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Research Paper

Effect of Salbutamol on Respiratory Muscle Strength in Spinal Muscular Atrophy

Sonia Khirani PhD [a,b], Ivana Dabaj MD [c,d,e], Alessandro Amaddeo MD [b,f,g], Jorge Olmo Arroyo MSc [b], Jacques Ropers PharmD [h], Stéphane Tirolien RT [c], Véronique Coudert MD [c], Brigitte Estournet MD [c,d,e], Brigitte Fauroux MD, PhD [b,f,g], Susana Quijano-Roy MD, PhD [c,d,e,i,*]

[a] *ASV Santé, Gennevilliers, France*
[b] *AP-HP, Hôpital Necker-Enfants Malades, Pediatric Noninvasive Ventilation and Sleep Unit, Paris, France*
[c] *AP-HP, Hôpital Raymond Poincaré, Hôpitaux Universitaires Paris-Ile-de-France Ouest, Pôle pédiatrique, Service de Pédiatrie, Garches, France*
[d] *Centre de Référence Maladies Neuromusculaires Garches-Necker-Mondor-Hendaye (GNMH), Garches, France*
[e] *Réseau National de Maladies Neuromusculaires FILNEMUS, Garches, France*
[f] *INSERM U955, Créteil, France*
[g] *Paris Descartes University, Paris, France*
[h] *Hôpitaux Universitaires Paris Île-de-France Ouest, Unité de Recherche Clinique, Boulogne, France*
[i] *INSERM U1179, Université Versailles Saint-Quentin (UVSQ), UFR des sciences de la santé Simone Veil, Montigny, France*

ABSTRACT

BACKGROUND: Oral salbutamol has shown clinical benefits in spinal muscular atrophy (SMA). We studied its effect on the respiratory muscle strength in children with different types of SMA. **METHODS:** Lung and respiratory muscle functions were assessed in children receiving daily oral salbutamol for at least one year. The respiratory data of age-matched SMA II historical control subjects were compared with data of SMA II patients receiving salbutamol. **RESULTS:** Seven children (6.4 ± 2.0 years old, range four to ten; one SMA I, five SMA II, and one SMA III) treated with salbutamol (duration 23 ± 8 months) were assessed. Maximal static inspiratory pressure, sniff nasal inspiratory pressure, and slow vital capacity were significantly better in the salbutamol-treated SMA II group compared with control subjects ($P < 0.05$). **CONCLUSIONS:** Long-term oral salbutamol showed benefits in respiratory function in children with SMA and appeared to increase the strength of the inspiratory muscles in a small cohort of SMA II patients.

Keywords: spinal muscular atrophy, salbutamol, respiratory muscles, lung function, motor function

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Introduction

Spinal muscular atrophy (SMA) is a common genetic neuromuscular disease caused by a deficit of the survival motor neuron (SMN) protein encoded by two closely related genes, *SMN1* and *SMN2*, located on chromosome 5q13. Patients have usually no functional *SMN1* gene, although they retain at least one copy of the *SMN2* gene.[1] The clinical severity of the disease depends on the amount of SMN protein,[2,3] and this is related to the number of copies of *SMN2* gene.[4] SMA represents the second most common cause of mortality from a recessive genetic disorder.

Owing to the wide phenotypic presentation, patients are classified in different types based on age of onset, severity of disease, and achieved motor milestones,[5,6] but there is a continuous spectrum of severity with intermediary forms.[7]

SMA causes a predominantly bilateral proximal muscle atrophy and weakness.[5,8] The respiratory muscles are also

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* Communications should be addressed to: Prof. Quijano-Roy; Service de Pédiatrie; AP-HP; Hôpital Raymond Poincaré; 92380 Garches, France.
E-mail address: susana.quijano-roy@aphp.fr

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involved with weakness of the intercostal muscles and a relatively spared diaphragm.[9] This respiratory muscle weakness translates into an impairment of cough, resulting in poor clearance of airway secretions and recurrent pulmonary infections, restrictive lung disease because of poor or insufficient chest wall and lung growth, nocturnal hypoventilation, and finally respiratory failure in the most severe patients.[8]

Short-acting β-adrenergic agonists have been showed to increase skeletal muscle strength in healthy humans[10,11] and in subjects with muscle weakness because of acute or chronic conditions.[12-14] Recent studies have suggested that salbutamol may induce a rapid and significant increase in *SMN2* full-length messenger RNA and SMN protein in human SMA fibroblasts.[15,16] Moreover, it has been shown that the significant increase in full-length *SMN2* transcript levels was directly proportional to *SMN2* gene copy number.[16] However, the mechanism of action of β2 agonists on human skeletal muscle is not completely understood, requiring further investigations.

A few studies have also evidenced motor and respiratory function improvements in SMA patients after treatment with oral salbutamol,[17,18] but no study has addressed its effect on respiratory muscle function. The lack of defined respiratory outcome measures was specifically pointed out recently in an international SMA workshop.[7] The aim of this study was to assess the effect of oral salbutamol on respiratory muscle strength and to describe the clinical effects in a series of SMA children.

Materials and Methods

Patients

We retrospectively reviewed the charts of all SMA patients followed in our multidisciplinary pediatric neuromuscular clinic who received daily treatment with oral salbutamol (GlaxoSmithKline, UK) started before December 2013. The diagnosis of SMA had been confirmed by mutation analysis of the *SMN*1 gene. We used the functional criteria of the International SMA Consortium to classify the patients[19]: type I SMA (SMA I): early infantile onset, patients are never able to sit without support; type II SMA (SMA II): onset before 18 months, patients are able to sit but are unable to walk unaided; type III SMA (SMA III): onset during childhood, patients are able to walk unsupported.

