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The novel histone deacetylase inhibitor 4-Me_2N-BAVAH differentially affects cell junctions between primary hepatocytes

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Abstract

Histone deacetylase (HDAC) inhibitors show great pharmaceutical potential, particularly in relation to cancer. However, very little is known about their biological outcome on hepatocytes, the major executors of xenobiotic biotransformation in the organism. The current study was set up to investigate the effects of the newly synthesized HDAC inhibitor 5-(4-dimethylaminobenzoyl)-aminovaleric acid hydroxamate (4-Me_2N-BAVAH) on hepatocyte gap junctions and adherens junctions, being main guardians of liver homeostasis. For that purpose, freshly isolated rat hepatocytes were cultivated for 7 days either in the absence or presence of 50 μM 4-Me_2N-BAVAH. Gap junction activity became promoted upon exposure to 4-Me_2N-BAVAH, which was associated with elevated Cx32 protein levels. By contrast, both Cx26 and Cx43 protein levels were negatively affected. The modifications in connexin protein content were not reflected at the transcriptional level. Finally, neither the expressions nor the cellular localizations of the adherens junction building stones E-cadherin, β-catenin and γ-catenin were altered by 4-Me_2N-BAVAH, a finding that is in contrast to what is commonly observed in tumor cells following exposure to HDAC inhibitors.

Keywords: Histone deacetylase inhibitor; 5-(4-Dimethylaminobenzoyl)-aminovaleric acid hydroxamate; Gap junction; Adherens junction; Primary hepatocytes

1. Introduction

It is well established that histone deacetylase (HDAC) inhibitors have great therapeutic potential, especially for the treatment of cancer. HDAC inhibitors indeed display pleiotropic antitumor properties, including the induction of cell cycle arrests, differentiation and apoptosis, as well as the inhibition of angiogenesis and metastasis (Drummond et al., 2005; Papeleu et al., 2005a; Vanhaecke et al., 2004a; Vinken et al., 2006a). While many of them have currently entered clinical tri-

Abbreviations: 4-Me_2N-BAVAH, 5-(4-dimethylaminobenzoyl)-aminovaleric acid hydroxamate; Cx, connexin; GJIC, gap junctional intercellular communication; HDAC, histone deacetylase; LDH, lactate dehydrogenase; PBS, phosphate-buffered saline solution; SAHA, suberoylanilide hydroxamic acid; TBS, Tris-buffered saline solution; TSA, Trichostatin A

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als, still little is known about HDAC inhibitor activity towards (healthy) non-tumorous cells. In particular, their biological outcome on hepatocellular homeostasis has been poorly documented. Such knowledge, however, is indispensable, since hepatocytes, the main effectors of xenobiotic biotransformation in the body, are highly exposed to drugs, and thereby represent primary targets of toxicity (Papeleu et al., 2005a; Vanhaecke et al., 2004a).

Cell junctions are major gatekeepers of liver homeostasis. In fact, anchoring cell junctions, with adherens junctions as prototypes, and their communicating counterparts, represented by gap junctions, play key roles in virtually all aspects of the hepatocyte's life cycle, ranging from cell growth to cell death (Vinken et al., 2006b). Adherens junctions, connecting cells one to another, arise from the interaction between transmembrane cadherins and cytosolic catenins (Wheelock and Johnson, 2003). In liver, hepatocytes express E-cadherin, β-catenin and γ-catenin, which are typical building stones of epithelial adherens junctions (Schmelz et al., 2001; Vinken et al., 2006c). Gap junctions, on the other hand, are composed of two connexons, in turn built up by six connexin proteins (Saez et al., 2003). Hepatocyte gap junctions mainly consist of Cx32 and Cx26 (Vinken et al., 2006c,d). These communicating cell junctions mediate the direct intercellular exchange of small and hydrophilic molecules, a flux called gap junctional intercellular communication (GJIC) (Saez et al., 2003; Vinken et al., 2006b,c).

