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Preformulation, formulation, and in vivo efficacy of topically applied Apomine

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ABSTRACT

Apomine is a novel compound that inhibits the mevalonate/isoprenoid pathway of cholesterol synthesis and may prove effective as a skin cancer chemoprevention therapy. This research was focused on the development of a new delivery approach for chemoprevention of melanoma using topically delivered Apomine. This included evaluating the effect of several factors on the stability of Apomine in solution, utilizing these to develop a topical formulation, and conducting efficacy studies with the developed topical formulation in the TPras mouse model. Preformulation included the influence of pH, buffer species, ionic strength, and organic solvents on the stability of Apomine at four different temperatures. Apomine was determined to undergo apparent first-order degradation kinetics for all conditions evaluated. Apomine undergoes base-catalyzed degradation. Less than 15% degradation is observed after >200 days under acidic conditions. Long-term stability studies were performed on two different topical cream formulations and indicate that both formulations are chemically stable for over 1 year at both 4 and 23 °C. The efficacy of topically applied Apomine, from ethanol and developed 1% cream, was evaluated in vivo against the incidence of melanoma. Regardless of delivery vehicle Apomine treatment caused a significant reduction in tumor incidence. Ethyl alcohol application of Apomine resulted in a greater tumor incidence reduction when compared to the development cream formulation; however, this difference was not significant.

1. Introduction

Apomine (Fig. 1), [2-(3,5-di-tert-butyl-4-hydroxy-phenyl)-1-(diisopropoxy-phosphoryl)-ethyl]-phosphonic acid diisopropyl ester, is a novel antineoplastic agent, which is one of a family of compounds that inhibits the mevalonate/isoprenoid pathway of cholesterol synthesis (Flach et al., 2000; Nguyen et al., 2002; Roitelman et al., 2004; Lewis et al., 2006; Roelofs et al., 2007). Apomine accelerates the degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) (Edwards et al., 2007). HMGR is an enzyme that catalyzes the first step of the mevalonate pathway, leading to the syntheses of cholesterol and other isoprenoid compounds. As a result of this mechanism, Apomine inhibits the growth of cancer cells from a number of cancer types, including lung, colon, breast and skin (Pourpak et al., 2007). Additionally, it induces apoptosis in tumor cell lines derived from leukemia, colon, liver, ovary, and breast cancers (Flach et al., 2000). Early clinical data suggest Apomine may be clinically active in humans (Alberts et al., 2001).

According to the American Cancer Society, more than one million cases of skin cancer go unreported annually. For 2008, an estimated 62,480 new cases will be the most serious form of skin cancer, melanoma, resulting in an estimated 11,200 deaths (American Cancer Society, 2008). Based on the increasing yearly incidence of skin cancer, there is an increasing need for effective chemopreventive drugs (Stratton et al., 2006). Here we investigate Apomine as a potential chemopreventative agent for melanoma.

Apomine's physiochemical properties, calculated octanol/water partition coefficient (CLogP) of 6.09 (ClogP, 1995), and low water solubility, calculated to be $3.98 \times 10{-}7$ molar, by the General Solubility Equation of Yalkowsky (Yalkowsky, 1999; Jain and Yalkowsky, 2001), are desirable for formulation in a topical cream base. Despite its known activity against the aforementioned cancers, little is known about the stability of Apomine as a function of pH, temperatures, and in different solvents. These data are necessary in determining if Apomine may be utilized in potential formulations. To this end, the stability of Apomine was determined in a variety of different aqueous and non-aqueous systems. Concurrently, with the low calculated aqueous solubility, solubility was explored as a function of different cosolvent concentrations. Based on these data, a topical formulation was developed for stability

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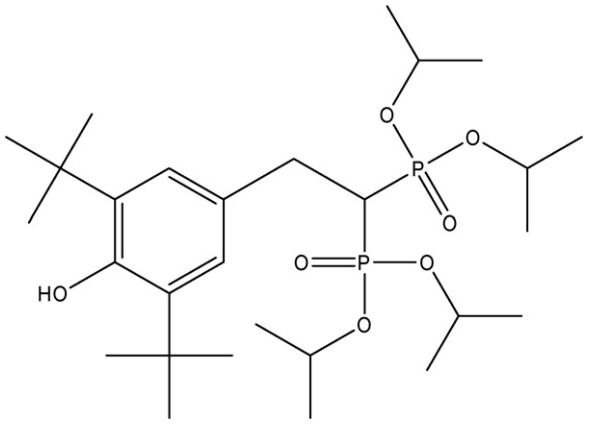

Fig. 1. Structure of Apomine.

determination. The stability of this formulation was evaluated prior to long-term efficacy evaluation.

Following the development of a suitable topical formulation of Apomine the efficacy was evaluated in vivo in the TPras mouse model. In this model the efficacy of a topically applied 1% Apomine formulation was evaluated as a potential chemoprevention and/or therapeutic agent against DMBA (7,12-dimethylbenz-[a]anthracene)-induced melanomas.