Treatment with oral salbutamol was initiated after clinical and cardiological assessment (electrocardiogram and 24-hour holter electrocardiogram) to rule out contraindications. A first test dose was performed (1 mg if weight was under 10 kg, 2 mg otherwise) to assess tolerance, heart rate, and blood pressure, each hour for 3 hours. The salbutamol dose was progressively increased over several weeks or months to a maximal dose of 6 mg/day (2 mg three times per day),[18] provided it was well tolerated. Patients continued their usual

TABLE 1.
Clinical and Genetic Data of SMA Patients

Patient (Gender)	Genetics	SMA Type Initial	Age (year) Weight (kg) At Drug Onset	Feeding Problem at Drug Onset	Initial VC	TT	Time to Full Drug Dose (months)	Duration of Drug TT (months)	Age at Test (year)
1 (M)	0 SMN1/3 SMN2	I Sitting supported	6.1 15.4	Severe dysphagia	53% (640 mL)	Brace IPPB HFIPV NIV nasogastric-tube	8	31	8.6
2 (M)	0 SMN1/3SMN2	II	3.1 12.4	Mild dysphagia	124% (840 mL)	Brace IPPB HFIPV	2	31	5.6
3 (F)	0 SMN1/3 SMN2	II	3.3 12.3		90% (510 mL)	Brace IPPB	8	13	4.4
4 (M)	0 SMN1/3SMN2	II	3.9 12.5	Fatigue, long meals	104% (810 mL)	Brace IPPB	6	30	6.3
5 (F)	0 SMN1/3SMN2	II	7.8 13.0		62%	Brace NIV	19	23	9.7
6 (M)	0 SMN1/4SMN2	II Walking steps supported	4.8 12.3		106% (810 mL)	Brace IPPB	8	20	5.8
7 (F)	1 SMN1mut/1 SMN2	III	5.2 12.4		157% (910 mL)	None	6	14	5.3

Abbreviations:
At test = At the time of the respiratory muscle tests
HFIPV = High-frequency intrapulmonary percussive ventilation
IPPB = Intermittent positive pressure breathing
MFM = Motor function measure
NA = Not available
NIV = Noninvasive ventilation
SMA = Spinal muscular atrophy
SMN = Survival motor neuron
SMN1mut = Point mutation in SMN1
TT = Treatments
VC = Vital capacity

treatment, including trunk and limb orthosis, motor and respiratory physical therapy, intermittent positive pressure breathing or high-frequency intrapulmonary percussive ventilation, and/or noninvasive ventilation (NIV) when indicated. They were followed by the clinical team at least every 6 months, and height, weight, heart rate, and blood pressure were systematically recorded. Lung function tests and motor function tests, using motor function measurement (MFM) scores MFM 32,[20] were performed when possible. In children younger than seven years, the short version (MFM 20) was used.[21]

The study was approved by the institutional review board of the French learned society for respiratory medicine "Société de Pneumologie de Langue Française," and all patients and parents gave their informed consent.

Lung function and respiratory muscle tests

All the patients had a complete assessment of the lung function and respiratory muscle tests between March 2014 and January 2015 when they were clinically stable. This evaluation was performed after at least one year with salbutamol treatment and at least four months with maximal daily dose.

Spirometry

The patients were asked to perform at least three acceptable slow vital capacity (SVC) curves and the curve with the highest SVC was used for the analysis, as per American Thoracic Society/European Respiratory Society standards.[22] Predicted SVC was calculated.[23] The measurements were done without a brace. Only measurements in the supine position were available for all patients and were therefore considered for subsequent analysis.

Respiratory muscle tests

Nonvolitional tests. An esogastric catheter was inserted pernasally after local anesthesia (lidocaine 2%; AstraZeneca, Rueil-Malmaison, France).[24] This 2.1 mm external diameter catheter mounted pressure transducer system (Gaeltec, Dunvegan, Isle of Skye, UK) has two integral transducers mounted 1 (for the gastric pressure [Pgas]) and 21 cm (for the esophageal pressure [Pes]) from the distal tip. Appropriate placement of the catheter was checked.[25] Transdiaphragmatic pressure (Pdi) was obtained by subtracting on line the Pes signal from the Pgas signal.

The Pgas to Pes swing ratio (ΔPgas/ΔPes) was used to assess the relative contribution of the respiratory muscles to tidal breathing.[26-29] A value ranging between −1 and 1 indicates an ever-increasing contribution of the ribcage and expiratory muscles, compared with the diaphragm, to tidal breathing. This ratio becomes equal to 1 in case of complete diaphragmatic paralysis.[29]

The patient's global inspiratory muscle and diaphragmatic effort during quiet breathing were assessed by calculating the esophageal (PTPes) and diaphragmatic pressure-time products (PTPdi), respectively. The PTPes per breath (PTPes/breath) was obtained by measuring the area under the Pes signal between the onset of inspiratory effort and the end of inspiration and was referred to the chest wall static recoil pressure-time relationship according to a methodology adapted from Sassoon et al.[30] The PTPdi/breath was obtained by measuring the area under the Pdi signal from the onset of its positive deflection to the end of inspiratory flow. Both PTPes and PTPdi were expressed per minute (PTPes/minute and PTPdi/minute).[31]

Height (cm) Weight (kg) At Test	Drug Dose (mg/kg/day)	Change in SMA Type	VC Change (Time After Full Dose)	MFM Change (Time After Full Dose)	Weight Change (kg/year)	Nutrition Change
130 26	0.23	II Sitting without support	23% (+540 mL) after 20 months	10% (D2 +25%) after 20 months	5	Gastrostomy canceled
117 17	0.35	No	No change	NA (too young)	2	No dysphagia, normal feeding
90 10	0.50	No	3%, (+90 mL) after 4 months	NA (too young)	1.5	
117 18	0.33	No	32% (+440 mL) after 2 months 24% (+660 mL) after 16 months	3% (D2 +8%) after 16 months	2	No feeding fatigue
125 22	0.27	No	18%	19% (D2, D1 +25%) after 14 months	2	
108 14	0.42	III Walk without support	No change	14% (D1, D3 +20%) after 7 months	0.3	
114 15	0.40	No	No change	No change	1.5	

