

Volatile molecules for COVID-19: a possible pharmacological strategy?

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Abstract

COVID-19 is a novel viral pneumonia with a higher incidence of bilateral pneumonia and pleural effusion. The high pulmonary tropism and contagiousness of the virus SARS-CoV-2 should stimulate new approaches to combat its widespread diffusion. In the development of new pharmacological strategies, the volatility of molecules is argued to add as much value as the desired antiviral and anti-inflammatory effect. Volatile molecules are characterized by a high vapour pressure and are consequently easily exhaled by the lungs. This feature could be exploited from a pharmacological point of view, reaching the site of action in an uncommon way but allowing for drug delivery. In this way, a hypothetical candidate molecule for COVID-19 must have a balance between its lung exhalation characteristics and antiviral and anti-inflammatory pharmacological action. Here, the feasibility, advantages and disadvantages of a therapy based on volatile molecules will be discussed. Known aerosolized antiviral drugs and volatile molecules are briefly reviewed, and a complete evaluation of the latter is provided in view of a possible clinical use.

SUMMARY

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1. INTRODUCTION

Since December 2019, a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been causing a pandemic pneumonia named COVID-19. Infection started in Wuhan, the largest city of Hubei Chinese province, and rapidly spread throughout the world. SARS-CoV-2 is a coronavirus belonging to the β -coronavirus cluster, which includes MERS-CoV, the causative agent of Middle East respiratory syndrome (MERS). The mortality rate of COVID-19 has been reported to range from 3.4% (WHO) up to 14% (Wu et al, 2020) depending on different authors and modalities of data collection.

The unstoppable COVID-19 has forced countries to develop new strategies to ensure both constant monitoring of the epidemic and an active response in terms of reorganization of intensive care (Remuzzi et al, 2020) and to discover possible experimental drug therapies (fig. 1). In this way, many authors are sharing clinical

experience using different pharmacological approaches, already on the market, in COVID-19 patients. A brief summary of pharmacological therapies currently under investigation is reported in table 1.

Fig. 1: Anti-COVID-19 strategy.

Scientists and pharmaceutical companies are starting to develop several types of vaccines. However, it seems that many time is required for the results to establish a reasonable level of safety. Effectiveness of vaccines is another important aspect because of the high amount of variability, linked to the mutation rate of the novel virus, which could make a previously helpful vaccine or new antiviral molecule ineffective. Government regulatory agencies are called upon to guarantee an accurate and careful evaluation process to satisfy all the necessary safety, quality and efficacy requirements, avoiding any shortcuts in assessment (Jiang, 2020).

In the absence of a known efficient pharmacological therapy and because of the lack of time due to this public health emergency, it is reasonable to explore any possible strategy of pharmacological intervention. Here, an approach is discussed considering the possibility of slowing the engraftment of the virus at the level of the pulmonary alveoli through a pharmacological mechanism of action. The approach would preferably be one that involves non-specific mechanisms of action at the site of infection instead of researching specific mechanisms of action to target the virus. To make an urgent decision on how to focus drug research, it may be useful to focus efforts on a therapy with known effects and safety profiles. This has already happened with the choice of tocilizumab, approved for rheumatoid arthritis, to block the massive release of cytokine IL6, induced by the coronavirus at the cellular level, and thus prevent its lethal effects. In China, more than 80 clinical trials are testing both the newest molecules and the oldest remedies, even those from Traditional Chinese Herbal Medicine (Maxmen, 2020), as reported in table 1. The situation that is developing is one in which there are many studies with therapeutic possibilities but limited time.

The lung is an internal organ with a high total capacity of 6 litres, an average surface area of approximately 50 m², and peculiar pharmacokinetics involving the absorption, metabolism and elimination of substances in its lumen. Understanding the functioning mechanisms of this vital organ, especially when targeted as in the case of COVID-19 pneumonia, can be useful for outlining a possible pharmacological strategy. Considering the above, here data from inhaled antiviral aerosol studies have been reviewed and the potential use of volatile molecules, easily eliminated and exhaled by the lungs, has been argued. This last pharmacological strategy has never considered before in clinical treatment and could be analysed with some limitations in this time of crisis.

Table 1: COVID-19 pharmacological therapies registered on clinicaltrial.gov (accessed at 8 april 2020).

