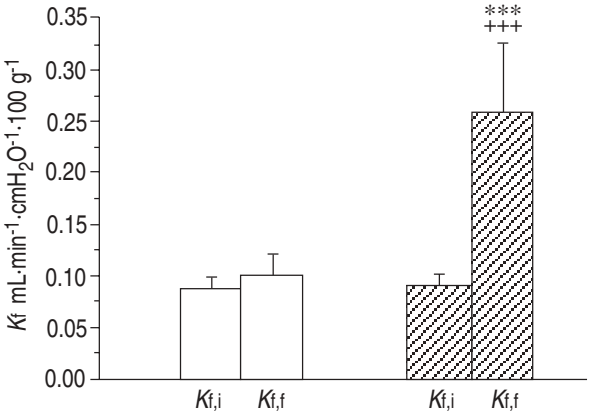

Fig. 2. – Effect of Cl_2 exposure on the filtration coefficient (K_f). $K_{f,i}$: initial filtration coefficient; $K_{f,f}$: final filtration coefficient. ***: $p<0.001$, compared to corresponding baseline value; $^{+++}$: $p<0.001$, compared to value in the control group. ▭: control group (n=7); ▬: chlorine-exposed group (n=7).

Filtration coefficient

There was no significant difference in baseline filtration coefficients ($K_{f,i}$) ($mL \cdot min^{-1} \cdot cmH_2O \cdot 100\ g^{-1}$) between the two groups. In the control group, $K_{f,f}$ did not differ from $K_{f,i}$. In contrast, in the Cl_2-exposed group, K_f increased ($p<0.001$) almost threefold compared to $K_{f,i}$ (fig. 2).

Effect of Cl_2 inhalation on tracheal pressure

Inspiratory peak pressure increased ($p<0.05$) by the end of Cl_2 exposure (8.3±2.5 *vs* 7.2±2.3 cmH_2O) and to 12.4±2.9 cmH_2O after an additional 30 min. Also, mean intratracheal pressure increased significantly 30 min after the end of Cl_2 exposure (5.4±0.7 *vs* 4.2±0.6 cmH_2O). The above increases in tracheal pressure were compatible with a minor degree of bronchial obstruction and/or with alveolar pulmonary oedema. No changes in tracheal pressures occurred in the control group.

Histological findings

Microscopic changes varied from discrete congestion of the alveolar capillaries to large areas of intra-alveolar oedema with little or no fibrin (fig. 3a and b). No thrombi in the small vessels, necrosis of the alveolar walls, or interstitial infiltration by polynuclear leucocytes were noticed (fig. 3a). Bronchial epithelium was altered, resulting in necrosis and desquamation but no associated inflammation (fig. 3b).

Discussion

This study was designed to investigate the pathogenesis of pulmonary Cl_2 exposure in order to obtain a more general understanding of the effects of the inhalation of toxic gases, with implications for preventing and treating lung injury. An isolated rabbit lung preparation, in which the bronchial microcirculation is not perfused