Volitional tests. To determine the strength of the inspiratory muscles, the patient was asked to perform 10 to 20 short, sharp maximal sniffs and the maximum sniff nasal inspiratory pressure (SNIP) was recorded.[32] Predicted values were calculated.[33] Concomitantly, maximal sniff Pes (Sniff Pes) and sniff Pdi (Sniff Pdi) were measured. The strength of the expiratory muscles was measured by asking the patient to perform a maximal cough. The peak Pgas value among at least five maximal coughs was measured (Pgas Cough).[34]

When possible, maximal static inspiratory pressure (MIP) and maximal static expiratory pressure (MEP) were measured from residual volume and total lung capacity, respectively. Patients were asked to perform at least five maneuvers until two reproducible maneuvers were obtained. The best maneuvers were retained for analysis and the mean value that could be maintained for one second was calculated, otherwise the peak value was used.[35,36] Predicted values were calculated.[35,36]

The diaphragmatic tension time index (TTdi), which estimates the endurance of the diaphragm, was calculated as TTdi = (Pdi/Sniff Pdi) × Ti/Ttot, where Pdi = mean Pdi during quiet spontaneous breathing, Sniff Pdi = Pdi during a maximal sniff, Ti = inspiratory time, and Ttot = total breath time during quiet breathing.[37] The esophageal tension time index (TTes), which estimates the overall endurance of the inspiratory muscles, was calculated as TTes = (Pes/Sniff Pes) × Ti/Ttot, where Pes = mean Pes during quiet spontaneous breathing and Sniff Pes = Pes during a maximal sniff.

SMA historical control subjects

To have age-matched control data to compare with the data of the salbutamol-treated SMA II children, we selected 21 longitudinal respiratory test data performed in 11 children with SMA II (ratio of one case data versus four control data).[38] The control patients were followed in our center and did not receive oral salbutamol. However, some patients received daily riluzole during two years, but this treatment did not show significant difference when compared with placebo in a previous therapeutic trial (Evaluate the Efficacy of Riluzole in Children and Young Adults With Spinal Muscular Atrophy [ASIRI] trial, data not published). Intermittent positive pressure breathing devices, high-frequency intrapulmonary percussive ventilation, or NIV was used when required. No historical control patient used NIV at the time of these measurements.

Statistical analysis

Population variables were expressed as the mean ± S.D. Comparison between SMA II control subjects and salbutamol-treated SMA II patients was performed using the *t* test, in case of normally distributed populations or a positive equal variance test, otherwise the Mann-Whitney rank sum test was used. The correlation between the time spent with salbutamol and the different respiratory measures was assessed using the Pearson product moment correlation or Spearman rank order correlation tests. A P value <0.05 was considered as statistically significant.

Results

Nine patients treated with oral salbutamol were identified. Two patients were not included in the study (one adolescent and a seven-year-old girl who was ventilated via a tracheostomy refused the respiratory muscle assessment). Therefore seven patients (one SMA I who was able to sit with support, five SMA II [among which one was able to stand and walk some steps with aid] and one SMA III) aged four to ten years, at the time of respiratory muscle tests, were included. None had scoliosis surgery. Concerning the genetic background, six patients carried the classic exon 7 deletion at the homozygous state. The number of *SMN2* gene copies in these patients is that expected for the clinical presentation and severity (the higher the number of *SMN2* copies, the milder the phenotype). On the contrary, Patient 7 has not the habitual homozygous deletion but instead, in addition to an exon 7 deletion, she presented a heterozygote mutation in *SMN1* gene (NM_000344.3:c5C>G (p.Ala2Gly)). She only carried one copy of *SMN2* gene, which is usually associated with a very severe phenotype. However, given the fact that the phenotype of this patient is the mildest of our series (SMA III), the heterozygous mutation seems to have milder pathogenic consequences than classic exon 7 deletion. Table 1 summarizes the genetic data and the clinical course from the onset of treatment until the performance of the respiratory muscle tests. Patient 3 was the youngest patient who performed the tests. Salbutamol was taken for 23 ± 8 months (range 13 to 31 months) with a maximal dose taken for 11 ± 9 months (range 4 to

TABLE 2.
Respiratory Status Before and After Salbutamol Use

Patient	Before Salbutamol	During Salbutamol Use
1	Triple antibiotic therapy as prophylaxis 1 hospitalization per year for respiratory infection	Antibiotics prophylaxis ongoing At 6 months: 1 respiratory infection without hospitalization At 12 months: no respiratory infection, no hospitalization At 18 months: 1 respiratory infection without hospitalization
2	2 Respiratory infections per year 1-2 Hospitalizations per year	At 6 months: 1 respiratory infection and 1 hospitalization At 12 months: no respiratory infection, no hospitalization From 18 months: no respiratory infection, no hospitalization
3	No respiratory infections No hospitalization	Stable under treatment
4	NA	At 12 months: 1 respiratory infection No hospitalization for respiratory infection
5	NA	NA
6	No respiratory infection No hospitalization	At 6 months: 1 hospitalization for respiratory infection At 12 months: no respiratory infection, no hospitalization At 18 months: no respiratory infection, no hospitalization
7	Recurrent respiratory infections No hospitalization for respiratory infections	Recurrent respiratory infections No hospitalization for respiratory infections

Abbreviation:
NA = not available

TABLE 3.
Lung Function and Respiratory Muscle Output, Endurance, and Strength of the SMA Patients Treated With Oral Salbutamol

