

## EXTENDED THERAPY WITH INTRAVENOUS ARGININE BUTYRATE IN PATIENTS WITH $\beta$ -HEMOGLOBINOPATHIES

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**Abstract Background.** Enhanced production of fetal hemoglobin lessens the severity of  $\beta$ -thalassemia and sickle cell disease. Intravenous infusion of arginine butyrate can increase the number of reticulocytes containing fetal hemoglobin in patients with these disorders, and it has induced a substantial increase in hemoglobin in one patient with thalassemia. We therefore tested the efficacy of this agent in patients with  $\beta$ -hemoglobinopathies.

**Methods.** We treated 10 patients with severe  $\beta$ -thalassemia or sickle cell disease with arginine butyrate at an initial dose of 500 mg per kilogram of body weight per day (final dose, 2000 mg per kilogram per day), 6 days per week, for a mean ( $\pm$ SD) of  $10 \pm 1.2$  weeks (range, 9 to 13). A hematologic response was defined as an increase in the hemoglobin concentration of at least 2 g per deciliter

in patients with thalassemia and as a twofold increase in fetal hemoglobin in patients with sickle cell disease.

**Results.** There were increases in  $\gamma$ -globin messenger RNA and in reticulocytes containing fetal hemoglobin, but no increases in hemoglobin, in the patients with thalassemia. A small, unsustained increase in fetal hemoglobin was observed in two patients with sickle cell disease. Drug toxicity was minimal at standard doses. One patient had a grand mal seizure after inadvertently receiving 2000 mg of arginine butyrate per kilogram over a period of six hours.

**Conclusions.** Ten weeks of intravenous arginine butyrate did not produce a hematologic response in 10 patients with either severe  $\beta$ -thalassemia or sickle cell disease. (N Engl J Med 1995;332:1606-10.)

ENHANCED production of fetal hemoglobin ( $\alpha_2\gamma_2$ ) lessens the severity of the two major  $\beta$ -hemoglobinopathies,  $\beta$ -thalassemia and sickle cell disease. In homozygous  $\beta$ -thalassemia, reduced or absent production of  $\beta$  chains results in an excess of unpaired  $\alpha$ -globin chains, which precipitate within the red cell, thus causing ineffective erythropoiesis and severe anemia. After the first year of life, when the switch from the production of  $\gamma$  chains to  $\beta$  chains normally occurs, most patients with homozygous  $\beta$ -thalassemia begin to require regular transfusions of red cells. Increased synthesis of  $\gamma$  chains reduces the imbalance between  $\gamma$  chains and  $\beta$  chains in thalassemia by increasing the synthesis of non- $\alpha$  chains. Increased synthesis of fetal hemoglobin reduces the severity of sickle cell disease through a different mechanism: it inhibits the polymerization of sickle hemoglobin and reduces sickling in vivo.<sup>1-6</sup> The investigational use of chemotherapeutic agents to stimulate the production of fetal hemoglobin<sup>7-22</sup> has aroused concern about the long-term effects of these drugs in nonmalignant disorders. A group of nonchemotherapeutic compounds, the butyric acid analogues, might be used as an alternative therapy for the  $\beta$ -hemoglobinopathies. The rationale for their use comes from reports of a delay in the normal switch from production of  $\gamma$  globin to  $\beta$  globin in newborn infants of diabetic mothers,<sup>23,24</sup> a finding subsequently attributed to elevated plasma concentrations of  $\alpha$ -amino-

*n*-butyric acid.<sup>25,26</sup> Studies of the effects of butyrate on fetal-globin genes in vitro<sup>26-29</sup> and in animals<sup>25,30,31</sup> were followed by a pilot trial<sup>32</sup> in which the administration of arginine butyrate to one patient with  $\beta$ -thalassemia increased the total hemoglobin concentration by 6 g per deciliter, a response that stimulated much enthusiasm for butyrate in the treatment of these disorders.<sup>33</sup> We now report the results of a study of extended administration of arginine butyrate to patients with  $\beta$ -thalassemia or sickle cell disease.