It has been shown on many occasions that HDAC inhibitors act as potent modulators of cell junctions in tumor cells, which frequently lack these structures (Vinken et al., 2006a). With respect to adherens junctions, sodium butyrate and Trichostatin A (TSA) were reported to upregulate productions of β-catenin and E-cadherin (Masuda et al., 2000), and γ-catenin (Chiba et al., 2004), respectively, in human liver cancer cells. In human and rat glioblastoma cells, 4-phenylbutyrate was found to increase connexin protein levels and GJIC (Ammerpohl et al., 2004; Asklund et al., 2004). Similar observations were made in *ras*-transformed rat WB-F344 liver epithelial cells treated with suberoylanilide hydroxamic acid (SAHA) (Ogawa et al., 2005), and in human prostate cancer cells exposed to TSA (Hernandez et al., 2006). Our group previously showed that TSA, a naturally occurring hydroxamate HDAC inhibitor, alters connexin protein levels in primary cultured hepatocytes, resulting in enhanced GJIC (Vinken et al., 2006d). Here, we test whether this also holds for 5-(4-dimethylaminobenzoyl)-aminovaleric acid hydroxamate (4-Me_2N-BAVAH), a TSA-like synthetic HDAC inhibitor with a more favourable pharmaco-toxicological profile (Elaut et al., 2004; Papeleu et al., in press). Moreover, we investigate for the first time the effects of 4-Me_2N-BAVAH on hepatocyte adherens junctions.

2. Materials and methods

2.1. Chemicals

4-Me_2N-BAVAH (5-(4-dimethylaminobenzoyl)-aminovaleric acid hydroxamate) (purity ≥96%) (Fig. 1) was synthesized as described elsewhere (Van Ommeslaeghe et al., 2003) and was kept in 30 mM stock solution, prepared in absolute ethanol (Merck, Germany). All other chemicals were commercially available products of analytical grade. Primary antibodies came from several suppliers and are listed in Table 1.

2.2. Cell cultures

Procedures for the housing of rats, and isolation and cultivation of hepatocytes were approved by the local ethical committee of the Vrije Universiteit Brussel. Male outbred Sprague–Dawley rats (200–250 g; Charles River Laboratories, Belgium) were kept under controlled environmental conditions (12 h light/dark cycle) with free access to food (Animalabo A04) and water. Hepatocytes were isolated by use of a two-step collagenase method and cell viability was assessed by trypan blue exclusion (Papeleu et al., 2005b). Viable (>85%) hepatocytes were plated at a density of 0.56×10^5 cells/cm^2 in Dulbecco's Modified Eagle Medium containing 0.5 I.E./ml insulin, 7 ng/ml glucagon, 292 mg/ml L-glutamine, antibiotics (7.3 I.E./ml benzyl penicillin, 50 μg/ml kanamycin monosulfate, 10 μg/ml sodium ampicillin, 50 μg/ml streptomycin sulfate) and 10% (v/v) fetal bovine serum. After 4 h, the medium was removed and replaced by fresh medium supplemented with 25 μg/ml hydrocortisone hemisuccinate and 0.25 μg/ml amphotericin B. After 24 h, the medium was renewed daily by serum-free medium. Conditions used were as follows: (i) cultures treated with 50 μM 4-Me_2N-BAVAH, dissolved in absolute ethanol (final concentration 0.05% (v/v)), (ii) cultures treated with 0.05% (v/v) absolute ethanol (referred to as "control" or "−"), and (iii) untreated cultures. No differences

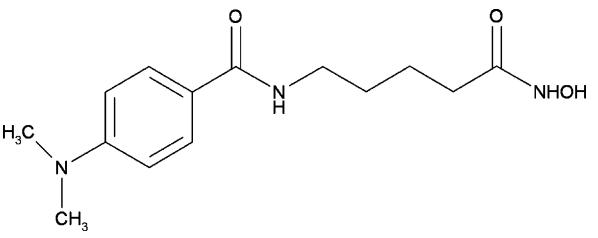

Fig. 1. Chemical structure of 5-(4-dimethylaminobenzoyl)-aminovaleric acid hydroxamate (4-Me_2N-BAVAH).