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feature



Vaporization, bioactive formulations and a marine natural product: different perspectives on antivirals

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This article examines three aspects of antivirals, such as hydroxychloroquine, chloroquine, and remdesvir, as they might relate to the treatment of a viral infection such as COVID-19: (i) the use of vaporization for the delivery of antivirals, with the bulk constituents having mild antiviral efficacy; (ii) the application of a marine natural product extract as opposed to a single molecule as an antiviral agent; and (iii) a counter intuitive approach to formulation that is, in part, based on delivering multiple species that fall into three categories: building blocks for the virus to accelerate replication; an energy source for the infected cell to boost its immune response; and the species that antagonize or provide toxicity to the virus.

Introduction

Bioactive vapors composed of organic species have the potential to deliver pharmaceutical agents efficiently and economically [1,2]. This article focuses on the vaporization and delivery of hydroxychloroquine and bryostatin (two compounds with antiviral activity), their bioactive formulations and some details about the applications of a marine natural product as an antiviral agent. The administration of the drug is matched with host-specific bioactive formulations. We discuss why the marine natural product bryostatin should be tested as a collection of molecular species found in nature and not a single active structure. Vaping for the delivery of nicotine and species found in 'vape juice' is a tremendous health risk; however, a similar device could be tested for antiviral drug

delivery, incorporating the same bulk solutions for the treatment of viral infections.

Hydroxychloroquine and chloroquine, both antimalarial agents, are being investigated as potential treatments of COVID-19 [3]. The coronavirus results in severe acute respiratory syndrome (SARS) [4], indicating an inhalation route using an aerosol or vapor might offer a more effective treatment method compared with a pill, i.v. or an implant, assuming the drugs can be delivered at acceptable doses with little or no side effects.

The Methodological Framework

A formulation that consists of three bulk constituents: 50% propylene glycol, 25% glycerin and 25% ethanol by volume (and 80% PG, 10% glycerin, 10% ethanol), was tested as a method

to deliver a unique set of formulations with anti- and pro-viral activities. Glycerin has been used to fix viruses for decades, propylene glycol has been shown to inactivate viruses and ethanol is widely used as a viral disinfectant. Ethanol is also added to increase the solubility of some of the polar species. Ammonium chloride, which can have a negative impact on the viral replication rate, is included at low levels [5]. Fatty acids including stearic, oleic, linoleic and/or arachidonic acids are included at <1% of the total mass. The state (s, l) and solubility of the fatty acids in the solvents must be considered. It has been demonstrated that specific fatty acids can impact the replication rate of the coronavirus [6]. Human serum albumin provides a source of amino acids for the virus. When suspended in the solution, ethanol denatures the structure

allowing it to decompose into its basic building blocks with less effort. ssDNA is added for its building blocks, which can be utilized by the virus. The two macromolecules comprise no more than 1% of the total mass. Fructose or glucose are included as an energy source for the infected cells, increasing their metabolic processes along with the uptake rate of the drug that attacks the viral population in an infected cell at a mass <1%. Hydroxychloroquine was tested at levels as high as 6% of the bulk solvent mass. In a typical formulation, the hydroxychloroquine or bryostatin extract was 2–4% of the bulk solvent mass and the other species a maximum of 3% of the solvent mass. Hydroxychloroquine can be replaced with chloroquine or Remdesivir in the evaporation experiment.

In our experiments the bulk solvent contains different mixtures of the proposed antiviral and the bioactive ingredients are vaporized by a commercially available vape device (<US\$35). The vapor is pulled through a 1 cm ID tube ~0.6 m in length to a 1 l flask. A vacuum was pulled for ~2 s and stopped, trapping the vapor in the flask. The flask contained 50 ml of high purity water and the static vapor was in contact with the aqueous phase for ~5 s before the vacuum was applied again. This cycle was performed five times and the water was removed and analyzed by LC–MS. Each species was confirmed using its parent ion and at least one fragment.

When a coronavirus infects the lungs, specifically the primary alveolar epithelial cells, there is a rapid response by the cell's immune system. The monosaccharides provide additional localized energy for the cell. The protein and nucleic acids suspended in the solvent provide building blocks for the viral replication process. The goal is to maintain or accelerate viral replication improving the efficacy of the drug during this vulnerable phase. At the same time, the fatty acids, ethanol and glycerin provide antagonistic activity toward the virus at the molecular level. In the vaporization process, droplets composed of glycerin and propylene glycol can be in the tens to several hundreds of nanometers diameter range [7]. These nanoparticles will rapidly release their bioactive species in the lungs owing to the high surface-area:volume ratio. The multitude of agents potentially interacting with a virus in a short timespan presents a unique conundrum for its replication and survival. The inhalation approach enables lower doses to be administered.

