Chemistry & Biology, Vol. 11, 1489–1493, November, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.chembiol.2004.08.024

Indoprofen Upregulates the Survival Motor Neuron Protein through a Cyclooxygenase-Independent Mechanism

Brief Communication

Mitchell R. Lunn,[1,2] David E. Root,[7] Allison M. Martino,[1,2] Stephen P. Flaherty,[1,2] Brian P. Kelley,[1,2] Daniel D. Coovert,[3] Arthur H. Burghes,[3] Nguyen thi Man,[4] Glenn E. Morris,[4] Jianhua Zhou,[5] Elliot J. Androphy,[5] Charlotte J. Sumner,[6] and Brent R. Stockwell[1,2,*]

[1]Department of Biological Sciences
[2]Department of Chemistry
Columbia University
Fairchild Center, MC 2406
1212 Amsterdam Avenue
New York, New York 10027
[3]Department of Molecular and Cellular Biochemistry and Department of Neurology
Ohio State University
Columbus, Ohio 43210
[4]Centre for Inherited Neuromuscular Disease
RJAH Orthopaedic Hospital
Oswestry, Shropshire SY10 7AG
United Kingdom
[5]Department of Medicine
University of Massachusetts Medical School
Worcester, Massachusetts 01605
[6]Neurogenetics Branch
National Institute of Neurological Disorders and Stroke
NIH
Building 35, Room 2A1010
35 Convent Drive
Bethesda, Maryland 20892

Summary

Most patients with the pediatric neurodegenerative disease spinal muscular atrophy have a homozygous deletion of the survival motor neuron 1 (*SMN1*) gene, but retain one or more copies of the closely related *SMN2* gene. The *SMN2* gene encodes the same protein (SMN) but produces it at a low efficiency compared with the *SMN1* gene. We performed a high-throughput screen of ~47,000 compounds to identify those that increase production of an *SMN2*-luciferase reporter protein, but not an *SMN1*-luciferase reporter protein. Indoprofen, a nonsteroidal anti-inflammatory drug (NSAID) and cyclooxygenase (COX) inhibitor, selectively increased *SMN2*-luciferase reporter protein and endogenous SMN protein and caused a 5-fold increase in the number of nuclear gems in fibroblasts from SMA patients. No other NSAIDs or COX inhibitors tested exhibited this activity.

*Correspondence: stockwell@biology.columbia.edu
[7]Current address: Broad Institute of Harvard and Massachusetts Institute of Technology, 320 Charles Street, Cambridge, Massachusetts 02141.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by rapid degeneration of lower motor neurons in the anterior horn of the spinal cord due to reduced survival motor neuron (SMN) protein. SMA is the leading genetic cause of infant mortality in the United States and Western Europe, with an incidence of 1 in 6000 live births and a carrier frequency of 1 in 40 [1–7]. There is no treatment for this orphan genetic disease. Studies of the underlying molecular pathology of SMA have the potential to reveal essential aspects of motor neuron function, aspects of SMN protein function and regulation, and therapeutic candidates for the disease [8–10].

We developed a high-throughput assay to detect reporter protein production from *SMN1*- and *SMN2*-minigene-reporter constructs, denoted *SMN1*-luc and *SMN2*-luc. We screened ~47,000 compounds from diverse compound libraries for those that could affect production of the SMN protein [11]. We discovered that two compounds—aclarubicin and 2-[4-(1-oxo-2-isoindolinyl)-phenyl]propionic acid (indoprofen)—selectively upregulated *SMN2*-reporter activity. Aclarubicin has been previously shown to modulate the splicing of *SMN2* [12]. Here, we describe the SMN regulating activity of indoprofen, a nonsteroidal anti-inflammatory drug.

Results

Indoprofen Selectively Increases Luminescence from an *SMN2*-Minigene-Reporter Construct

We developed a high-throughput reporter assay designed to detect SMN production. We used C33a cells stably expressing an *SMN*-minigene with luciferase reporter [13] that consisted of exons 6 through 8 and intervening introns, for either *SMN1* or *SMN2*. Luciferase, the reporter gene's product, is only produced when proper splicing and translation occurs.