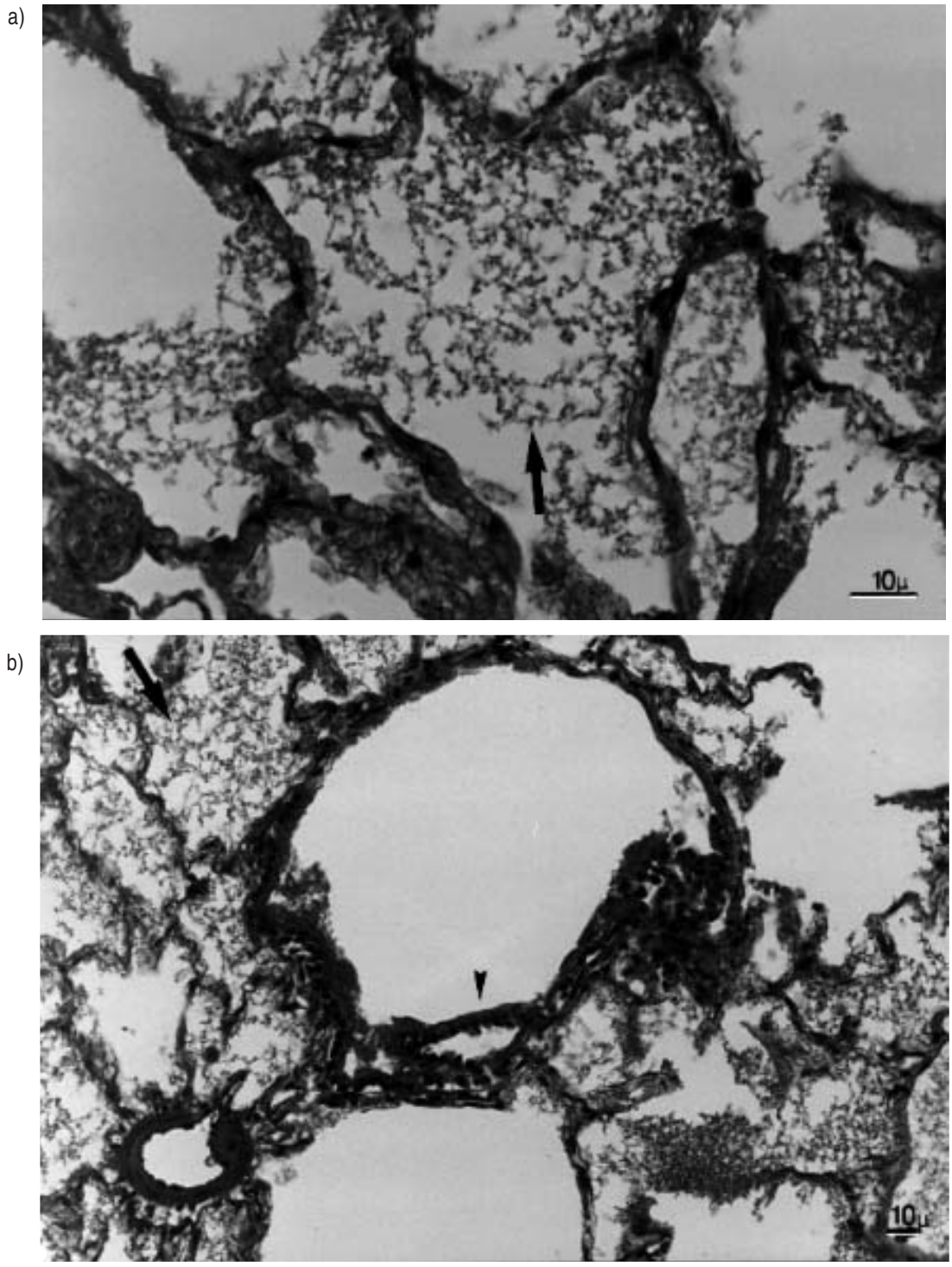

Fig. 3. – Photomicrographs of lung sections from Cl_2-injured lung. a) showing congestion of the alveolar capillaries, alveolar oedema (arrow), and absence of neutrophil infiltration of the alveolar walls. (Haematoxylin and eosin stain; internal scale bar = 10 µm). b) showing alteration of bronchial epithelium resulting in necrosis and desquamation (arrowhead), and alveolar oedema (arrow). (Haematoxylin and eosin stain; internal scale bar = 10 µm).

and does not contribute to pulmonary oedema, was used to characterize the action of acute Cl_2 exposure solely on the alveolar blood gas barrier and the pulmonary microcirculation. Our results indicate that the pulmonary oedema induced by Cl_2 exposure results mainly from an increased permeability of the pulmonary microcirculation.

Effects of Cl_2 exposure on lung transvascular filtration and capillary pressure

Cl_2 exposure increased weight gain 30 min after exposure compared to control lungs. We assumed that the rate of change in lung weight ($\Delta w/\Delta t$) reflected the rate of change of net transvascular fluid filtration, since

lymphatic drainage was obstructed by ligation of all connections between the lung and other thoracic structures.