2. Materials and methods

2.1. Materials

Apomine was supplied by ILEX Oncology (San Antonio, TX, USA). Dibasic potassium phosphate, monobasic sodium phosphate, sodium chloride (NaCl), and 37% hydrochloric acid were purchased from Sigma (St. Louis, MO, USA). Citric acid was received from Spectrum (New Brunswick, NJ, USA). Boric acid was obtained from Aldrich (Milwaukee, WI, USA). Sodium hydroxide (NaOH) was obtained from EM Science (Darmstadt, Germany). A Millipore water purification system with a 0.22-μm filter was utilized for water. Tetrahydrofuran (THF) and isopropyl alcohol (IPA), both of HPLC grade, were purchased from Burdick and Jackson (Muskegon, MI, USA). HPLC grade acetonitrile (ACN) was purchased from EMD (Gibbstown, NJ, USA). Ethyl alcohol (EtOH), 200 proof, was received from Aaper Alcohol and Chemical Co. (Shelbyville, KY, USA). Dermabase cream was purchased from Paddock laboratories, Inc. (Minneapolis, MN, USA).

2.2. High performance liquid chromatography (HPLC) method

The HPLC system consisted of a Waters 2690 separation module (Waters, Milford, MA, USA) coupled with a Waters 996 Photodiode array (PDA) detector. Separation was achieved on a C_{18} column head at 30 °C with detection at 210 nm. Instrument control, data acquisition, processing, and peak symmetry was analyzed using Millenium32 Chromatography Manger Software (Waters, Milford, MA, USA).

Stability for Apomine in the topical formulation was conducted via a gradient chromatographic method in order to separate Apomine from its degradation product and the cream excipients (Kuehl et al., 2006). Aqueous samples and organic stability were analyzed via initial isocratic conditions (63:35, CAN–H_2O) for 12 min, with a flow rate of 0.6 ml/min (Kuehl et al., 2006).

2.2.1. Specificity and selectivity

The specificity and selectivity of the assay for Apomine was confirmed with both mass spectrometry and library spectra matching, using a PDA detector. The initial isocratic conditions were used to analyze a standard of Apomine by liquid chromatography–mass spectrometry (LC–MS) (Finnigan MAT TSQ 7000, in positive ion mode). Further confirmation of peak purity was conducted using data collected from a 996 PDA detector and analyzed with Millenium32 Chromatography Manger Software. A library spectrum was used to check for peak purity and confirm the absence of co-eluting species.

2.3. pH measurements

All pH measurements were conducted on a Hanna Instruments Model HI 221 (Woonsocket, RI, USA) pH/mV meter with a model HI 1083 pH probe for aqueous samples, while cream samples were analyzed with a ThermoOrion, model 8135BN (Beverly, MA, USA), flat surface electrode. The pH of aqueous solutions was measured at the beginning, throughout the stability run, and upon completion of each stability trial, while cream pH measurements were acquired at each pull point. The instrument was calibrated each day with a two-point curve bracketing the expectant pH values.

2.4. Solubility studies

The solubility of Apomine was determined as a function of pH, from pH 2.0 to 12.0, at intervals of 1.0-pH units. Solubility between pH 2.0 and 5.0 was determined in a 0.1-M citrate buffer, between pH 5.0 and 9.0 with a 0.1-M phosphate buffer, and between pH 9.0 and 12.0 with a 0.1-M borate buffer. All buffers had an ionic strength of 0.2 M, adjusted with NaCl. Due to the fact that aqueous solubility is below the limit of detection (LOD), ACN was used as a cosolvent at 20% (v/v). The solubility of Apomine was also evaluated as a function of various cosolvents. EtOH, ACN, and IPA were mixed with water in volume fractions of 5, 10, 15, 20, 25, and 30%. All solubility samples were run in duplicate and allowed to equilibrate at 23 °C for at least 48 h. Prior to analysis via HPLC, samples were filtered with a 0.2-μm PTFE filter.

2.5. Stability studies

2.5.1. Effect of pH

The effect of pH on the stability of Apomine was evaluated with a citrate buffer (0.1 M) in the pH range of 2.0–5.0, a phosphate buffer (0.1 M) in the range of 5.0–9.0 and a borate buffer (0.1 M) in the range of 9.0–12.0. Buffers were pH adjusted with NaOH and HCl as needed, while ionic strength was adjusted to 0.2 M with NaCl. The influence of ionic strength was tested by using a citrate buffer at pH 5.0, a phosphate buffer at pH 7.0, and with a borate buffer at pH 9.0. The ionic strength of these samples was adjusted to 0.2, 0.3, and 0.5 M with NaCl. Stability was determined using HPLC. In order to achieve adequate solubility levels aqueous samples were prepared with ACN as a cosolvent at 25% (v/v).

2.5.2. Effect of temperature

Temperature influences on the degradation rates of Apomine were determined at four different temperatures (4, 23, 37, and 48 °C). Analysis of the degradation rates of Apomine at different temperatures affords the assessment of activation energy.

2.5.3. Effect of organic solvents

The influence of organic solvents on the stability of Apomine was tested with ACN, IPA, and EtOH at four different temperatures (4, 23, 37, and 48 °C).

In order to accurately determine the effect of each condition on the degradation of Apomine, samples were typically analyzed for a minimum of six sample points, spanning three to six half-lives. In order to reduce the potential for self-catalysis samples were