We tested 20,000 compounds from a combinatorial library, 1040 compounds from a National Institute of Neurological Disorders and Stroke (NINDS) library, 2337 compounds from our Annotated Compound Library [14], and 23,685 compounds from our TIC Library, which is a composite of compounds purchased from TimTec, IBS, and ChemBridge that were selected for specific properties, including stereochemical complexity [11]. We discovered that indoprofen significantly increased reporter activity from *SMN2*-luc cells relative to *SMN1*-luc cells (Figures 1 and 2). The most effective concentration, 2.8 μg/ml (~10 μM, MW = 281.3), resulted in a 3-fold increase in luminescence. The raw data is available in the Supplemental Data of our companion paper in this issue [11] (available online at http://www.chembiol.com/cgi/content/full/11/11/1489/DC1/).

To find related active compounds, we tested those with structures similar to indoprofen, including NSAIDs, and found none that increased luminescence. Com-

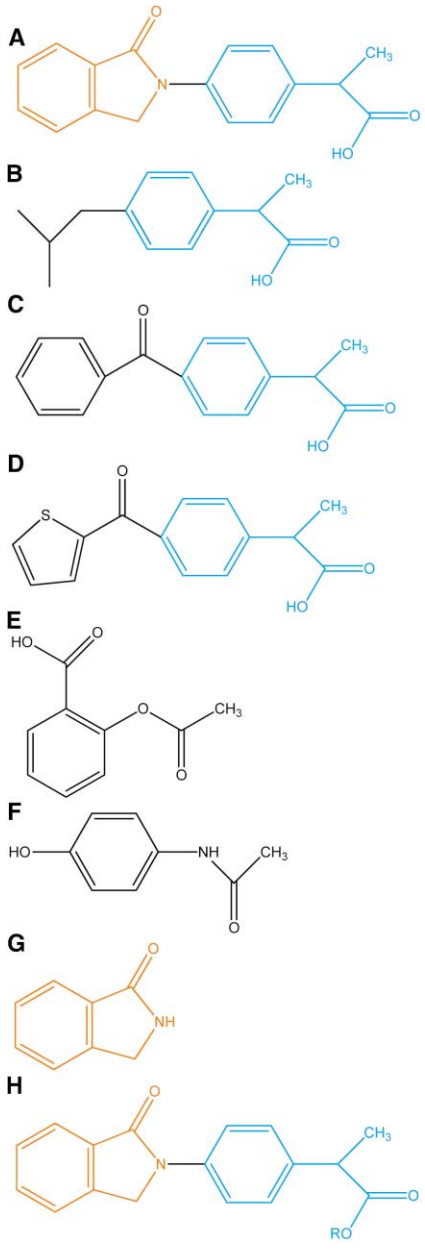

Figure 1. Indoprofen and Related Compounds: Chemical Structures and Maximum Percent Luminescence Enhancement from *SMN1* and *SMN2* Minigene-Reporter Constructs for Treated versus Untreated Cells

(A) Indoprofen; *SMN1*: 44%, *SMN2*: 184%. (B) Ibuprofen; *SMN1*: 5.6%, *SMN2*: −0.8%. (C) Ketoprofen; *SMN1*: −30%, *SMN2*: −21%. (D) Suprofen; *SMN1*: −4.0%, *SMN2*: −5.0%. (E) Aspirin; *SMN1*: −5.1%, *SMN2*: −3.0%. (F) Acetaminophen; *SMN1*: −17%, *SMN2*: −9.5%. (G) Isoindolinone; *SMN1*: 18%, *SMN2*: 21%. (H) Esterified indoprofen derivatives. R = methyl; *SMN1*: 38%, *SMN2*: 72%. R = ethyl; *SMN1*: 18%, *SMN2*: 98%. R = isopropyl; *SMN1*: 18%, *SMN2*: 70%. The isoindolinone group ("indo") is shown in orange, and the phenylpropionic acid group ("profen") is shown in blue. All chiral compounds were racemic mixtures.

pounds with only the 2-phenylpropionic acid group or the isoindolinone group did not enhance luminescence. Methyl, ethyl, and isopropyl esters of indoprofen retained some degree of activity and selectivity, but this may have been due to simple hydrolysis in cells and generation of indoprofen (Figure 1).