Whilst an increase in lung weight indicates pulmonary oedema, it does not necessarily reflect the magnitude either of transvascular fluid filtration or accumulation of water in the extravascular spaces, because it may also reflect an accumulation of blood in lung tissue. We therefore chose to express lung water as extravascular lung water/blood free dry weight (EVLW/BFDW) to have a better estimation of oedema formation.

The EVLW/BFDW value reported in the control lungs (4.0±0.5 $g \cdot g^{-1}$ dry weight (DW)) was similar to that reported in intact rabbit lung by BHATTACHARYA *et al*. [17]. In contrast, the EVLW/BFDW doubled in the Cl_2-exposed lungs (8.6±1.6 $g \cdot g^{-1}$ DW). Because Cl_2-injured lungs had increased DW, a frequent finding in permeability oedema [18], the increase in extravascular lung water expressed per gram blood-free dry lung was probably underestimated. These results suggest that the increase in lung weight following Cl_2 exposure reflected accumulation of water rather than blood, and that a substantial interstitial and alveolar oedema was present in the lungs exposed to Cl_2. Light microscopy confirmed that no haemorrhages were present.

Using the three occlusion measurements of capillary pressure 5 min after Cl_2 exposure, we identified an increase in the pressure drop across the venous microvessels ($\Delta P_{c,v}$). Since venous pressure and blood flow were constant, this increase in pressure across the walls of venous microvessels could potentially increase pulmonary filtration pressure, as observed previously in other lung injuries [19]. However, this increase was transient. Therefore, Cl_2 exposure appeared to induce pulmonary oedema by some cause other than an increase in filtration pressure.

Effects of Cl_2 exposure on the permeability of the blood/gas barrier

The effects of Cl_2 exposure can be put in perspective with a simplified model of the blood/gas barrier. The usual model includes a tight alveolar barrier [20] in series with a permeable capillary endothelial barrier and in parallel with the interstitial-lymphatic pathway [21]. Could the increase in extravascular fluid following Cl_2 exposure have resulted from an alteration of the hydraulic conductance of the alveolar epithelium without an alteration of the hydraulic conductance of the endothelium? Although the present study does not provide direct evidence for or against this hypothesis, previous studies shed some light on this issue. PETERSON *et al*. [22] increased lung epithelial permeability in anaesthetized sheep with an aerosol of *Pseudomonas aeruginosa* elastase, without increasing pulmonary lymph flow and, therefore, endothelial permeability. These workers observed no increase in extravascular lung water as long as pulmonary vascular pressures did not increase [22]. On the other hand, GRIMBERT *et al*. [23] observed, in anaesthetized dogs, that hydrochloric acid instillation in the airways was followed not only by an increase in lung water but also by a doubling of lymph flow. Because capillary pressure was maintained constant, lung lymph flow was unlikely to have increased without an increase in endothelial permeability. We suggest that, in the present study, the increase in $\Delta w/\Delta t$, during the 30 min of observation following Cl_2 injury, can be explained only by an increase in capillary hydraulic conductance, since changes in capillary pressure were minimal during this time. Furthermore, we suggest that alveolar oedema formation is facilitated by epithelial injury.

The most important finding of this study is that, for similar increase in venous pressure, Cl_2 exposure was followed by an almost threefold increase in K_f. Indeed, K_f, which measures the hydraulic conductance, is the product of the vascular filtration surface area and the hydraulic conductivity of the microvascular exchange barrier [15]. In the present study, the influence of a change in surface area was minimized as lung perfusion was characterized by a condition typical of zone 3 ($P_a > P_v >$ alveolar pressure (P_a)), a high blood flow (100 $mL \cdot min^{-1} \cdot kg^{-1}$), and stable blood gas values. Alterations in hydraulic conductivity were, therefore, primarily responsible for the increase in hydraulic conductance. The mechanism of these alterations of hydraulic conductivity remains unclear: it may be a combination of an initial direct chemical injury and a late indirect neutrophil-mediated injury.

