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The investigation of resveratrol and analogs as potential inducers of fetal hemoglobin

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ABSTRACT

Beta-thalassemia, is a hemoglobinopathy characterized by reduced beta-globin chain synthesis, leading to imbalanced globin chain production, ineffective erythropoiesis and anemia. Increasing gamma-globin gene expression is a promising therapeutic approach as it reduces this imbalance by combining with the excess alpha globin chains and producing fetal hemoglobin (HbF). Furthermore, increased iron absorption and repeated blood transfusions lead to iron overload and tissue damage secondary to reactive oxygen species. Compounds exhibiting both antioxidant and HbF inducing activities are, therefore, highly desirable therapeutic agents. Resveratrol, a natural phytoalexin, combines these two activities but is also cytotoxic. Nine hydroxystilbenic resveratrol derivatives were synthesized in an attempt to identify compounds that retain the HbF-inducing and antioxidant activities of resveratrol but exhibit reduced cytotoxicity. Three derivatives (P1, P4 and P11) exhibited similar hemoglobin-inducing properties to resveratrol in K562 cells, however, only P11 showed reduced cytotoxicity. All three derivatives demonstrated variable HbF-inducing activity in primary erythroid progenitor cells from healthy donors. Resveratrol and P11 increased HbF induction significantly, with P11 having the highest activity. Additionally, P4 significantly increased progenitor numbers. A combinatorial treatment in K562 cells using resveratrol and decitabine resulted in a statistically significant increase in hemoglobin-inducing activity only above the level shown by resveratrol alone.

1. Introduction

Beta-thalassemia, a global health burden, is an inherited disorder of beta globin chain production. The disease is characterized by reduced synthesis of functional β-globin chains, leading to imbalanced globin chain ratio, ineffective erythropoiesis and anemia [1]. As a result, thalassaemic patients require regular blood transfusions with regular iron chelation therapy as a means of managing the disease. However, this treatment is not curative and still has substantial morbidity. A very promising therapeutic approach for β-thalassemia is the production of fetal hemoglobin (HbF) through pharmacological reactivation of endogenous gamma-globin genes as γ-globin can substitute for the absent or reduced adult β-globin [2,3]. The currently available HbF-inducing chemical agents have limited clinical application due to their moderate therapeutic properties and potential cytotoxic effects [4,5]. Therefore, identification of novel agents with higher HbF inducing activity and lower cytotoxicity has been one of the major challenges over the past few years.

Oxidative stress has been observed in various types of thalassemia as well as in other hereditary and acquired hemolytic anemias. This can be mainly attributed to iron overload due to increased iron absorption in the gastrointestinal tract [6], multiple blood transfusions as well as to increased intracellular denaturation of imbalanced hemoglobin subunits resulting in dissociation of heme from globin and iron from heme [7,8]. Labile non-transferrin bound iron can be involved in chemical reactions that generate reactive oxygen species (ROS) that affect various cellular components, particularly the cell membrane, damaging vital organs including the heart, liver and endocrine system [9]. In addition, endogenous antioxidant mechanisms are depleted due to the increased need to neutralize the oxidative stress [10]. Oral administration of vitamin E in transfusion-independent beta-thalassemia patients was shown to decrease ROS production and increase glutathione reductase levels in red blood cells [11]. Moreover, natural antioxidants such as curcuminoids [12] and fermented papaya preparation [13] were studied for their potential use towards elimination of antioxidant stress.

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Resveratrol is a natural phytoalexin found in a variety of human dietary products such as in the skin of red grapes, peanuts and red wine as well as in medicinal plants where it is produced in response to infection or other stresses. Resveratrol was originally isolated in 1940 from the root of *Veratrum grandiflorum*, a poisonous medicinal plant [14] known for its antimicrobial activity. However, it was not until the beginning of 1990 that research into resveratrol intensified due to evidence of the cardioprotective effects of red wine [15]. Since the 1990s, growing evidence from several studies has been accumulating showing resveratrol to have many biological activities including anti-inflammatory [16], anti-proliferative [17], chemopreventive [18,19], antioxidant [20,21] and lifespan enhancing activities [22–24], while having the ability to limit or prevent progression of cerebral ischemic injuries, cardiovascular injuries [25,26], cancer progression [11,18,27], arthritis [28,29], diabetes [30], neurodegernerative disorders [31,32] and a number of other aging-associated and stress resistance disorders. The mechanisms by which resveratrol is able to exert such a wide range of effects is not fully understood, but studies have led to the identification of a large number of direct targets for this compound. However, a number of its biological actions have been attributed to its antioxidant properties.

Apart from its antioxidant activities, resveratrol was found to inhibit ribonucleotide reductase in the same manner as hydroxyurea, the well known HbF inducer, as well as to promote erythroid differentiation [33]. Fibach et al. [34] were the first to show that resveratrol, in addition to its antioxidant activity, can also stimulate the expression of γ-globin genes and increase fetal hemoglobin production. In the current study, we investigated the ability of resveratrol and nine new hydroxystilbenic derivatives as potential HbF-reactivating agents as well as the combined effect of resveratrol and decitabine, another known HbF-inducer, as potential therapeutic approaches for β-thalassemia.