Despite the noteworthy increase in *SMN2*-minigene-reporter activity, real-time RT-PCR using SMA patient fibroblasts failed to show an increase in the ratio of full-length to truncated transcripts, or in the absolute level of transcripts following indoprofen treatment (data not shown). Considering indoprofen's selective activity for *SMN2*-luc, it is unlikely that indoprofen is acting post-translationally, as both *SMN1*-luc and *SMN2*-luc encode the same protein. We propose that indoprofen has a pre- or cotranslational effect on protein production from *SMN2*, through a cyclooxygenase-independent mechanism. For example, it is possible that indoprofen, resembling a nucleotide, binds to the *SMN2* pre-mRNA and displaces proteins that reduce the rate of translation. We sought evidence as to whether indoprofen also affects the level of endogenous SMN protein in human cells.

Indoprofen Increases SMN Protein Level in Human Type I SMA Patient Fibroblasts

To assess whether indoprofen treatment affected SMN protein production, we treated type I SMA patient fibroblasts (3813) with indoprofen and assessed protein level by Western blotting (Figure 2). We treated 3813 cells with 5 and 20 μM indoprofen for 3 days with daily media and compound changes, even though liquid chromatography-mass spectroscopy (LC-MS) revealed that there was no significant degradation of indoprofen over 4 days in cell culture medium (data not shown). Both concentrations of indoprofen treatments yielded similar effects on SMN protein increase, although occasionally higher levels were observed from either treatment concentration (Figure 2). Combining data from both treatments, indoprofen-treated cells resulted in a mean 13% increase in SMN protein production versus untreated cells (independent, one-tailed t test; $n = 17$ [9 treated samples, 8 control samples], $\nu = 15$, $p < 0.014$).

Indoprofen Increases Number of Nuclear Gems in Human Type I SMA Patient Fibroblasts

The increase in SMN protein production led us to inquire about the effect of indoprofen on the overall number of gems—punctate structures in the nucleus. The number of gems (short for "Gemini of coiled [Cajal] bodies") directly correlates with SMN protein production [5], and they are found in many adult cell types (especially neurons) and in all fetal tissues. Fibroblasts from normal patients, SMA carriers, and type I SMA patients have ~80 gems, ~40 gems, and ~1 to 2 gems per 100 nuclei, respectively [5].

We treated human type I SMA fibroblasts (2806) with indoprofen (5 and 15 μM) to observe changes in gem count. Both indoprofen treatments yielded a significant increase in gem count (5 μM: independent, one-tailed t test; $n = 17$ [8 treated, 9 untreated], $\nu = 15$, $p = 6.9 \times 10^{-4}$ and 15 μM: independent, one-tailed t test; $n = 16$ [7 treated, 9 untreated], $\nu = 14$, $p = 1.7 \times 10^{-4}$) (Figure 2). Pooling the two treatments also generated a significant result (independent, one-tailed t test; $n = 24$ [15 treated,

