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Novel Valproic Acid Derivatives with Hemoglobin F Inducing Activity

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Pharmacological induction of hemoglobin F expression may be a promising approach for the treatment of β-thalassemia and sickle cell disease. Valproic acid, a drug frequently used for the treatment of seizure disorders, has been shown to enhance fetal hemoglobin synthesis in erythroid cells. However, this effect is only modest and requires relative high concentrations. Therefore, the drug appears not to be applicable for the treatment of β-globin chain disorders. Here, we describe the identification of novel valproic acid derivatives with potent hemoglobin F inducing activities at concentrations that presumably can be obtained in vivo. Am. J. Hematol. 81:374–376, 2006. © 2006 Wiley-Liss, Inc.

Key words: HbF; hemoglobin F; VPA; valproate; valproic acid; thalassemia; K562

INTRODUCTION

β-Thalassemia is caused by a defective expression of the adult β-globin gene causing excess of α-globin chains and ineffective erythropoiesis. The fetal γ-globin genes are structurally intact but are developmentally silenced. If these could be reactivated, functional hemoglobin synthesis could be maintained during adulthood, ameliorating the severity of the disease [1]. Clinical observations reported increased hemoglobin F (HbF) levels in patients treated with valproic acid (VPA) for epilepsy [2–4]. However, this effect was only weak, reflecting the relative modest HbF inducing potency of VPA in erythroid cells in vitro [5,6]. We therefore have tested a series of novel VPA derivatives and related chemical structures with respect to their HbF-augmenting activities in K562 erythroid cells.

MATERIALS AND METHODS

K562 cells were cultured and treated as has been described previously [5]. VPA derivatives were synthesized as described before [7–9]. Determination of HbF synthesis was performed using a specific ELISA [5].

RESULTS AND DISCUSSION

We have treated human erythroid K562 cells with a series of novel valproic acid derivatives and related chemical structures. The structure of VPA has been modified by introduction of unsaturated carbon bonds, variation of the aliphatic chain length, and derivatization of the carboxylic acid function. Additional compounds investigated in this study focused on the fatty acid nature of VPA. After treatment for 4 days, HbF concentrations in total cellular extracts were determined using a specific HbF-ELISA (Fig. 1). Of the 13 compounds tested, 4 exhibited significant HbF inducing activity: (±)-2-ethyl-4-methylpentanoic acid, (*S*)-2-pentyl-4-pentynoic acid as well as its racemate (±)-2-pentyl-4-pentynoic acid, and (±)-2-hexyl-4-pentynoic acid. These compounds were active at 125–500 μM concentrations, which can be expected to be achievable in patients, because the VPA plasma concentrations in patients treated for epilepsy are 300–700 μM. Furthermore, the HbF inducing derivatives did not exhibit cytotoxic effects in our culture model (results not