The dose of inhaled Cl_2 was approximately 70 µmol in the present study. BARROW *et al*. [24] reported that $Cl_2 + H_2O \rightarrow HOCl + HCl$ is the most probable reaction of Cl_2 plus H_2O under biological conditions (pH 7.4 and 37°C). Addition of 50 µmol of hypochlorous acid (HOCl) to the perfusate of isolated perfused rat lungs produces an immediate 2.8 fold increase in microvascular permeability related to a rapid shortening of the cytoskeletal actin microfilaments and the retraction of adjacent cells from each other [25]. The direct effects of HOCl also led to intracellular protein oxidation of endothelial cells [25]. Therefore, these observations support a role for HOCl in the first hour following Cl_2-induced lung injury. The initial increase in the permeability of alveolar capillaries to proteins induced by the aspiration of 300 µM of hydrochloric acid (HCl) in lungs of intact rats appears to result from the direct chemical effect of acid [26], rather than from recruitment and activation of neutrophils, which occurs later with subsequent release of oxidants and proteases [27]. In the present study, infiltration of neutrophils into the interstitial and alveolar spaces was not noticeable 60 min after Cl_2 injury (fig. 3). These microscopic observations are similar to those reported by WINTERNITZ *et al*. [10] in dogs dying 2–4 hours after Cl_2 exposure, and by DEMNATI *et al*. [28] in rats sacrificed 1 h after 10 min exposure to 1,500 ppm Cl_2. Other indirect effects of Cl_2 exposure, such as the release of substance P by a peripheral axon reflex in the isolated lung, remain speculative. When given as an *i.v.* bolus, substance P increases K_f in the isolated rabbit lung [29].

The effects of Cl_2 exposure (500 ppm for 10 min) on hydraulic conductance are similar to those observed by KENNEDY *et al*. [26] in rabbits following phosgene exposure (150 ppm for 10 min). Indeed, although they did not calculate K_f, an approximately 2.5 fold increase in K_f can be estimated from their data on weight gain curves when left atrial pressure was elevated at a high level. Because changes in surface area are minimized at high levels of left atrial pressure, phosgene may be

estimated to be three times more effective than Cl_2 in increasing hydraulic conductivity. This threefold greater potential toxicity of phosgene on the blood/gas barrier has been attributed to the low water solubility of phosgene, which allows it to reach the distal pulmonary structures [30]. Based on the above observations, Cl_2 exposure in isolated lung may be a convenient model of the toxicity of choking agents on the blood/gas barrier, its odour threshold being substantially below the toxic limit. The present study may, thus, offer a model for further investigation of therapies for choking agent induced lung injury.

While therapy for the direct injury may not seem feasible, one possible form of therapy is to decrease the capillary pressure and/or surface exchange area of the injured lung in order to minimize the consequences of alterations in microvascular permeability on filtration. Although its consequences on the surface exchange area may differ depending on whether the lung injury is heterogeneous (*e.g.* acid aspiration), or homogeneous (*e.g.* Cl_2 exposure), the danger of an increase in capillary pressure in the injured lung is always present. In dogs, we previously calculated that a rise of 3 mmHg of capillary pressure multiplies the normal filtration rate by a factor of 8 in the areas affected by acid instillation, whereas this factor is only 2 in intact areas [23]. In the present study, the ~7 cmH_2O rise in capillary pressure necessary for the measurement of K_f increased $\Delta w/\Delta t$ by 1.65±0.56 $mL \cdot min^{-1} \cdot 100\ g^{-1}$ in Cl_2-injured lungs, but only 0.67±0.22 $mL \cdot min^{-1} \cdot 100\ g^{-1}$ in control lungs ($p<0.001$). The above observations suggest that pulmonary capillary pressure should be kept below normal following Cl_2 exposure.

In conclusion, we have shown that exposure of blood-perfused isolated rabbit lung preparations to high concentrations of Cl_2 causes an almost threefold increase in the filtration coefficient within 30 min and a continuous increase in lung weight gain. The primary cause of oedema was most likely altered pulmonary capillary permeability (*i.e.* increased hydraulic conductivity because surface area changes were minimal in this model). The pathogenesis of Cl_2 exposure may be characterized by: rapid onset, direct injury of the epithelial and endothelial membranes and increased permeability of the pulmonary capillary walls leading to increased transport of fluid and protein into the alveolar compartment due to the alveolar epithelial injury. This results in pulmonary oedema formation.

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