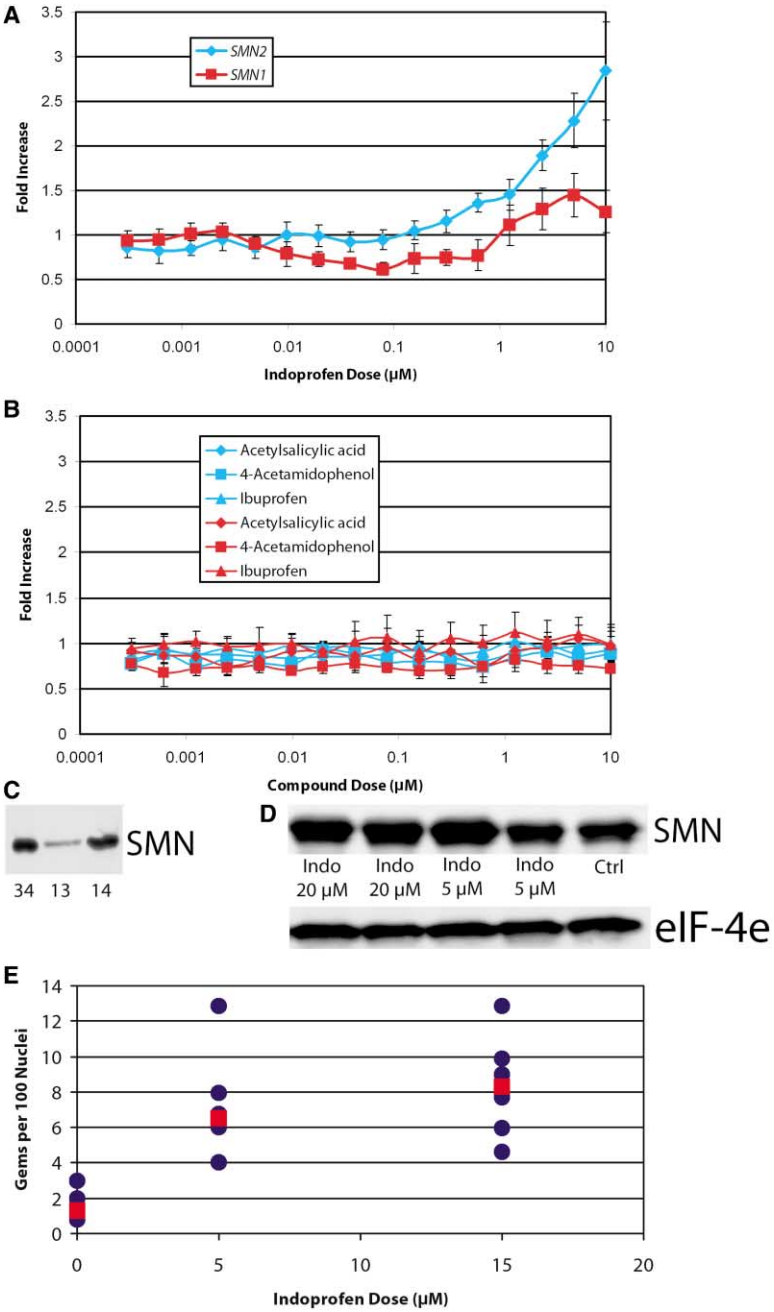

Figure 2. Indoprofen Increases Minigene-Reporter Luminescence, SMN Protein Level, and Number of Nuclear Gems

(A) C33a cells with a minigene-reporter construct containing either *SMN1* (red) or *SMN2* (blue) treated with indoprofen. A similar result was obtained in a mouse neuronal cell line (NSC34) (data not shown).

(B) Aspirin (acetylsalicylic acid), acetaminophen (4-acetamidophenol), and ibuprofen were tested in c33a cells with the same constructs as in (A).

(C) Type I SMA patient fibroblasts (13) with no *SMN1* have less SMN protein than carrier fibroblasts (14) that have 1 copy of *SMN1*. NSC34, a mouse neuronal cell line, cells (34) with two copies of *SMN1* exhibit an effect that is visually indistinguishable from carrier fibroblasts.

(D) Indoprofen-treated cells had a mean 13% increase in SMN protein production over untreated cells (independent, one-tailed t test; $n = 17$, $\nu = 15$, $p < 0.0139$) as determined by densitometric analysis. Indoprofen-treated samples and control sample are noted. An initiation factor (eIF-4e) is shown as a loading control.

(E) Indoprofen treatment of type I SMA patient fibroblasts (2806) results in an increase in gem count when compared to nontreated cells. Blue circles represent samples within each treatment. Red squares represent the mean number of gems per 100 nuclei within each treatment (0 μM: 1.3; 5 μM: 6.5; and 15 μM: 8.3).

9 untreated], $\nu = 22$, $p = 3.2 \times 10^{-7}$]. Additionally, we tested one other type I SMA fibroblast cell line (3813) and one mouse fibroblast cell line (25). These cell lines yielded comparable results to 2806 (see Supplemental Data available online at http://www.chembiol.com/cgi/content/full/11/11/1489/DC1/).

