Longitudinal Monitoring of Lung Injury in Children after Acute Chlorine Exposure in a Swimming Pool

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Online data supplement

Methods

Subjects and study design

On 17 February 2004, 18 children attending a swimming pool were accidentally exposed to chlorine gas after an erroneous servicing procedure: the children were having a swimming lesson when the water turned yellow due to an excessive quantity of chlorine being added to the pool. The children began to feel ill, with coughing, vomiting, dyspnea, burning eyes and throat.

Ten children (5 male, age range 6 to 12 years) were taken to Padova hospital; 4 were immediately admitted to the Pediatric Intensive Care Unit. A child needed mechanical ventilation for 4 days, during which time a bronchoscopy with bronchoalveolar lavage (BAL) was performed. The others 6 children were admitted to the Pediatric Ward.

After admission, the children were evaluated using standard medical procedures (physical examination, blood chemistry, chest X-ray, transcutaneous oximetry). In addition FE_{NO}, spirometry and EBC analysis were performed and serum stored.

FE_{NO}, spirometry and EBC collection were done in the first 24 hours after exposure in 9 children, and on day 4 (after extubation) in the child needing mechanical ventilation.

The children were then reassessed periodically over the following 15 months:

-spirometry was performed on days 0-3-4-8-15 and months 2-4-8-15;

-FE_{NO} measurements were performed on days 0-8-15 and months 2-4-8-15;

-EBC collection was performed on days 0-8 and months 2-8.

At month 8, the children also took an exercise challenge test on a treadmill.

The follow-up protocol was approved by the local ethical committee and both the parents and the children gave their informed consent to take part in the study; at least one parent was asked to attend all diagnostic procedures.

Exhaled nitric oxide (FE_{NO})

Fractional exhaled nitric oxide (FE_{NO}) was measured with the NIOX system (Aerocrine, Stockholm, Sweden), using a single breath online method according to ATS/ERS recommendations (E1). Children inhaled NO-free air through the mouth to total lung capacity and exhaled through a dynamic flow restrictor with a target flow of 50 ml/sec for at least 6-7 seconds. No nose clip was used. Visual incentives provided feedback for flow-rate compliance.FE_{NO}, expressed as ppb, was calculated as the mean of three measurements that agreed to within 10% of the mean value.

Pulmonary function test

Lung function was analyzed by flow-volume spirometry. Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and forced expiratory flow rate between 25% and 75% ($FEF_{25-75\%}$) of forced vital capacity were measured and expressed as percentages of predicted reference values (% pred.) according to international recommendations (E2,E3). The best of three maneuvers was recorded. Spirometry was also performed, within 3 days of the intoxication, after administering 300 µg of inhaled salbutamol by metered dose inhaler with a spacer device (Aerochamber, Trudell, Canada). Reversibility to β_2-agonists was defined as a more than 12% increase in FEV_1 after salbutamol inhalation.

Exhaled breath condensate (EBC)

EBC was collected with a home-made condenser consisting of: *a*) a mouthpiece set up to work as a saliva trap too; *b*) a T shape polypropylene valve; *c*) a 10-cm Tygon tube (Nalgene 890 FEP tubing; Nalge Nunc International, Rochester, New York, USA); *d*) a 50-mL polypropylene vial; and *e*) a Dewar flask refrigerated with ice. The five disposable components are easy to assemble, giving rise to a simple, portable

apparatus that is essentially composed of two parts: *a*) a disposable part, which is kept at room temperature and consisted of the mouthpiece, the non-rebreathing valve and the tube (connecting the valve to the vial); and *b*) a condensing part, which includes a disposable vial immersed in the ice inside the Dewar flask. Exhaled air condenses along the internal surface of the vial.

Without using a nose clip, the children were instructed to breathe tidally through their mouths via the non-rebreathing valve for 15 minutes sitting comfortably. They maintained a dry mouth during collection by periodically swallowing excess saliva.

On condensation, the EBC droplets collect at the bottom of the tube. Samples were stored at –80 °C in polypropylene tubes until analytical determinations.