Patients	SVC (L) (%pr)	PTPes/minute (cmH_2O.s/minute)	PTPdi/minute (cmH_2O.s/minute)	TTes	TTdi	MIP (cmH_2O) (%pr)	SNIP (cmH_2O) (%pr)	Sniff Pes (cmH_2O)	Sniff Pdi (cmH_2O)	MEP (cmH_2O) (%pr)	Pgas Cough (cmH_2O)
1	1.07 (62%)	300	475	0.04	0.06	—	−62 (63%)	−72	99	—	28
2	0.98 (97%)	190	362	0.01	0.05	—	−61 (69%)	−62	102	—	37
3	0.60 (93%)	151	339	0.02	0.08	—	−38 (45%)	−33	50	—	30
4	1.31 (100%)	—	—	—	—	−50 (86%)	−90 (99%)	−94	117	63 (90%)	52
5	0.87 (58%)	296	718	0.06	0.12	−45 (74%)	—	−39	71	30 (34%)	41
6	1.46 (132%)	203	369	0.01	0.02	−130 (235%)	−85 (95%)	−93	149	93 (139%)	74
7	0.77 (70%)	—	—	—	—	—	−52 (59%)	−54	73	—	72

Abbreviations:
MEP = Maximal static expiratory pressure
MIP = Maximal static inspiratory pressure
Pgas Cough = Gastric pressure during a maximal cough
%pr = Percent of predicted value
PTPdi = Diaphragmatic pressure-time product
PTPes = Esophageal pressure-time product
Sniff Pdi = Maximal sniff transdiaphragmatic pressure
Sniff Pes = Maximal sniff esophageal pressure
SNIP = Maximal sniff nasal inspiratory pressure
SVC = Slow vital capacity
TTdi = Diaphragmatic tension time index
TTes = Esophageal tension time index

30 months). All the patients were receiving the maximal dose of salbutamol at the time of the respiratory muscle tests. Patient 5 was the oldest patient who received salbutamol. Oral salbutamol was well tolerated by all the patients, with no major adverse effects. All patients gained weight from 0.3 to 5 kg/year. Table 2 shows the respiratory status before and during salbutamol use.

Mean ΔPgas/ΔPes was −0.70 ± 0.32 indicating an increased contribution of the ribcage and expiratory muscles to tidal breathing, although one SMA II patient (Patient 5) had a normal tidal breathing. Table 3 presents the parameters of lung function and respiratory muscle output, endurance, and strength for all the salbutamol-treated SMA patients. Mean SVC %predicted (%pr) value was within normal ranges (87 ± 26%). PTPes/minute values were only slightly greater than normality, whereas PTPdi/minute values were all greater than normal with a very high value observed in Patient 5. Respiratory muscle and diaphragmatic endurances were within the normal ranges. Inspiratory muscle strength

TABLE 4.
Anthropometric Data of the Historical Type II SMA Control Subjects

Patient	Gender	Height (cm)	Weight (kg)	BMI (kg/m^2)	Age (years)	Pharmacologic Treatments
1	F	114	15	11.5	7.5	Riluzole
		124	17	11.1	9.5	
2	F	115	35	26.5	8.2	Riluzole was stopped
		136	—	—	10.2	
		141	17	8.6	12.2	
3	F	125	30	19.2	7.6	
		132	35	20.1	9.1	
4	M	140	32	16.3	9.4	Riluzole
		152	40	17.3	10.8	
5	M	100	20	20.0	3.2	
		114	20	15.4	3.8	
		138	34	17.9	6.5	
6	F	113	20	15.7	7.9	Riluzole
7	F	122	24	16.1	8.5	
8	F	136	33	17.8	14.1	
9	M	—	—	—	12.3	
10	M	110	13	10.7	3.9	
11	F	108	14	12.0	6.8	
		—	—	—	8.7	
		133	21	11.9	11.8	
		158	30	12.0	13.0	
Mean ± S.D.		127 ± 16	25 ± 9	15.6 ± 4.4	8.8 ± 3.0	

Abbreviations:
BMI = Body mass index
SMA = Spinal muscular atrophy

TABLE 5.
Lung Function and Respiratory Muscle Output, Endurance, and Strength of the Type II SMA Historical Control Subjects

Patient	SVC (L) (%pr)	PTPes/minute ($cmH_2O.s$/minute)	PTPdi/minute ($cmH_2O.s$/minute)	TTes	TTdi	MIP (cmH_2O) (%pr)	SNIP (cmH_2O) (%pr)	Sniff Pes (cmH_2O)	Sniff Pdi (cmH_2O)	MEP (cmH_2O) (%pr)	Pgas Cough (cmH_2O) (%pr)
1	1.08 (85%)	381	469	0.09	0.09	−57 (102%)	−52 (55%)	−55	121	29 (38%)	66
	1.23 (75%)	107	139	0.02	0.08	−20 (35%)	−48 (48%)	−53	85	10 (11%)	34
2	0.83 (55%)	159	507	0.05	0.16	—	−32 (33%)	−38	58	—	49
	0.75 (33%)	111	296	0.03	0.13	−29 (65%)	−28 (27%)	−36	58	50 (55%)	32
	0.61 (24%)	113	244	0.06	0.32	−22 (38%)	−14 (13%)	−15	16	33 (32%)	17
3	0.90 (51%)	—	—	—	—	—	−54 (57%)	—	—	—	—
	0.59 (28%)	108	382	0.02	—	—	−41 (41%)	−26	—	—	30
4	1.36 (47%)	142	381	0.03	0.13	−30 (44%)	−38 (38%)	−48	93	40 (46%)	79
	1.22 (39%)	113	368	0.05	0.22	−20 (27%)	−28 (26%)	−28	56	28 (30%)	48
5	—	172	—	0.06	—	—	−30 (37%)	−39	42	—	30
	—	—	—	—	—	—	−26 (32%)	—	—	20 (36%)	—
	—	268	596	0.05	0.10	—	−40 (44%)	−60	93	—	45
6	0.73 (58%)	179	416	0.06	0.06	—	−19 (20%)	−25	87	—	61
7	0.56 (36%)	124	492	0.06	0.17	−19 (30%)	−41 (42%)	−43	93	40 (49%)	60
8	1.30 (76%)	187	509	0.02	0.04	−66 (95%)	−40 (34%)	−66	95	55 (49%)	36
9	—	81	209	0.05	0.04	−13 (29%)	−19 (17%)	−24	64	25 (24%)	34
10	—	171	333	0.07	0.16	—	−27 (33%)	−30	53	—	19
11	—	—	—	—	—	—	−44 (48%)	−55	78	—	30
	—	238	210	0.04	0.13	—	−45 (46%)	−55	73	37 (45%)	35
	1.51 (56%)	—	—	—	—	−27 (45%)	−36 (33%)	−60	—	32 (32%)	—
	1.85 (54%)	138	209	0.03	0.13	−36 (54%)	−42 (37%)	−60	81	38 (36%)	34
Mean ± S.D.	1.04 ± 0.39 (51 ± 18%)	164 ± 74	360 ± 134	0.05 ± 0.02	0.13 ± 0.07	−31 ± 17 (51 ± 26%)	−35 ± 11 (36 ± 12%)	−43 ± 15	73 ± 25	34 ± 12 (37 ± 12%)	41 ± 17