Effect of Indoprofen in a Mouse Model of SMA

In an attempt to find a maximum effective dose, the pharmacokinetics of indoprofen in mice were studied. Pregnant mice were treated with a single dose of 20 mg/kg of indoprofen, using either intraperitoneal (IP) injection or oral gavage, to determine how much indoprofen enters the brain, blood, and embryo after treatment. Consistent with previous rodent studies using ^{14}C-labeled indoprofen [15], liquid chromatography-mass spectrometry (LC-MS) revealed that no indoprofen was present in brain tissue 4 hr after treatment (data not shown). Indoprofen was found in the both the plasma of the pregnant mice and their embryos one hour after treatment. Both routes of administration resulted in ~5.3 μM indoprofen plasma concentration, while the IP method was shown to be more effective than gavage at delivering indoprofen to embryonic tissue. An average concentration of ~3.0 μM indoprofen was found in embryos of IP-treated mice at embryonic day 13 (E13).

We tested the effect of indoprofen on the viability of SMA model mice, which lack murine *Smn* but contain

Table 1. Numbers of Non-SMA and SMA Litters and Embryos from Untreated and Indoprofen-Treated Mother Mice

Litters		
	Non-SMA	SMA
Untreated	7	0
Indoprofen treated	4	3
Embryos		
	Non-SMA	SMA
Untreated	57	0
Indoprofen treated	39	3

a human *SMN2* transgene (*i.e.*, *Smn*$^{-/-}$; *TgSMN2*$^{+/-}$) [16]. To generate litters that contained 25% *Smn*$^{-/-}$; *TgSMN2*$^{+/-}$ mice, we mated *Smn*$^{+/-}$; *TgSMN2*$^{+/+}$ mice with *Smn*$^{+/-}$; *TgSMN2*$^{-/-}$ mice. Our SMA model mice were derived from the published transgenic model created in C57BL/6 and crossed with FVB (Taconic Labs) [16]. These mice were backcrossed once with a wild-type FVB mouse to generate our C57BL/6/FVB mice. We found that in this strain of *Smn*$^{-/-}$; *TgSMN2*$^{+/-}$, embryos die at approximately embryonic day 11 (E11).

To determine whether indoprofen could increase the viability of these SMA model embryos, we treated such pregnant mice twice daily for the first 14 days of pregnancy by IP injection with 5 mg/kg indoprofen in phosphate buffered saline, the maximum dose that exhibited no toxicity within the 14 days. On embryonic day 14 (E14), we genotyped the embryos and ascertained the number of *Smn*$^{-/-}$; *TgSMN2*$^{+/-}$ (SMA genotype) embryos (Table 1). At E14, none of the 7 untreated litters harbored any SMA genotype embryos, whereas 3 of 7 indoprofen-treated litters harbored SMA genotype embryos (Fisher Exact test, $\nu = 1$, $p = 0.096$). Thus, there was a trend in which indoprofen increased the viability of SMA model mice. As expected, indoprofen treatment significantly increased the mean litter size from 5.7 embryos to 6.9 embryos (independent, one-tailed t test; $n = 14$, $\nu = 12$, $p = 0.040$). There was also a trend in which indoprofen increased the number of SMA embryos (Fisher Exact test, $\nu = 1$, $p = 0.073$).

Discussion

Most SMA patients have a homozygous deletion of *SMN1* and are consequently left with an insufficient level of SMN protein to prevent disease. This protein has been further implicated in many cellular processes including snRNP biogenesis [17–21], mRNA splicing [22–26], apoptosis [27–30], and axonal transport [31–33].

We have discovered a nonsteroidal anti-inflammatory drug (indoprofen) that produces an increase in luminescence in *SMN2*-luc cells compared to *SMN1*-luc and untreated cells. This *SMN2*-selective activity indicates that indoprofen is not simply acting on the construct's promoter. Indoprofen treatment of type I SMA patient fibroblasts results in a small increase in SMN protein level, and in the number of nuclear gems. Finally, indoprofen treatment caused a trend in which embryonic viability in SMA model mice was increased.

