Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells

C Angelozzi, F Borgo, F D Tiziano, A Martella, G Neri and C Brahe

J. Med. Genet. 2008;45;29-31; originally published online 11 Oct 2007; doi:10.1136/jmg.2007.051177

Updated information and services can be found at:
http://jmg.bmj.com/cgi/content/full/45/1/29

These include:

References
This article cites 21 articles, 9 of which can be accessed free at:
http://jmg.bmj.com/cgi/content/full/45/1/29#BIBL

Rapid responses
You can respond to this article at:
http://jmg.bmj.com/cgi/eletter-submit/45/1/29

Email alerting service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

To order reprints of this article go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to Journal of Medical Genetics go to:
http://journals.bmj.com/subscriptions/
Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells

C Angelozzi, F Borgo, F D Tiziano, A Martella, G Neri, C Brahe

ABSTRACT
Spinal muscular atrophy (SMA) is an inherited neuromuscular disorder caused by homozygous absence of the survival motor neuron gene (SMN1). All patients have at least one, usually two to four copies of the related SMN2 gene which, however, produce insufficient levels of functional SMN protein due to the exclusion of exon 7 in the majority of SMN2 transcripts. Here, we show that salbutamol, a β2-adrenoceptor agonist, determines a rapid and significant increase in SMN2-full length mRNA and SMN protein in SMA fibroblasts, predominantly by promoting exon 7 inclusion in SMN2 transcripts. These data, together with previous clinical findings, provide a strong rationale to investigate further the clinical efficacy of salbutamol in SMA patients.

RESULTS
Salbutamol increases SMN2 transcript levels
SMA fibroblast cell lines were treated with 0.05 μM salbutamol for time periods ranging from 5 min to 4 h. In all three cell lines a significant increase in SMN2-full length (SMN2-fl) transcript levels (p < 0.0005) was found relative to the respective untreated cultures (fig 1). Surprisingly, an approximately twofold increase in SMN2-fl transcript levels was detected already after 5 min of treatment. The maximum increase in SMN2-fl transcripts (>200%) was observed after 30–60 min, followed by a slow decline of the SMN2 mRNA levels.

The rapid increase in SMN2-fl transcript levels led us to hypothesise that salbutamol may affect exon 7 inclusion during SMN2 mRNA maturation rather than promoter activation. Therefore, we measured both SMN2-fl and exon7-deleted (SMN2-del7) transcript levels simultaneously. Indeed, we found in parallel to the increase in SMN2-fl mRNA, a significant decrease (p < 0.02) in SMN2-del7 transcript levels (fig 1). After 5 min of treatment, SMN2-del7 transcript levels were reduced by 16.5–23% relative to the untreated cultures. After 15 min of salbutamol exposure, all three cultures showed the maximum decrease in SMN2-del7 transcripts, ranging from 30–40%; thereafter transcript levels slowly returned towards baseline.

To determine whether salbutamol may also activate transcription the total SMN2 transcript levels were measured in the three cell lines. A slight increase in total SMN2 transcript levels of up to a maximum of 50% at 15–30 min of treatment was observed, suggesting an effect of salbutamol also on promoter activation (fig 1).

As shown in fig 1, the time curves were strikingly similar for the three cell lines. Moreover, the increase in SMN2-fl transcript levels triggered by salbutamol was almost exactly...
concomitant to a decrease in SMN2-del7 levels, with the exception that the maximum decrease in SMN2-del7 transcripts slightly anticipated the peak increase in SMN2-fl mRNA (at 15 and 30 min, respectively). These data, together with the finding of a relatively modest increase in total SMN mRNA, strongly suggest that salbutamol enhances SMN2-fl transcript levels mainly by stimulating inclusion of exon 7 into the mature transcripts.