2. Aerosolized antiviral drugs

Inhalation is the preferred route of administration for many drugs that have a direct effect on the airways, particularly in conditions such as asthma and chronic obstructive pulmonary disease COPD (Berger, 2009). The inhalation route is also used to facilitate systemic administration in other pathologies (e.g., to avoid daily insulin injections). The major advantage of the inhalation route is the administration of the drug to the airways in doses that are effective and have a direct action on the site but with a much lower risk of systemic side effects. The size of the particles administered by inhalation is of critical importance in determining the deposit site within the respiratory tract. The optimal size for airway deposition is 2-5 μ m of mass median aerodynamic diameter (MMAD). Larger particles tend to settle in the upper airways, and smaller particles remain suspended and then exhaled (Sturton et al. 2008).

There are numerous ways of administering inhaled drugs (Virchow et al. 2008):

- * pressurized metred inhalers
- * expansion chambers
- * powder inhalers

* nebulizers

* gas

Some data have suggested the use of aerosolization in antiviral therapy or anti-symptomatic treatment, and this is confirmed by some trials recently registered for COVID-19 treatment.

Debs et al. (1988) conducted in vivo studies examining the effect of oral of aerosol administration of the antiviral agent ganciclovir in an experimental model of murine cytomegalovirus (MCMV) pneumonia. The authors reported the same outcome for the two groups but suggested a more specific inhibition of replication of MCMV in the lungs with the aerosolized drugs. A more recent in vivo study has tested through aerosol an experimental synthetic ligand (PUL-042) for Toll-like receptor (TLR) 2/6 and TLR 9 in a mouse pneumonia experimental model. The use of this aerosolized immune stimulant co-administered with aerosolized antiviral oseltamivir has resulted in a greater rate of survival in patients with influenza pneumonia compared to controls (Leiva-juarez et al., 2018). The interest in this new type of inhaled immune stimulant that targets the TLR pathway has resulted in a very recent registration on clinicaltrial.gov of a new trial with PUL-042 to reduce the severity COVID-19 pneumonia in SARS-CoV-2 positive patients (ClinicalTrial.gov id: NCT04312997 and NCT04313023).

It has already been argued that aerosol delivery of antiviral drugs or vaccines may lead to some advantages in safety and efficacy in treating influenza (Wong et al., 2010). For antiviral drugs, the main advantage of the inhalation route is the lack of first pass metabolism, which leads to increased bioavailability. For example, the old drug ribavirin (RBV) has been proposed for aerosol therapy in critical care situations, but it is without strong recommendation and is restricted to high-risk patients (Diot et al., 2016; Velkov et al., 2015). A recent comparative retrospective cohort analysis has found no significant differences in clinical outcome between oral and inhaled ribavirin therapy but a higher cost for the aerosol therapy (Trang et al., 2018). However, the inhaled RBV therapy combined with intravenous immunoglobulin is applied in bone marrow transplant patients in cases of viral pneumonia because of its poor systemic absorption, protecting against haemolytic anaemia frequently noted after oral administration (Velkov et al., 2015).

One of the most well-known antiviral drugs, zanamivir (Relenza[®], GlaxoSmithKline), has showed a low oral bioavailability: to solve this problem a new inhaled formulation has been approved, and 15% of the inhaled dose reaches the lower respiratory tract (Peng et al. 2000). A comparative clinical study has underlined the greater effect of aerosol compared to oral oseltamivir in reducing symptoms of influenza A or B (Kawai et al., 2008).

Even though IFN- γ is not an antiviral drug, it is helpful in the treatment of some respiratory diseases due to its immunomodulatory pharmacological activity. Recently, a novel nebulized formulation of interferon gamma (IFN- γ) has been tested using special vibrating mesh-type nebulizers. This experiment was conducted following the regulatory standard requirements of methodologies for the assessment of pulmonary drug delivery. Applying this new technology to a nebulizer system has improved the delivery of this large molecule achieving optimal bioavailability in the lower respiratory tract, while maintaining its pharmacological activity. Aerosolized IFN- γ has been tested in clinical trials showing great tolerability with some improvement in the reduction of cavity lesion size and bacterial loads (Moss et al., 2005; Condos et al., 2004; Condos et al., 1997).

3. Drug exhalation by the lungs

In pharmacology, drug metabolism and elimination in the lungs are not well studied. However, it is possible to consider the elimination process from a “drug delivery” rather than a “drug elimination” point of view.