Other candidate drugs for treating SMA patients have shortcomings, such as severe side effects [34], extremely short half-life [35, 36] or nonselectivity of mechanism [37–39]. Indoprofen is an NSAID with minimal side effects [40, 41] that may be worthy of study as a therapeutic agent for SMA. As indoprofen is not modifying splicing, possible additive effects may result from combination treatment with other drugs. In addition, it may serve as a chemical probe to identify proteins that regulate the production of SMN protein.

Significance

Chemical genetic screening approaches can be used to discover both novel therapeutic agents and chemical tools for genetic diseases. We describe such an approach for the pediatric genetic disease spinal muscular atrophy (SMA). We developed a high-throughput assay that detects production of an SMN-luciferase reporter protein, and we used this assay to identify compounds that increase production of SMN protein in cells. In a screen of ~47,000 compounds, we discovered that a nonsteroidal anti-inflammatory drug (indoprofen) increases endogenous SMN protein level in type I SMA patient fibroblasts. Additionally, we found that indoprofen caused a 5- to 6-fold increase in the number of nuclear gems, an indicator of greater intracellular SMN protein concentration. No other NSAIDs displayed this ability to upregulate SMN protein level. This suggests that SMN upregulation is not caused by inhibiting COX activity (the known target of NSAIDs). Finally, we found that indoprofen caused a trend toward increased viability of SMA model mice. Indoprofen may prove to be a treatment for SMA, and it may serve as a chemical tool to identify novel proteins regulating SMN protein production.

Supplemental Data

Detailed experimental procedures are available as Supplemental Data at http://www.chembiol.com/cgi/content/full/11/11/1489/DC1/.

Acknowledgments

We thank Jill Heemskerk for valuable suggestions and Harmen Bussemaker for advice on statistical tests. This research was funded by grants from Andrew's Buddies and Families of SMA. B.R.S. is supported in part by a Career Award at the Scientific Interface from the Burroughs Wellcome Fund.

Received: July 10, 2004
Revised: August 12, 2004
Accepted: August 19, 2004
Published: November 29, 2004

References

1. Melki, J., Lefebvre, S., Burglen, L., Burlet, P., Clermont, O., Millasseau, P., Reboullet, S., Benichou, B., Zeviani, M., Le Paslier, D., et al. (1994). De novo and inherited deletions of the 5q13 region in spinal muscular atrophies. Science *264*, 1474–1477.
2. Melki, J., Abdelhak, S., Sheth, P., Bachelot, M.F., Burlet, P., Marcadet, A., Aicardi, J., Barois, A., Carriere, J.P., Fardeau, M., et al. (1990). Gene for chronic proximal spinal muscular atrophies maps to chromosome 5q. Nature *344*, 767–768.
3. Pearn, J. (1980). Classification of spinal muscular atrophies. Lancet *1*, 919–922.
4. Lefebvre, S., Burglen, L., Reboullet, S., Clermont, O., Burlet, P.,

Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., et al. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. Cell *80*, 155–165.