The second pharmaceutical tested is the marine natural product bryostatin. Bryostatin has been shown to have various levels of efficacy

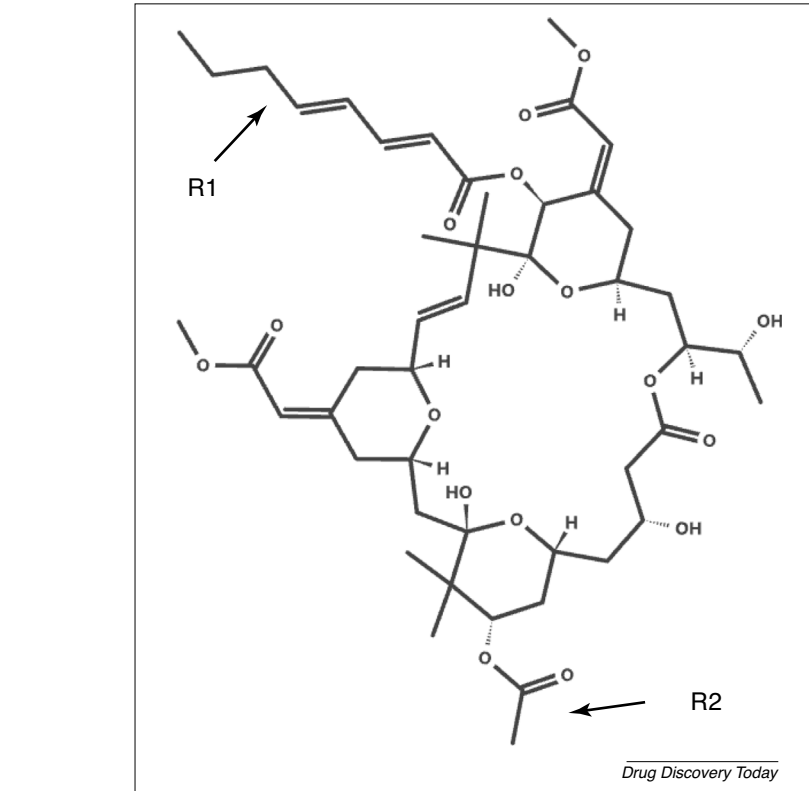


FIG. 1

The structure of bryostatin-1 has a bryophan ring and R_1 (C_8) and R_2 (C_2) functional groups.

against different types of cancers [8,9], neurological diseases [10,11] and antiviral activity [12,13]. A protein kinase C modulator in medicinal applications, its limitations in large-scale studies are cost and availability. Bryostatin is naturally produced by marine bacteria having a symbiotic relationship with the bryozoan *Bugula neritina*. Bryostatin provides a chemical defense against predators seeking the *Bugula* larvae [14]. Although the interaction between viral and bacterial species in the world's oceans is a developing field, it is estimated that marine viruses outnumber marine bacteria by ratios that range from 20:1 to 50:1. This suggests that marine bacterium can interact with multiple viral species and would need a broad-spectrum antiviral defense to survive. Marine creatures such as Beluga whales [15] and bottlenose dolphins [16] are infected with coronaviruses and their defense systems could involve symbiotic bacterium that produce a small molecule(s) with antiviral properties.

The bryostatin group is characterized by a bryophan ring with two R groups (Fig. 1). There have been 20 bryostatin structures isolated from *Bugula* and published that have different R_1 and R_2 constituents. There are probably many more variations of the bryophan ring in nature. In

analysis of marine extracts, there is a complex mix of structures that can be correlated with the bryophan ring having differences in the R_1 and R_2 structures. Simple marine creatures, such as fire coral, use complex poisons for different survival tasks [17]. This mixture has been noted in the literature by several groups [18,19]. The complex mixtures extracted from *Bugula* suggest the bacterial defense system is a multi-pronged system and not a single structure.

QSAR calculations of bryostatin-1 and its substructures (Bryo-1: $R_1 = C_8$, $R_2 = C_2$; $R_1 = OH$, $R_2 = C_2$; $R_1 = OH$, $R_2 = OH$; $R_1 = H$, $R_2 = OH$; $R_1 = H$, $R_2 = H$) give a range of physical values (Lipinski's rules: logP, TPSA, H-bonds possible, etc.) and biological activities: G-protein-coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. Typically, many natural product chemists isolate and focus on a single molecule with medicinal activity.

In some of our vaporization experiments we replaced hydroxychloroquine with bryostatin extracts and found the species with a higher water solubility appeared in the aqueous phase ($R_1 = OH$, $R_2 = OH$; $R_1 = H$, $R_2 = OH$; $R_1 = H$, $R_2 = H$). The ester bonds that link R_1 and R_2 to the bryophan ring are exposed to numerous

naturally occurring carboxylates in the marine environment.

We completed preliminary work with a standard ‘vape’ instrument that is used worldwide for the delivery of nicotine in a vapor form. This indicates the technology is available on a large and economical scale and the conditions under which it works are well understood. It typically uses two primary fluids in the vaporization process: glycerin or glycerol (C₃H₈O₃) and propylene glycol (C₃H₈O₂), which are used in this study. At this preliminary stage, the goal was to show the vaping equipment could be used to transport various species, as a vapor, to the flask. Despite a minimum exposure time, the various species were measured in the aqueous phase.

Concluding remarks

Our group has developed a complex for treating different forms of tuberculosis. First- and second-line antibiotics were repurposed attacking the microbe with an additional antagonist [Cu(II)] and accelerating its cellular processes with an energy source [20,21]. We have used a similar repurposing approach with cancer drugs using sucrose and glucose to provide additional energy to the cancer cells, copper to increase the concentration of a limiting nutrient in the angiogenesis process and the inclusion of fatty acids and proteins to provide nutrients and increase the serum lifetime [22–24]. We have also been exploring an ocean-centered synthesis of bryostatin and explored many of the chemical reactions possible in this environment [25–27]. These interdisciplinary activities enabled us to explore nontraditional approaches that were economical and often efficient in independent *in vitro* studies.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: <https://doi.org/10.1016/j.drudis.2020.04.010>.

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