Abbreviations:
MEP = Maximal static expiratory pressure
MIP = Maximal static inspiratory pressure
Pgas Cough = Gastric pressure during a maximal cough
%pr = Percent of predicted value
PTPdi = Diaphragmatic pressure-time product
PTPes = Esophageal pressure-time product
Sniff Pdi = Maximal sniff transdiaphragmatic pressure
Sniff Pes = Maximal sniff esophageal pressure
SNIP = maximal sniff nasal inspiratory pressure
SVC = Slow vital capacity
TTdi = Diaphragmatic tension time index
TTes = Esophageal tension time index

was within normality, whereas expiratory muscle strength was decreased.

The mean age of the five salbutamol-treated SMA II patients was 6.4 ± 2.0 years. It was not significantly different from the mean age of the SMA II control subjects (8.8 ± 3.0 years, $P = 0.100$) at the time of their respiratory muscle evaluations. Tables 4 and 5 present the anthropometrics data and respiratory data of the SMA II control subjects. When comparing the respiratory data of the historical control subjects with the SMA II patients receiving salbutamol, the mean values of MIP (−31 ± 17 vs −75 ± 48 cmH_2O, respectively, $P = 0.017$), MIP %pr (51 ± 26 vs 132 ± 90 %pr, respectively, $P = 0.015$), SNIP (−36 ± 11 vs −69 ± 24 cmH_2O, respectively, $P = 0.022$) (Fig 1A), SNIP %pr (36 ± 12 vs 77 ± 25%, respectively, $P = 0.007$) (Fig 1B), and SVC %pr (51 ± 18 vs 96 ± 26%, respectively, $P < 0.001$) (Fig 1C) were all significantly better in the salbutamol-treated SMA II. No differences were observed for expiratory muscle strength (Fig 1D). There was a clear trend for improvement when considering mean values of MEP and MEP %pr in salbutamol-treated SMA II and II-III patients compared with SMA II control subjects; however, data were available only in three salbutamol-treated SMA II patients with a large variability. Figure 2 shows the relationships of SNIP, SNIP %pr, and Pgas Cough with age in salbutamol-treated SMA II patients and historical control subjects.

No correlations were observed between the duration of salbutamol treatment (total duration or duration with maximal dose) and absolute and %predicted values of MIP, SNIP, or SVC. However, there was a trend for a better SNIP %predicted with longer duration of salbutamol treatment (Fig 3).

The most remarkable changes in the clinical course of the series were observed in two patients who acquired sitting and walking ability without aid, respectively, whereas they were able to be seated or walk only with support before the treatment was started (Patient 1, SMA I and Patient 6, SMA II) (cf. Supplementary material).

Discussion

Our study assessed the respiratory muscle strength and clinical changes in young children with SMA taking daily oral salbutamol for more than one year. A significant