5. Coovert, D.D., Le, T.T., McAndrew, P.E., Strasswimmer, J., Crawford, T.O., Mendell, J.R., Coulson, S.E., Androphy, E.J., Prior, T.W., and Burghes, A.H. (1997). The survival motor neuron protein in spinal muscular atrophy. Hum. Mol. Genet. *6*, 1205–1214.
6. Lefebvre, S., Burlet, P., Liu, Q., Bertrandy, S., Clermont, O., Munnich, A., Dreyfuss, G., and Melki, J. (1997). Correlation between severity and SMN protein level in spinal muscular atrophy. Nat. Genet. *16*, 265–269.
7. McAndrew, P.E., Parsons, D.W., Simard, L.R., Rochette, C., Ray, P.N., Mendell, J.R., Prior, T.W., and Burghes, A.H. (1997). Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. Am. J. Hum. Genet. *60*, 1411–1422.
8. Stockwell, B.R. (2002). Chemical genetic screening approaches to neurobiology. Neuron *36*, 559–562.
9. Stockwell, B.R. (2000). Chemical genetics: ligand-based discovery of gene function. Nat. Rev. Genet. *1*, 116–125.
10. Stockwell, B.R. (2000). Frontiers in chemical genetics. Trends Biotechnol. *18*, 449–455.
11. Kelley, B.P., Lunn, M.R., Root, D.E., Flaherty, S.P., Martino, A.M., and Stockwell, B.R. (2004). A flexible data analysis tool for chemical genetic screens. Chem. Biol. *11*, this issue, 1495–1503.
12. Andreassi, C., Jarecki, J., Zhou, J., Coovert, D.D., Monani, U.R., Chen, X., Whitney, M., Pollok, B., Zhang, M., Androphy, E., et al. (2001). Aclarubicin treatment restores SMN levels to cells derived from type I spinal muscular atrophy patients. Hum. Mol. Genet. *10*, 2841–2849.
13. Zhang, M.L., Lorson, C.L., Androphy, E.J., and Zhou, J. (2001). An in vivo reporter system for measuring increased inclusion of exon 7 in SMN2 mRNA: potential therapy of SMA. Gene Ther. *8*, 1532–1538.
14. Root, D.E., Flaherty, S.P., Kelley, B.P., and Stockwell, B.R. (2003). Biological mechanism profiling using an annotated compound library. Chem. Biol. *10*, 881–892.
15. Goldaniga, G.C., Montesanti, L., and Valzelli, G. (1980). Tissue distribution of 14C-indoprofen in the rat. Arzneimittelforschung *30*, 1659–1661.
16. Monani, U.R., Sendtner, M., Coovert, D.D., Parsons, D.W., Andreassi, C., Le, T.T., Jablonka, S., Schrank, B., Rossol, W., Prior, T.W., et al. (2000). The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(−/−) mice and results in a mouse with spinal muscular atrophy. Hum. Mol. Genet. *9*, 333–339.
17. Buhler, D., Raker, V., Luhrmann, R., and Fischer, U. (1999). Essential role for the tudor domain of SMN in spliceosomal U snRNP assembly: implications for spinal muscular atrophy. Hum. Mol. Genet. *8*, 2351–2357.
18. Carvalho, T., Almeida, F., Calapez, A., Lafarga, M., Berciano, M.T., and Carmo-Fonseca, M. (1999). The spinal muscular atrophy disease gene product, SMN: a link between snRNP biogenesis and the Cajal (coiled) body. J. Cell Biol. *147*, 715–728.
19. Fischer, U., Liu, Q., and Dreyfuss, G. (1997). The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell *90*, 1023–1029.
20. Lefebvre, S., Burglen, L., Frezal, J., Munnich, A., and Melki, J. (1998). The role of the SMN gene in proximal spinal muscular atrophy. Hum. Mol. Genet. *7*, 1531–1536.
21. Pellizzoni, L., Charroux, B., and Dreyfuss, G. (1999). SMN mutants of spinal muscular atrophy patients are defective in binding to snRNP proteins. Proc. Natl. Acad. Sci. USA *96*, 11167–11172.
22. Kashima, T., and Manley, J.L. (2003). A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. Nat. Genet. *34*, 460–463.
23. Miyaso, H., Okumura, M., Kondo, S., Higashide, S., Miyajima, H., and Imaizumi, K. (2003). An intronic splicing enhancer element in survival motor neuron (SMN) pre-mRNA. J. Biol. Chem. *278*, 15825–15831.
24. Mourelatos, Z., Abel, L., Yong, J., Kataoka, N., and Dreyfuss, G. (2001). SMN interacts with a novel family of hnRNP and spliceosomal proteins. EMBO J. *20*, 5443–5452.