2. Material and methods

2.1. Materials

Resveratrol (3,4′,5-trihydroxystilbene) was purchased from Sigma (St Louis, USA). The nine resveratrol analogs were designed and synthesized by Perkins condensation following the procedure described elsewhere [35]. The purity of each compound (>97%) was controlled by HPLC.

2.2. Preparation of agents

Stock solutions of all compounds were prepared in 100% methanol and kept at −20 °C. The working solutions were prepared in 50% methanol. The chemical structures of the compounds tested are shown in Fig. 1.

2.3. Culture of human K562 cell line and analysis of cell differentiation

The human erythroleukemic cell line K562 was maintained in RPMI medium (Gibco, Invitrogen Inc., Paisley, UK) enriched with 10% FBS (Gibco, Invitrogen Inc., Paisley, UK), 0.6% Glutamine (Gibco, Invitrogen Inc., Paisley, UK) and 50 U/ml of Penicillin/Streptomycin (Gibco, Invtrogen Inc., Paisley, UK). The cells were kept at 37 °C with 5% CO_2 in a humified environment. Varying concentrations of each agent under investigation were added in 2×10^4 cells/ml. Cell survival measured by Trypan blue (Sigma, St. Louis, USA) and the Hb inducing ability of each agent measured by Benzidine staining were investigated for all derivatives after 5 days of treatment. Un-treated cells were used as negative control and cells treated with 150 μM Hydroxyurea as positive

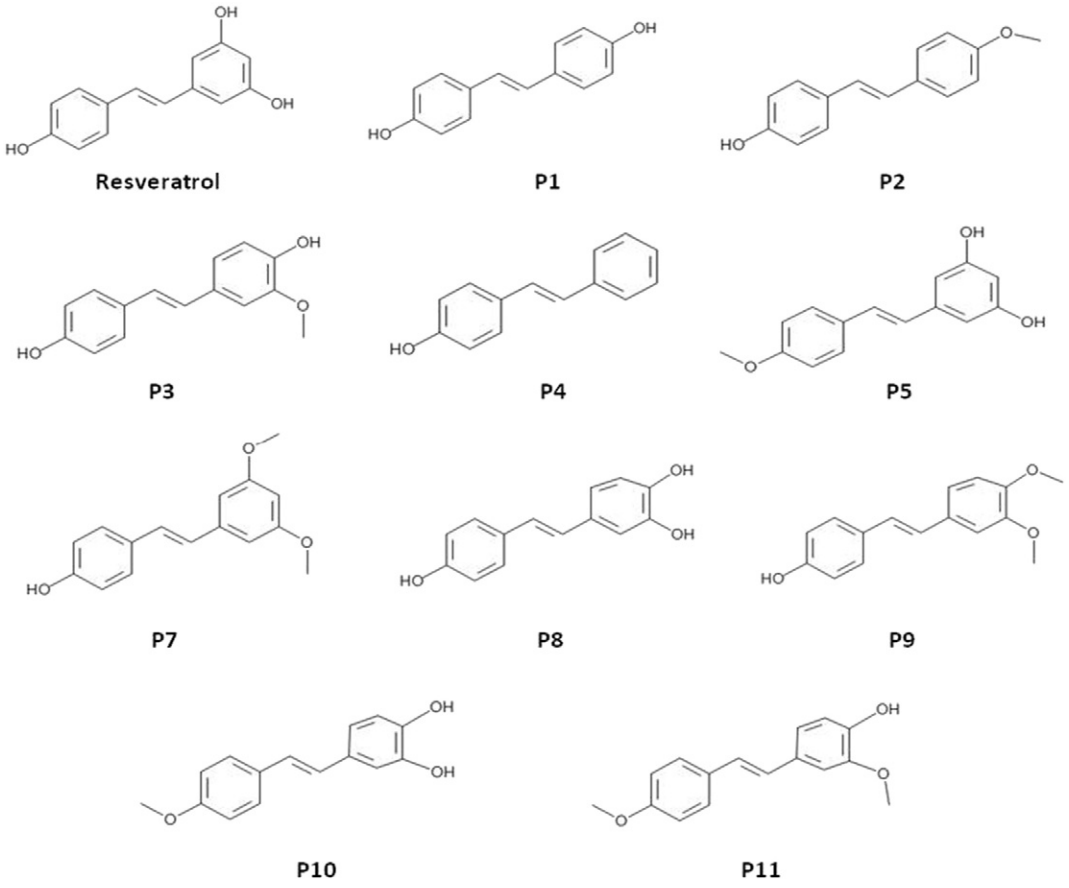

Fig. 1. Structures of the nine hydroxystilbenic derivativeanalogs to resveratrol along with resveratrol, the parent compound, which is commercially available.