### METHODS

#### Patients

Ten patients (mean [ $\pm$ SD] age,  $15.1 \pm 10.5$  years; range, 2.6 to 38), were admitted to the hospital for the administration of intravenous arginine butyrate (Table 1). Five patients had sickle cell disease: four of them had sickle cell anemia and one had compound heterozygosity for hemoglobin S and  $\beta^0$ -thalassemia. Of the five patients with thalassemia, four had homozygous  $\beta$ -thalassemia and one had compound heterozygosity for hemoglobin E and  $\beta^0$ -thalassemia. Seven patients had received red-cell transfusions, which were discontinued 4 to 25 weeks before the start of the study. Mutations in the  $\alpha$ -globin and  $\beta$ -globin gene clusters were determined as previously described.<sup>34</sup> The nucleotide substitution of threonine for cysteine 158 base pairs downstream from the 5' end of the G $\gamma$ -globin gene was determined by analysis with the restriction enzyme *Xmn*I.<sup>35</sup> The presence of this substitution is noted as *Xmn*I+, and its absence as *Xmn*I- (Table 1).

Arginine butyrate was administered at a dose of 500 mg per kilogram of body weight during the first 24 hours of treatment. In nine patients, the dose was increased over the subsequent 48-hour period to a maximum of 2000 mg per kilogram per day. In the 10th patient the maximal dose was 1500 mg per kilogram per day because of severe nausea with any further increase in the dose. Arginine butyrate was infused through a central venous catheter 24 hours a day 5 to 6 days a week for a mean ( $\pm$ SD) of  $10 \pm 1.2$  weeks (range, 9 to 13). In two patients (Patients 6 and 9) this regimen was followed by intermittent therapy (30 to 50 hours per week for another 16 and 5 weeks, respectively). All patients received daily folic acid.

The study was approved by the institutional review boards of the Hospital for Sick Children and Toronto Hospital, the Food and Drug Administration, and the Health Protection Branch, Ottawa,

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Table 1. Characteristics and Transfusion Histories of 10 Patients Receiving Arginine Butyrate.

PATIENT NO.	AGE (YR)	SEX	GENOTYPE*	TRANSFUSION HISTORY
<b><math>\beta</math>-Thalassemia</b>				
1	2.6	F	Homozygous $\beta$ -thalassemia (619-bp deletion) - $\alpha^{37}/\alpha\alpha$ <i>XmnI</i> -/-	No previous transfusions
2	5.0	F	Homozygous $\beta^+$ -thalassemia (IVS-1#6/IVS-1#110) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Irregular transfusions; last transfusion 5 wk before study entry
3	5.8	F	Homozygous $\beta^0$ -thalassemia (41/42[-CTTT]/A $\rightarrow$ T at codon 17) - $\alpha^{37}/\alpha\alpha$ <i>XmnI</i> -/-	Transfusion-dependent; last transfusion 4 wk before study entry
4	16.1	F	Homozygous $\beta^+$ -thalassemia (IVS-1#6/IVS-1#110) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Infrequent transfusions; last transfusion >1 yr before study entry
5	38.4	M	$\beta^0$ -thalassemia/hemoglobin E (41/42[-CTTT]/G $\rightarrow$ A at codon 28) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> +/-	No previous transfusions
<b>Sickle cell disease</b>				
6	8.8	F	Hemoglobin SS - $\alpha^{37}/\alpha\alpha$ <i>XmnI</i> -/-	No previous transfusions
7	16.9	M	Hemoglobin S/ $\beta^0$ -thalassemia (loss of CT at codon 5) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> not determined	Regular transfusions; last transfusion 3 mo before study entry
8	17.5	F	Hemoglobin SS $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> not determined	Frequent, irregular transfusions; last transfusion >6 mo before study entry
9	18.8	M	Hemoglobin SS $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Red-cell exchange 4 wk before study entry
10	21.3	F	Hemoglobin SS $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Infrequent transfusions; last transfusion >1 yr before study entry

\*The abbreviation bp denotes base pair, *XmnI*+ the presence of a C $\rightarrow$ T substitution 158 base pairs downstream from the 5' end of the G $\gamma$ -globin gene, and *XmnI*- the absence of this mutation.

Ontario. Each patient or a parent gave informed consent before the study.

A clinical response was defined as an increase in the hemoglobin concentration of at least 2 g per deciliter in patients with thalassemia and as a twofold increase in the percentage of fetal hemoglobin in patients with sickle cell disease. These were considered the primary and only clinically important end points of the study. Blood counts were monitored twice weekly. Fetal hemoglobin was measured by alkali denaturation and by densitometry when the concentration exceeded 15 percent of the total hemoglobin concentration. The absolute fetal hemoglobin concentration was calculated as the product of the percentage of fetal hemoglobin and the total hemoglobin concentration.