25. Pellizzoni, L., Kataoka, N., Charroux, B., and Dreyfuss, G. (1998). A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. Cell *95*, 615–624.
26. Skordis, L.A., Dunckley, M.G., Yue, B., Eperon, I.C., and Muntoni, F. (2003). Bifunctional antisense oligonucleotides provide a *trans*-acting splicing enhancer that stimulates SMN2 gene expression in patient fibroblasts. Proc. Natl. Acad. Sci. USA *100*, 4114–4119.
27. Iwahashi, H., Eguchi, Y., Yasuhara, N., Hanafusa, T., Matsuzawa, Y., and Tsujimoto, Y. (1997). Synergistic anti-apoptotic activity between Bcl-2 and SMN implicated in spinal muscular atrophy. Nature *390*, 413–417.
28. Schrank, B., Gotz, R., Gunnersen, J.M., Ure, J.M., Toyka, K.V., Smith, A.G., and Sendtner, M. (1997). Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc. Natl. Acad. Sci. USA *94*, 9920–9925.
29. Kerr, D.A., Nery, J.P., Traystman, R.J., Chau, B.N., and Hardwick, J.M. (2000). Survival motor neuron protein modulates neuron-specific apoptosis. Proc. Natl. Acad. Sci. USA *97*, 13312–13317.
30. Young, P.J., Day, P.M., Zhou, J., Androphy, E.J., Morris, G.E., and Lorson, C.L. (2002). A direct interaction between the survival motor neuron protein and p53 and its relationship to spinal muscular atrophy. J. Biol. Chem. *277*, 2852–2859.
31. Rossoll, W., Jablonka, S., Andreassi, C., Kroning, A.K., Karle, K., Monani, U.R., and Sendtner, M. (2003). Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of β-actin mRNA in growth cones of motoneurons. J. Cell Biol. *163*, 801–812.
32. Zhang, H.L., Pan, F., Hong, D., Shenoy, S.M., Singer, R.H., and Bassell, G.J. (2003). Active transport of the survival motor neuron protein and the role of exon-7 in cytoplasmic localization. J. Neurosci. *23*, 6627–6637.
33. Fan, L., and Simard, L.R. (2002). Survival motor neuron (SMN) protein: role in neurite outgrowth and neuromuscular maturation during neuronal differentiation and development. Hum. Mol. Genet. *11*, 1605–1614.
34. Case, D.C., Jr., Ervin, T.J., Boyd, M.A., Bove, L.G., Sonneborn, H.L., and Paul, S.D. (1987). Phase II study of aclarubicin in acute myeloblastic leukemia. Am. J. Clin. Oncol. *10*, 523–526.
35. Chang, J.G., Hsieh-Li, H.M., Jong, Y.J., Wang, N.M., Tsai, C.H., and Li, H. (2001). Treatment of spinal muscular atrophy by sodium butyrate. Proc. Natl. Acad. Sci. USA *98*, 9808–9813.
36. Daniel, P., Brazier, M., Cerutti, I., Pieri, F., Tardivel, I., Desmet, G., Baillet, J., and Chany, C. (1989). Pharmacokinetic study of butyric acid administered in vivo as sodium and arginine butyrate salts. Clin. Chim. Acta *181*, 255–263.
37. Phiel, C.J., Zhang, F., Huang, E.Y., Guenther, M.G., Lazar, M.A., and Klein, P.S. (2001). Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J. Biol. Chem. *276*, 36734–36741.
38. Brichta, L., Hofmann, Y., Hahnen, E., Siebzehnrubl, F.A., Raschke, H., Blumcke, I., Eyupoglu, I.Y., and Wirth, B. (2003). Valproic acid increases the SMN2 protein level: a well-known drug as a potential therapy for spinal muscular atrophy. Hum. Mol. Genet. *12*, 2481–2489.
39. Sumner, C.J., Huynh, T.N., Markowitz, J.A., Perhac, J.S., Hill, B., Coovert, D.D., Schussler, K., Chen, X., Jarecki, J., Burghes, A.H., et al. (2003). Valproic acid increases SMN levels in spinal muscular atrophy patient cells. Ann. Neurol. *54*, 647–654.
40. Bruni, G., Lavezzari, M., Perbellini, A., Battaglia, A., and Emanueli, A. (1982). Adverse reactions to indoprofen: a survey based on a total of 6764 patients. J. Int. Med. Res. *10*, 306–324.
41. Porro, G.B., Corvi, G., Fuccella, L.M., Goldaniga, G.C., and Valzelli, G. (1977). Gastro-intestinal blood loss during administration of indoprofen, aspirin and ibuprofen. J. Int. Med. Res. *5*, 155–160.