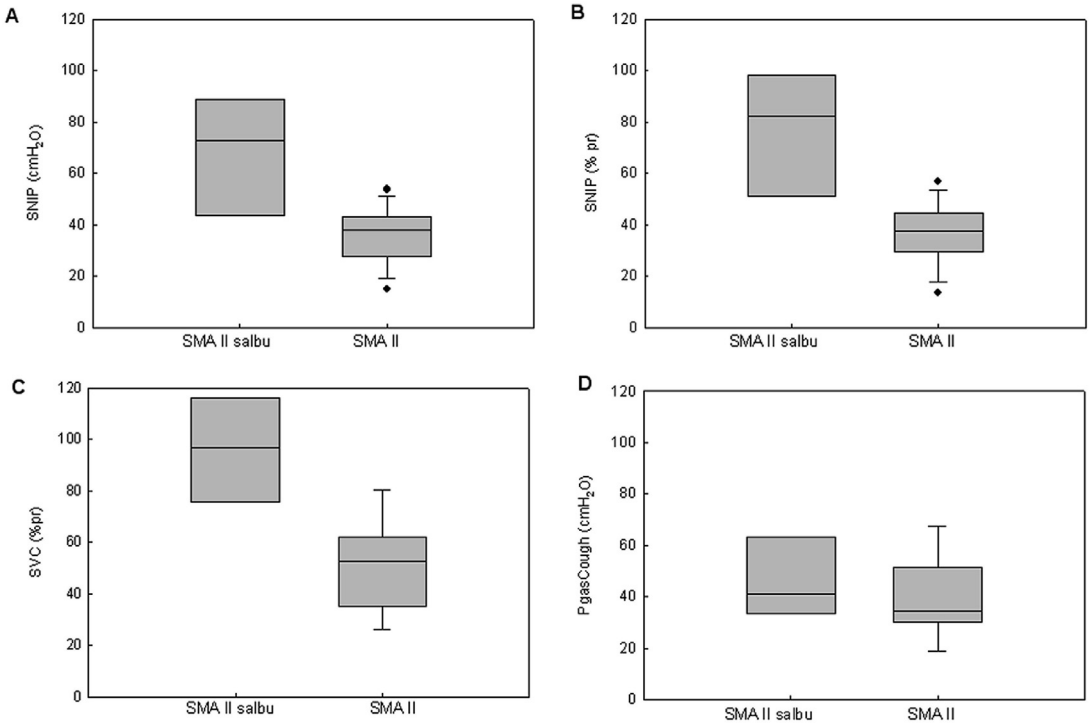

FIGURE 1.
Comparison of values of SNIP (A), SNIP %pr (B), SVC %pr (C), and Pgas Cough (D) between salbutamol-treated type II SMA patients and type II SMA historical control subjects. Median (middle line into the gray boxes), twenty-fifth to seventy-fifth percentiles and error bars are plotted. The black dots represent outliers. Pgas Cough, gastric pressure during a maximal cough; %pr, percent of predicted value; SMA, spinal muscular atrophy; SNIP, maximum sniff nasal inspiratory pressure; SVC, slow vital capacity.

increase in SVC and in the strength of the inspiratory muscles could be evidenced in the small subgroup of SMA II patients compared with mean age-matched SMA II historical control subjects. A marked clinical improvement in peripheral muscle strength and weight could also be evidenced.

Only a few studies have assessed the effects of salbutamol in SMA patients. Concerning respiratory function, Kinali et al.[17] found an improvement in forced vital capacity after 6 months of oral albuterol (SMA II and III). In the study by Pane et al.,[18] who followed 23 non-ambulant young SMA II children who received salbutamol for 12 months, families reported a subjective improvement in cough efficiency. We did not find a significant difference in the expiratory muscle strength between the treated and untreated groups estimated by an easy to perform cough maneuvers (Pgas Cough). MEP measurement, which is a complementary widespread test, was not available in all the patients because of the difficulty to perform this maneuver. A potential explanation for the lack of significant improvement in cough efficiency could be the concurrent weakness of abdominal wall muscles or the fact that measurements of expiratory pressures may underestimate cough efficiency (data not published), and therefore measurements of the peak cough flow should always be associated. Unfortunately, peak cough flow was available only in two of the five SMA II patients (data not shown) and not in the others because of technical problems or fatigue. As predicted values of inspiratory muscle strength and SVC increased by almost 100%, one can probably exclude inspiratory muscle weakness to explain the lack of improvement in cough efficiency. The sniff test represents an easy test to assess the inspiratory muscle strength, and therefore inspiratory muscle weakness, and can be performed in young children.[39] It should always be performed in complement to or in substitution of MIP, in children who present difficulty to perform MIP or in young children. The advantage of this test is that it could consent to assess the treatment effect on the inspiratory muscle strength in very young children. Endurance of the diaphragm also improved in treated SMA II children, although there was no significant difference with the historical control subjects. The treated SMA II patients had a higher inspiratory effort (PTPes and PTPdi) than historical control subjects, although not significant, mainly because of Patient 5 who was the oldest patient treated with salbutamol, having very weak respiratory muscles, and using NIV since age three years.

Oral salbutamol was followed in most of our patients by a marked clinical improvement. In particular, a striking increase in weight was observed in most of them, likely a secondary effect that could have multiple origins (increment in muscle strength or reduced fatigue for