Secondary end points of the study included biologic markers anticipated to change during butyrate therapy, including the concentration of messenger RNA (mRNA) of  $\gamma$  and  $\beta$  globin, ratios of globin-chain synthesis, and the number of reticulocytes containing fetal hemoglobin (F reticulocytes). The S1 nuclease protection assay was used to measure mRNA of  $\gamma$  and  $\beta$  globin in peripheral blood.<sup>25</sup> Total cellular RNA isolated by extraction with guanidium isothiocyanate was analyzed by hybridization to probes specific for human  $\gamma$  and  $\beta$  globin that were end-labeled with [ $\gamma^{32}$ P]ATP. All samples, run in parallel with a human cell line expressing  $\gamma$  globin (K562), were standardized by simultaneous hybridization to a  $\beta$ -actin probe. After digestion, protection products were subjected to electrophoresis in a

denaturing gel (6 M urea and 6 percent polyacrylamide), which was then dried and autoradiographed. We determined the ratio of  $\gamma$ -globin mRNA to  $\gamma$ -globin +  $\beta$ -globin mRNA before and during therapy. The value during therapy is expressed as a multiple of the pre-treatment ratio.

The ratios of globin-chain synthesis<sup>32</sup> and the proportions of F reticulocytes<sup>36</sup> were determined by assay as previously described. All systems were reviewed and a physical examination was conducted daily, and chemical profiles were obtained twice weekly in all patients.

## RESULTS

### Primary End Points

Overall, the response to butyrate therapy was disappointing. In no patient with thalassemia were significant changes in total hemoglobin concentration, fetal hemoglobin concentration, or the imbalance between  $\alpha$ -globin and non- $\alpha$ -globin chains observed. One patient with thalassemia had an apparent increase in fetal hemoglobin (Patient 3 in Table 2), but increases in the percentage of hemoglobin and the absolute fetal-hemoglobin concentration were consistent with a return of a  $\beta^0$ -thalassemia phenotype that had been suppressed by regular transfusions. In one patient red-cell survival, studied with <sup>51</sup>Cr-tagged autologous red cells before and after butyrate therapy, was unchanged from base line (half-life, 250 hours vs. 248 hours).

The increases in fetal hemoglobin in patients with sickle cell disease

were minor. Two patients had transient, moderate increases in fetal hemoglobin during butyrate therapy. In the first patient (Patient 7 in Table 2), fetal hemoglobin increased from 4.7 percent to 11.5 percent of the total hemoglobin concentration; the absolute fetal-hemoglobin concentration increased by 0.18 g per deciliter. In Patient 10, fetal hemoglobin increased from 6.9 to 17.3 percent, and the absolute fetal-hemoglobin concentration increased by 0.5 g per deciliter. An increase in fetal hemoglobin from 0 to 4.2 percent in Patient 9, in whom values of approximately 5 percent had been previously documented, was consistent with a return to a phenotype suppressed by transfusions. No significant increase in mean hemoglobin was observed in patients with sickle cell disease.

### Secondary End Points

Changes in the ratio of  $\gamma$ -globin mRNA to  $\gamma$ -globin +  $\beta$ -globin mRNA of 1.8-fold and 3.1-fold were observed in both patients whose fetal hemoglobin increased dur-

Table 2. Biologic Markers of Efficacy Measured before and during Treatment with Arginine Butyrate in Five Patients with  $\beta$ -Thalassemia and Five Patients with Sickle Cell Disease.\*