Salbutamol increases gem number and SMN protein levels

The SMN protein is localised in the cytoplasm and the nucleus where it is concentrated in dot-like structures, designated gems. The number of gems is notably reduced in SMA patients, therefore gem number count is considered a suitable test of the efficacy of a drug in enhancing SMN protein expression. For SMA type I and type II cultures, we found a 2.9-fold increase \( p(0.002) \) in the number of nuclei with gems after 1–2 h of salbutamol treatment and a maximum of 3.7- to 4.6-fold increase in the number of gems/100 nuclei after 2–4 h \( p(4.610^2) \). The SMA type III cell line, which had higher baseline gem numbers, reached a maximum of 2.3- and 2.6-fold \( p(1.310^2) \) increase in positive nuclei and gem number, respectively (Fig 2A,B). An increase in both positive nuclei and gem numbers was already detectable within 30 min of treatment in all three cell lines. Western blot analysis showed a significant increase in SMN protein levels in the salbutamol...

\[\text{Figure 1} \quad \text{Effect of salbutamol treatment on SMN2 full-length (SMN2-fl), exon 7-deleted (SMN2-del7) and total SMN2 transcript levels in fibroblast cell lines from a type I (A), a type II (B) and a type III (C) SMA patient. Each data point represents the average percentage increase/decrease in transcript levels in treated versus untreated cultures. Values represent averages of three separate experiments; for each experiment, real time PCR reactions were performed in triplicate and repeated at least two times. Error bars represent standard deviation.}\]

\[\text{Figure 2} \quad \text{Effect of salbutamol treatment on gem number and SMN protein levels in the three cell cultures. (A) Percentage of nuclei with gems and (B) number of gems/100 nuclei in salbutamol treated and untreated cell lines. (C) Representative western blot of SMN and \( \beta \)-tubulin. (D) SMN protein levels in salbutamol treated versus untreated cell cultures, quantified by densitometric analysis and normalised to \( \beta \)-tubulin used as loading control. Values represent the average \(( \pm \text{SD})\) of at least three independent experiments for each cell line.}\]
DISCUSSION

All SMA patients have at least one, usually two to four SMN2 genes, being higher copy numbers generally associated with milder phenotypes. The levels of full length SMN mRNA and functional protein produced by these genes are insufficient to prevent disease onset and progression due to alternative splicing of the majority of SMN2 transcripts. Thus, increasing the synthesis of full length mRNA from the SMN2 genes could be of invaluable therapeutic importance. Evidence has recently been provided that SMN2 gene expression can be enhanced by pharmacological treatment with different compounds. We and others have shown that the deacetylase inhibitors phenylbutyrate and valproic acid increase SMN2 levels both in vitro and in vivo, but their clinical efficacy remains unclear.1-8

No clinical data are yet available on other drugs that have been shown to affect SMN2 expression in vitro including aclarubicin, hydroxyurea, sodium vanadate, indoprofen and a quinazoline compound, and the side effects of some of them may preclude their use for SMA treatment.9-12

β2-agonists are widely used drugs, mainly for the treatment of asthma, and are well tolerated. Besides their known anabolic effects on skeletal muscle, several studies have documented also a neuroprotective effect of these drugs. For example, clenbuterol has been shown to cross the blood–brain barrier and to enhance regeneration of motor neuron axons in motor neuron degeneration (mnd) mice,17 to promote regeneration of peripheral nerves,18 and to ameliorate denervation-induced atrophy in rat models.19 Preliminary evidence for a clinical effect of β2-agonists in SMA patients has previously been reported and awaits confirmation by placebo controlled trials.19 We show here that salbutamol induces a significant and almost immediate increase in SMN2-fl mRNA in SMA fibroblasts and also a significant increase in SMN protein levels.12

The observed concomitant decrease in SMN2-de7 transcript levels suggests that salbutamol stimulates inclusion of exon 7 in SMN2 mRNA. It is known that the majority of SMN2 transcripts lack exon 7, but the percentage of full length and del7 transcripts may vary in different cell types. We and others have previously shown that in primary SMA fibroblasts between 50–80% of transcripts are del7.20 Thus, the observed 30–40% reduction of the level of SMN2-de7 transcripts in fibroblast cultures following salbutamol treatment is likely to account, at least in part, for the high increase (≥200%) in SMN2-fl transcripts. A fraction of the increase in SMN2-fl transcript levels may be ascribed to an effect of salbutamol on transcription activation, which may be related to the previously reported cAMP-responsive elements in the SMN promoter.21

To our knowledge this is the first evidence that a β2-agonist may influence the splicing pattern of a gene, which raises the possibility that splicing of other target genes could also be affected. How salbutamol mediates correct splicing of SMN2 transcripts is unknown. One possible mechanism could be the activation of specific SR-proteins which play a key role in splicing regulation. Efforts to elucidate the pathway involved in salbutamol-mediated SMN2 splicing are currently in progress.

REFERENCES