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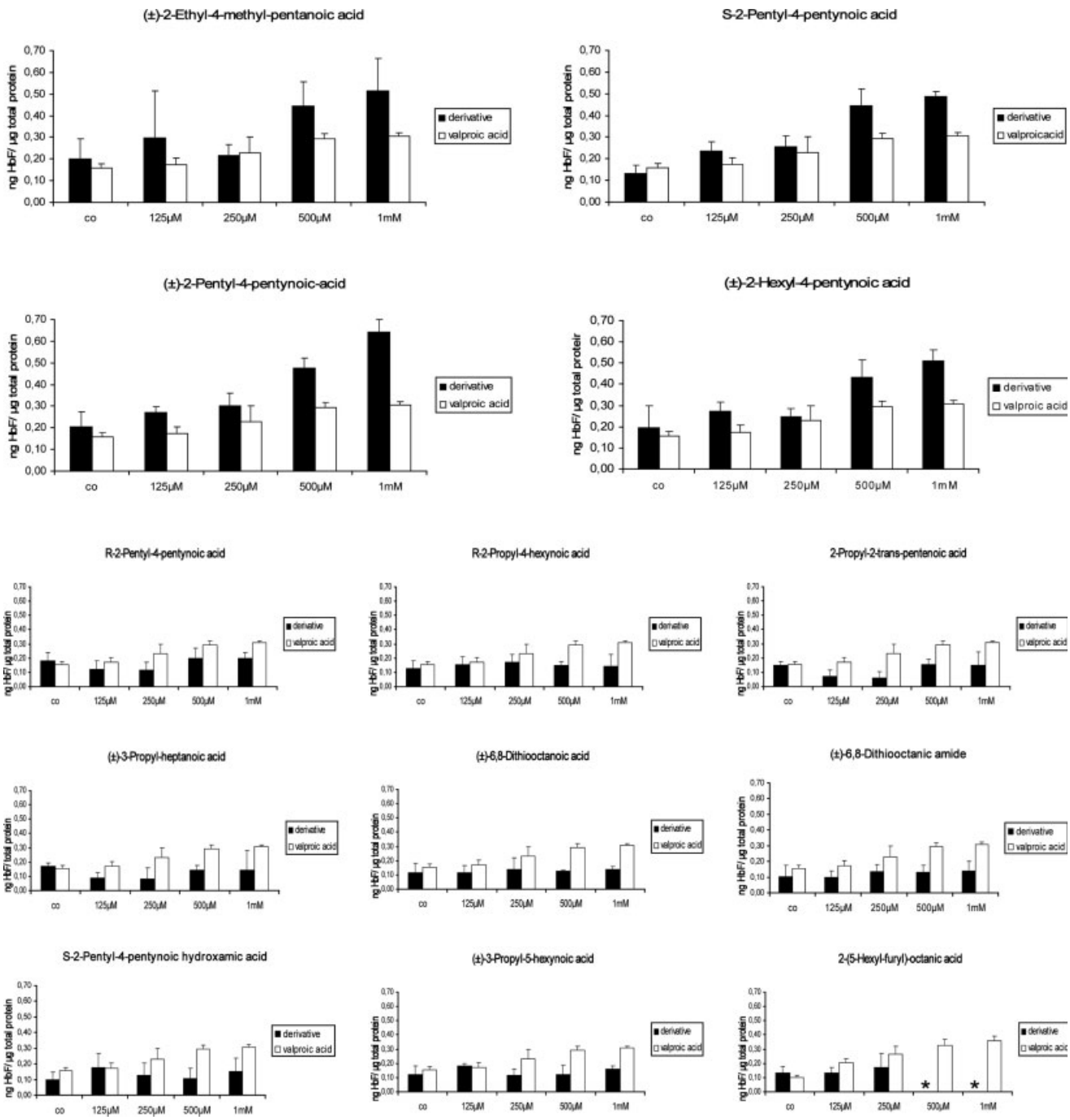

Fig. 1. K562 erythroid cells were treated with valproic acid (white bars) and valproic acid derivatives or related structures (black bars) at concentrations ranging from 125 µM to 1 mM. "Co" refers to solvent-treated control cells. Fetal hemoglobin (HbF) and total protein concentrations were determined in total cellular extracts. Shown are mean values and standard deviations of 3 independent experiments. *Cytotoxic effects of 2-(5-hexylfuryl)octanoic acid > 500 µM.

shown). The chemical modifications rendering VPA more active in terms of HbF induction are introduction of a triple bond at position 4, resulting in an unsaturated fatty acid derivative as well as an α hydrogen atom and an elongation of one side chain. The HbF inducing activity appears to be very specific since the carboxylic acid function of 2-pentyl-4-pentynoic acid requires S enantiomer configuration. The molecular mechanism of VPA action involves inhibition of histone deacetylases, since of all derivatives tested, only the HbF inducing compounds induced accumulation of acetylated histone proteins (results not shown).

Taken together, we have identified novel VPA derivatives with superior hemoglobin F inducing properties compared with valproic acid. These compounds act at concentrations, which presumably can be obtained in patients and require further in vivo evaluation.

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