PATIENT NO.	$\gamma$ -GLOBIN: $\gamma$ -GLOBIN + $\beta$ -GLOBIN mRNA <sup>†</sup>		$\alpha$ -GLOBIN CHAINS: NON- $\alpha$ -GLOBIN CHAINS		F RETICULOCYTES			FETAL HEMOGLOBIN			TOTAL HEMOGLOBIN		
	PEAK	MEAN	PRE	MEAN	PRE	PEAK	MEAN	PRE	PEAK	MEAN	PRE	PEAK	MEAN
	percent							percent			g/dl		
<b><math>\beta</math>-Thalassemia</b>													
1	1.00	0.96	4.83	4.13	67	76.0	74.3	98.0	98.0	98.0	8.6	8.2	7.6
2	1.16	0.93	2.85	2.87	56	69.1	58.5	18.1	48.4	31.6	9.3	7.0	5.8
3	1.06	0.99	4.24	4.33	0	98.6	93.3	2.2	15.1	13.1	9.0	6.7	5.3
4	1.14	1.05	2.68	2.76	65.0	78.0	67.4	58.4	60.5	57.0	8.3	9.3	8.4
5	1.53	1.15	3.87 $\ddagger$	2.53	24.2	65.4	55.5	49.6	55.0	46.8	7.8	7.3	6.7
Mean $\pm$ SD	1.18 $\pm$ 0.21	1.02 $\pm$ 0.09	3.69 $\pm$ 0.92	3.32 $\pm$ 0.84	42.3 $\pm$ 29.4	77.4 $\pm$ 12.9	69.8 $\pm$ 15.1	45.3 $\pm$ 37.3	55.4 $\pm$ 29.6	49.4 $\pm$ 31.9	8.6 $\pm$ 0.6	7.7 $\pm$ 1.1	6.8 $\pm$ 1.3
<b>Sickle cell disease</b>													
6	1.28	1.28	ND	0.99	2.0	24.7	17.6	6.8	7.5	7.2	8.8	8.8	8.6
7	1.84	1.45	2.44	2.71	21.0	28.7	26.1	4.7	11.5	7.9	6.0	8.0	6.1
8	0.79	0.58	0.90	1.22	19.3	20.7	18.8	6.9	7.3	6.6	8.0	9.1	7.4
9	1.02	0.91	1.16	1.25	ND	13.0	10.3	0	4.2	4.2	10.3	11.7	10.2
10	3.11	1.80	1.23	1.27	17.0	34.0	30.4	6.9	17.3	11.7	9.9	11.8	10.6
Mean $\pm$ SD	1.61 $\pm$ 0.93	1.20 $\pm$ 0.47	1.43 $\pm$ 0.67	1.49 $\pm$ 0.69	10.3 $\pm$ 10.2	24.2 $\pm$ 8.0	20.6 $\pm$ 7.8	5.1 $\pm$ 3.0	9.6 $\pm$ 5.0	7.5 $\pm$ 2.7	8.6 $\pm$ 1.7	9.9 $\pm$ 1.8	8.6 $\pm$ 1.9

\*Pre refers to data obtained before therapy with arginine butyrate, peak to the single highest value measured during therapy, and mean to the mean of all measurements obtained during therapy. ND denotes assay not done.

<sup>†</sup>We determined the ratio of  $\gamma$ -globin mRNA to  $\gamma$ -globin +  $\beta$ -globin mRNA before and during therapy. The value during therapy is expressed as a multiple of the pretreatment ratio. Base-line determinations were assigned a value of 1.00; see the Methods section for further explanation.

<sup>‡</sup>The determination of the ratio in this base-line sample may be inaccurate because of evidence of sample degradation. No other measurements were obtained before therapy with arginine butyrate was begun.

ing the study. In two other patients (Patients 5 and 6) very small (1.5-fold and 1.3-fold, respectively) increases were also observed, but without increases in fetal hemoglobin.

The percentage of F reticulocytes, determined by an assay that identifies any reticulocyte containing fetal hemoglobin in concentrations exceeding 2 to 3 pg per cell,<sup>36</sup> increased in two patients with thalassemia: from 0 to 98.6 percent in Patient 3, as a result of the increase in fetal-hemoglobin synthesis previously suppressed by transfusions, and from 24.2 to 65.4 percent in Patient 5 in the absence of an increase in the percentage of fetal hemoglobin. The percentage of F reticulocytes also increased in two patients with sickle cell disease: from 2.0 to 24.7 percent without an increase in fetal hemoglobin in Patient 6, and from 17.0 to 34.0 percent in parallel with the moderate increase in fetal hemoglobin observed in Patient 10.

Consistent increases in the mean red-cell volume and mean corpuscular hemoglobin concentration were observed in two patients, in parallel with an increase in F reticulocytes (Patient 5) and with increases in both F reticulocytes and fetal hemoglobin (Patient 10). In no patient were consistent decreases in plasma free hemoglobin, serum lactate dehydrogenase, or bilirubin observed — a finding consistent with the lack of improvement in erythropoiesis or hemolytic anemia.