EBC metabolites, i.e. leukotriene B4 (LTB-4) and cysteinyl leukotrienes (Cys-LTs), were quantified using specific enzyme immunoassay (EIA) kits (LTB4, Cayman Chemical Milan, Italy; $LTC_4/D_4/E_4$ Amersham Pharmacia Biotech, Milan, Italy;), according to the manufacturer's instructions, as previously described (E4,E5).

CC16 Serum analysis

Serum Clara cell-specific protein CC16 (collected 3-5 hours after chlorione exposure) was determined by latex immunoassay (E6).

Exercise challenge

Exercise testing was done on an electrically-driven treadmill (PK Morgan Ltd, Gillingham, Kent, UK) for 6 minutes under stable environmental conditions (temperature 20-22°C, humidity 50-55%). The workload was increased until 80% of the predicted maximal heart rate was reached. Spirometric measurements were performed with subjects at rest and 1,

3, 9, 15 and 20 min after the end of exercising using a 10-L bell spirometer (Biomedin, Padova, Italy). The best of three FEV_1 values was expressed as a percentage (%) of predicted reference values (E2). A 12% post-exercise fall in FEV_1 after exercise cut-off was considered suitable, since this is generally accepted to define EIB (E7). Heart rate was recorded continuously with a heart rate meter (Sport Tester® TM PE 3000; Polar Electro, Kempelehea, Finland) during exercise and recovery.

Ten healthy children, relatives of doctors and nurses of our hospital, were recruited as control group. They presented a negative past medical history and were matched for age and gender with the intoxicated patients (5 males, age range 6 to 12 years).

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM) for normally distributed data, and as median and interquartile range (IQR) for not normally distributed data. Lung function parameters, FE_{NO} levels and EBC markers were compared during the follow-up using Friedman's Repeated Measures Analysis of Variance on Ranks, followed by Student-Newman-Keuls Multiple Comparison Test. The Mann-Whiney U test was used to compare biomarker levels in exposed and control children. Correlations between variables were evaluated with Spearman's rank test. Statistical significance was assumed for p values of less than 0.05. Statistical analysis was performed using SigmaStat version 3.0.

Results

Table E1. Exhaled biomarkers at different sampling times**.**

	Acute phase	8th day	15th day	2nd month	4th month	8th month	15th month	Healthy controls
FE_{NO} (ppb)	4.7 [3.9-7.9] *	6,8 [5.2-9.2]*	7,1 [6.2-8.2] *	12.6 [11.4-15] †‡§	10,4 [7.4-14.1]	10.7 [8.7-9.7]	11.4 [9.9-17.4]	10,8 [8.9-12.2]
LTB_4 (pg/mL)	24.4* [22.5-24.9]	23.3* [22.4-25.8]	//	12 [9.3-17.1] * †	//	2.5 [0.5-4.4] §	//	4.7 [3-10.9]
Cys-LTs (pg/ml)	25.6 [13.1-38.3] *	33.1 [15.2-35.8]	//	2.0 [2.0-14.7] †§	//	4.3 [2.0-5.9]	//	7.2 [4-15.8]

Data are expressed as median [interquartiles]. Differences are evaluated with Friedman repeated measures analysis of variance followed by multiple comparison Student-Newman–Keuls test.

*p<0.05 compared to controls

†p<0.05 compared to admission

‡p=ns compared to 15th months

§p=ns compared to controls

Table E2: Spirometric values at admission and during the follow up

	Admission	3rd day	8th day	15th day	2nd month	4th month	8th month	15th month
FVC% pred.	51 [43-60]	84 [62-85]	85,5*†‡ [81-96]	97§ [82-108]	98.5 [93-109]	97.5 [85-113]	98.5 [87-106]	95.5 [86-104]
FEV_1% pred.	51 [46-60]	67 [56 -81]	88*†§ [72 -89]	92 [77-102]	97,5 [87-100]	95 [81-104]	93 [83-101]	91 [83-99]

Data are expressed as median [interquartiles] of percentage of predicted. Differences are evaluated with Friedman repeated measures analysis of variance followed by multiple comparison Student-Newman-Keuls test.

*p<0.05 compared to admission

†p<0.05 compared to 3rd day

‡p<0.05 compared to 15th months

§p=ns compared to 15th months

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