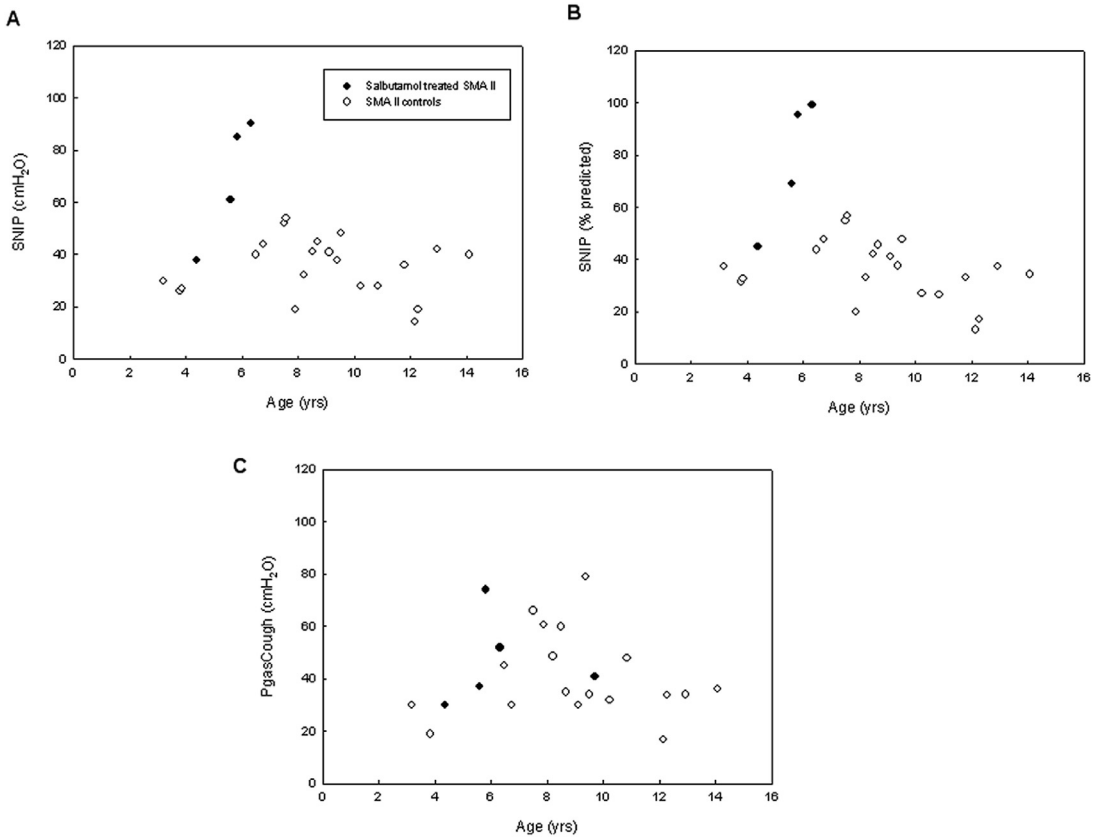

FIGURE 2.
Relationships of SNIP (A), SNIP %pr (B), and Pgas Cough (C) with age in salbutamol-treated type II SMA patients (black dots) and type II SMA historical control subjects (white dots). Pgas Cough, gastric pressure during a maximal cough; %pr, percent of predicted value; SMA, spinal muscular atrophy; SNIP, maximum sniff nasal inspiratory pressure.

swallowing or breathing, less number of infectious complications). Pane et al.[18] also found an improvement in the skeletal muscle function without significant adverse effects after 6 months of treatment in most of their children. All the families of the children who received the treatment for 12 months reported an increase in stamina and function (strength and endurance), which was confirmed by the clinical assessment showing an improvement on the used motor functional scale (Hammersmith Functional Motor Scale). Pane et al.[18] also reported that three children treated with salbutamol acquired the ability to walk independently for a few steps. In our study, we also observed a significant improvement in the motor abilities of two children, with one who acquired the ability to sit without support and another who walked without support in the year after the onset of treatment. These two patients could be therefore reclassified after salbutamol treatment as type II and type III. Concerning motor function measurements, we used the MFM scale, which has been validated for SMA patients.[40] This scale shows a better sensitivity to capture activities and possible changes in weak SMA patients compared with the Hammersmith Functional Motor Scale, including more items capturing axial and upper limb activities.[41]

Our preliminary study has several limitations. First, the timing of respiratory assessment after reaching the maximal dose of salbutamol differed among patients. Therefore one must be cautious in interpreting the effect of salbutamol on respiratory muscle function. Moreover, because the comparisons between treated and untreated patients were done with a previous SMA II cohort of children, we cannot be certain that the differences observed between both groups of patients were related only to salbutamol; also, results cannot be extended to other types of SMA. Second, the comparison was done between one salbutamol-treated patient against four historical control data (longitudinal data) and not against four individual control patients as age-matched data were not available. Overall, the treatment group consisted of a small population of patients, and results should be further confirmed in a larger population and in other types of SMA. Finally, because the respiratory assessment relies on volitional maneuvers, one should be aware of potential submaximal values. Furthermore, SVC measurements were probably not done in the same position in the two groups of patients.

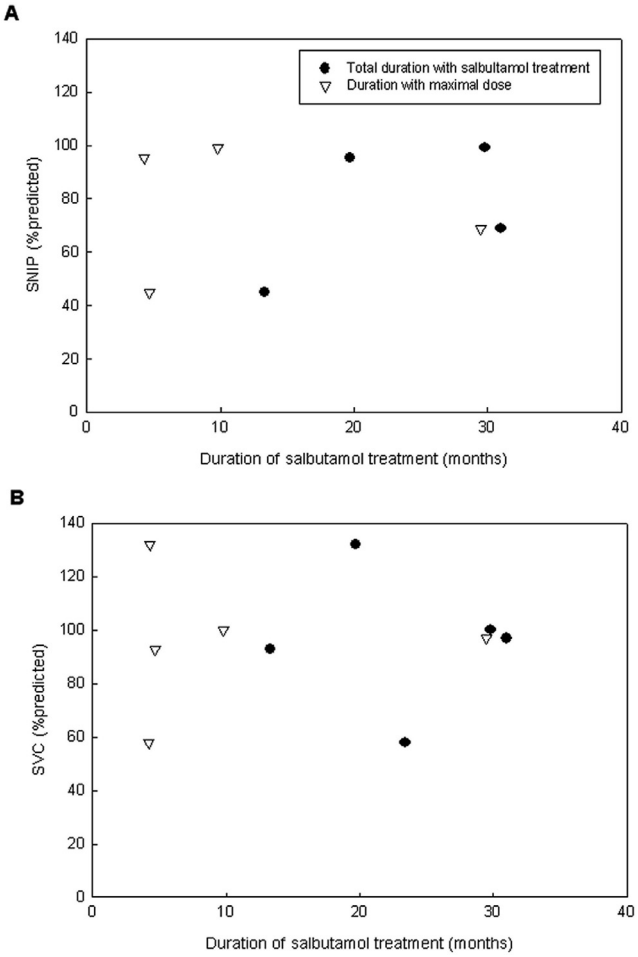

FIGURE 3.
Relationships between total duration with salbutamol (black dots) and duration with maximal dose of salbutamol (white triangles) and percent of predicted values of SNIP (A) and SVC (B). SNIP, maximum sniff nasal inspiratory pressure; SVC, slow vital capacity.

Conclusions

This preliminary study confirms the clinical interest of oral salbutamol in children with SMA and strongly suggests a selective improvement in the strength of the inspiratory muscles in SMA II patients. The results of our study are in line with previous reports and point toward a number of parameters of muscle strength, such as SVC, SNIP, and MIP, that could be used as outcome measures in future studies. This preliminary study offers the bases for further prospective studies to confirm the findings and extend to other forms of SMA. Future studies should propose a definite protocol for oral salbutamol intake and timing of the testing.