### Toxicity

Hypokalemia requiring oral potassium supplementation occurred in eight patients; nausea requiring parenteral antiemetics or anorexia was noted in nine patients. In one patient daily infusion of arginine butyrate

at a dose of 1500 mg per kilogram produced constant nausea; increases in the dose to a maximum of 2000 mg per kilogram per day resulted in intractable vomiting. Mean blood urea nitrogen concentrations increased from  $9.6 \pm 3.7$  to  $29.2 \pm 10.9$  mg per deciliter ( $P \leq 0.005$ ) during therapy, returning to normal within 24 hours after treatment with butyrate was stopped. Serum creatinine levels remained unchanged.

Because of a labeling error during the production of the study drug, one patient received a dose of 2000 mg of arginine butyrate per kilogram over a period of six hours, after which she suffered a grand mal seizure. The results of all metabolic investigations, computed tomography, and electroencephalography were normal. The patient recovered without sequelae and completed the study after a two-week butyrate-free interval.

### DISCUSSION

An increased synthesis of fetal hemoglobin lessens the severity of  $\beta$ -thalassemia and sickle cell disease. In patients with  $\beta^0$ -thalassemia, the absolute lack of the synthesis of  $\beta$ -globin chains upsets the normal balance between  $\alpha$ -globin and  $\beta$ -globin chains in erythrocytes. Augmenting the production of the  $\gamma$  chains of fetal hemoglobin reduces this imbalance, thus improving erythropoiesis and ameliorating anemia.<sup>37</sup> In patients who are homozygous for hemoglobin S, coinheritance of a determinant for high expression of fetal hemoglobin results in a relatively benign form of sickle cell disease.<sup>4,5,35,38,39</sup> Moreover, any increment of fetal hemoglobin reduces mortality in sickle cell disease.<sup>40</sup>

Several cell-cycle-specific agents, including azaciti-

dine, cytarabine, vinblastine, and hydroxyurea, stimulate the synthesis of  $\gamma$ -globin and fetal hemoglobin.<sup>7-22</sup> Because the toxicity of these drugs poses at least a theoretical long-term risk to patients with nonmalignant disorders,<sup>41</sup> noncytotoxic agents that augment fetal-hemoglobin production are of great interest. A pilot trial<sup>32</sup> showed that short-term infusions of arginine butyrate increased the number of F reticulocytes and the synthesis of  $\gamma$ -globin mRNA in a small number of patients with  $\beta$ -hemoglobinopathies. In this same study, an increase in total hemoglobin was observed in one patient with thalassemia. The present study aimed to determine the efficacy of extended administration of arginine butyrate in a larger group of patients, in whom hematologic response was defined as a clinically important increase in total hemoglobin or the percentage of fetal hemoglobin.

In contrast to the pilot study, this study found that extended administration of arginine butyrate did not increase total hemoglobin in patients with thalassemia, nor did it cause sustained increases in fetal hemoglobin in patients with sickle cell disease. The moderate increases in  $\gamma$ -globin chains in a few patients were not associated with sustained hematologic responses over a 10-week period. These findings seem inconsistent with the proposed effect of butyrate — augmentation of the expression of the  $\gamma$ -globin gene.<sup>27-31</sup> However, there is evidence that butyric acid may increase the expression of  $\alpha$  globin, although not to the same extent as that of  $\gamma$  globin.<sup>42</sup> Increases in the expression of both  $\alpha$ -globin genes and non- $\alpha$ -globin genes (including  $\gamma$  globin) would not fully reduce the imbalance between  $\alpha$ -globin chains and non- $\alpha$ -globin chains, which is the cause of the ineffective erythropoiesis in severe thalassemia.

One finding from the pilot study was confirmed in the present study: increases in F reticulocytes were observed in approximately half the patients. However, in two patients with thalassemia the increases were consistent simply with a return of the phenotype suppressed by regular transfusions, whereas the greatest increase in a patient with sickle cell disease was not associated with any hematologic response during six months of therapy. The importance of a butyrate-induced increase in F reticulocytes is unclear. This assay identifies any reticulocyte containing fetal hemoglobin in concentrations exceeding 2 to 3 pg per cell<sup>36</sup>; it is therefore possible that small increases in  $\gamma$ -globin mRNA may be insufficient to cause measurable increases in fetal hemoglobin.

Our findings, disappointing in view of an earlier pilot study, should stimulate an investigation of the possible influences of genotype; the usefulness of other short-chain fatty acids,<sup>43,44</sup> acylators, and butyrate analogues<sup>45-47</sup>; and the therapeutic role of these compounds in combination with other agents that augment fetal hemoglobin in patients with  $\beta$ -hemoglobinopathies.

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