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Appendix. Results

Nine patients treated with oral salbutamol were identified. Two patients were not included in the study, a teenager boy with spinal muscular atrophy type I (SMA I), because of irregular compliance of treatment and refusal to perform the respiratory muscle assessment, and a 7 year-old girl with SMA I (who was able to sit with support) because she was tracheostomized and refused the respiratory muscle assessment. Therefore seven patients (one SMA I who was able to sit with support, five SMA II [among which one was able to stand and walk some steps with aid] and one SMA III) from 3 to 8 years old at the onset of treatment and 4 to 10 years old at the time of respiratory muscle tests were included. None had scoliosis surgery. Concerning the genetic background, six patients carried the classic exon 7 deletion at the homozygous state. The number of *SMN2* gene copies in these patients is that expected for the clinical presentation and severity (the higher the number of *SMN2* copies, the milder the phenotype). On the contrary, Patient 7 has not the habitual homozygous deletion but instead, in addition to an exon 7 deletion, she presented a heterozygote mutation in *SMN1* gene (NM_000344.3:c5C>G (p.Ala2Gly)). She only carried one copy of *SMN2* gene, which is usually associated with a very severe phenotype. However, given the fact that the phenotype of this patient is the mildest of our series (SMA III), the heterozygous mutation seems to have milder pathogenic consequences than classic exon 7 deletion. Table 1 summarizes the genetic data and the clinical course from the onset of the treatment until the respiratory muscle tests were performed. Patient 3 was the youngest patient who performed the tests. Salbutamol was taken for 23 ± 8 months (range 13 to 31 months) with a maximal dose taken for 11 ± 9 months (range 4 to 30 months). All the patients were receiving a maximal dose of salbutamol of 6 mg/day at the time of the respiratory muscle tests. Patient 5 was the oldest patient who received salbutamol. Oral salbutamol was well tolerated by all the patients, with no major adverse effects. Heart rate was within normal ranges for age and three boys had an impact on their behavior, being very prone to talk and moving excessively, but all had good performances at school. All patients gained weight from 0.3 to 5 kg/year. Table 2 shows the respiratory status before and after salbutamol use.

Mean $\triangle$Pgas/$\triangle$Pes was −0.70 ± 0.32 indicating an increased contribution of the ribcage and expiratory muscles to tidal breathing, although one SMA II patient (Patient 5) had a normal tidal breathing. Table 3 presents the lung function and respiratory muscle output, endurance, and strength parameters of all the salbutamol-treated SMA patients. Mean slow vital capacity (SVC) %predicted was within normal ranges (87 ± 26%). PTPes/minute values were only slightly greater than normality, whereas PTPdi/minute values were all greater than normal with a very high value observed in Patient 5. Respiratory muscle and diaphragmatic endurances were within the normal ranges. Inspiratory muscle strength was within normality, whereas expiratory muscle strength was decreased.

Twenty-one respiratory data charts of 11 SMA II historical control patients were selected. Mean age was 8.8 ± 3.0 years (n = 21) and was not significantly different to the mean age of the five salbutamol-treated SMA II patients (6.4 ± 2.0 years, $P = 0.100$). Tables 4 and 5 present the anthropometrics data and respiratory data of the SMA II control subjects. When comparing the respiratory data of the historical control subjects with the SMA II patients receiving salbutamol, only the mean values of MIP (−31 ± 17 vs −75 ± 48 cmH_2O, respectively, $P = 0.017$), MIP %predicted (51 ± 26 vs 132 ± 90 %pr, respectively, $P = 0.015$), SNIP (−36 ± 11 vs −69 ± 24 cmH_2O, respectively, $P = 0.022$) (Fig 1A), SNIP %predicted (36 ± 12 vs 77 ± 25%, respectively, $P = 0.007$) (Fig 1B) and SVC % predicted (51 ± 18 vs 96 ± 26%, respectively, $P < 0.001$) (Fig 1C) were significantly better in the salbutamol-treated SMA II. No differences were observed for the expiratory muscle strength (Fig 1D). There was a clear trend for improvement when considering mean values of MEP and MEP %predicted in salbutamol-treated SMA II patients compared with SMA II control subjects; however, the data were available in only three salbutamol-treated SMA II patients with a large variability. Figure 2 shows the relationships of SNIP, SNIP %pr, and Pgas Cough with age in salbutamol-treated SMA II patients and SMA II historical control subjects.

No correlations were observed between the duration of salbutamol treatment (total duration or duration with maximal dose) and absolute and %predicted values of MIP, SNIP, or SVC. However, there was a trend for a better SNIP %predicted with duration of salbutamol treatment (Fig 3).

The most remarkable changes in the clinical course of the series were observed in two patients (Patients 1 and 6). Patient 1, an 8-year-old boy, was the most severe patient of the series. He was unable to sit without support before salbutamol intake and had frequent hospitalizations because of recurrent lung atelectasis, respiratory infections, and swallowing disturbances. He received nocturnal NIV from age 3 years. At age 6 years, he weighed 15.4 kg and gastrostomy was indicated but was refused by the parents and an nasogastric-tube was used to complete oral intake. Soon after the onset of salbutamol treatment, his global condition improved, nasogastric-tube was discontinued and gastrostomy was discarded. He gained 5 kg/year during the next 2 years. Motor function and respiratory function also improved and he was able to sit without support. A 23% improvement in SVC (increase of 540 mL) was observed 20 months after the onset of treatment and motor function measurement (MFM) 20 total score improved by 10%, with a 25% increment in D2 score. Patient 6, a 6-year old boy, was already able to walk with support and had normal SVC. After 1 year with salbutamol treatment, he was able to walk unsupported for more than 30 m. MFM total score increased by 14% after 7 months of treatment, with an increment of 20% in D1 and D3 scores.

Patients 2 and 3 were too young to perform MFM tests. Patients 2, 6, and 7 had already normal or subnormal SVC values.