The ability of a drug to cross cell membranes depends on its partition coefficient. The partition coefficient of a substance depends on its chemical-physical characteristics; hydrophilic groups refer to those that are capable of forming hydrogen bonds with water, such as the carboxylic, alcoholic, amino, aldehyde and ketone groups and electrically charged groups. The partition coefficient of a drug (i.e., its ability to cross cell membranes) can vary due to metabolization processes. Generally, the systemic metabolization processes lead

to the formation of more hydrophilic compounds with partition coefficients lower than those of the original drugs. Furthermore, it is important to remember that many drugs are organic molecules that contain acidic or basic residues, that is groups which, depending on the pH of the solution in which they are found, can be electrically neutral or charged. For these drugs, the partition coefficient is also dependent on the pH of the environment and the pKA of the reactive groups.

The diffusion of drugs follows Fick's law, so a drug with an adequate partition coefficient can diffuse through cell membranes. The laws governing the diffusion between two compartments separated by a membrane are described by Fick's law: molar flow = $(c_1 - c_2) \cdot D \cdot A/d$ wherein by molar flow is meant the speed (moles per second) of the passage of a solute from compartment 1 to compartment 2; c_1 and c_2 are the concentrations of the compound in the two compartments; D is the diffusion coefficient, which depends on the chemical-physical characteristics of solvent and solute (in the case of passage through biological membranes, D is mainly determined by the partition coefficient); A is the area of the membrane that separates the two compartments, and d is its thickness. In the case of the plasma membrane, d can be considered a constant; in the case of a tissue, d depends on the number of cell layers to be overcome. Knowledge of this physiological mechanism could be useful in the review of possible molecules with low pharmacodynamic specificity towards the virus but with a high tropism for pulmonary elimination. The site of action in this case becomes the first element of pharmacological advantage over the aetiopathological agent.

4. VOLATILE MOLECULES

The pulmonary elimination is inversely proportional to the blood solubility of a molecule. Other chemical characteristics, such as vapour pressure and molecular dimension, increase lung elimination via exhalation: higher lipophilic properties, higher vapour pressure and lower molecular weight contribute to easier elimination through exhalation. The main classes of volatile organic compounds that are exhaled by the lungs can be summarized as saturated (ethane, pentane and aldehydes) and unsaturated hydrocarbons (isoprene), and oxygen- (acetone), sulphur- (ethyl mercaptan and dimethyl sulphide) and nitrogen- (dimethylamine, ammonia) containing compounds (Dent et al., 2013).

The pharmacokinetic pathway of lung elimination is known, for example, for chloral hydrate a highly lipophilic and small molecule used as sedative. This drug is now used only in a few cases due to its narrow therapeutic index, but it had been preferred in the paediatric population for its easy oral administration and short half-life. Oral administration represents a clear advantage, increasing patient compliance. Another drug that stimulates indirect lung elimination is disulfiram, used in the treatment of alcohol dependence. Its metabolites, carbon disulphide (CS_2) and acetone, are transported from the blood into alveolar air and exhaled with the breath. Disulfiram increases the acetaldehyde blood concentration by aldehyde dehydrogenase inhibition of ethanol metabolism, with an increase also in lung exhalation and acetaldehyde toxicity (Torsten et al., 2017). The particular mechanisms of this drug cause many of the unwanted effects of a hangover immediately after alcohol consumption to break the ethanol addiction. Curiously, disulfiram has been tested in vivo against the viruses that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), but the studies did not provide adequate clinical evidence (Lin et al., 2018).

Many volatile molecules come from natural sources. The most common example of this, experienced by each of us, is the pulmonary excretion of garlic diallyl-derivatives, such as diallyl disulphide, and other organic sulphur compounds, such as allyl methyl sulphide and methyl mercaptan, in expired air and how they affect social relations. A number of molecules with typical volatile characteristics are the small terpenoid class of monoterpenes consisting of two isoprene units that can be linear (acyclic) or contain one or two rings.

Terpenoids represent the major component of essential oils, and the large number of pharmacological properties of each compound have already been discussed (Kozioł et al., 2014). The most well-known are α -pinene, limonene, γ -terpinene, terpinolene, arbanol, α -terpineol, linalool, thymol, menthol and carveol. Common pharmacological effects of different types of cyclic monoterpenes are antibacterial, antiviral and antifungal effects (Dehsheikh et al., 2020).

In consideration of the chemical-physical properties of eucalyptol (1,8-cineole), it is likely to think of significant levels of substance in the lungs. In addition, taking note of its characterized kinetics of pulmonary elimination and according to Fick's law, the time of persistence should also be sufficient for eucalyptol to perform a considerable pharmacological action. Furthermore, due to the negligible side effects, the risk/benefit ratio suggests that its pharmacological effects are worth testing.

4.1 Eucalyptol (1,8-cineole)

Eucalyptol (fig. 2) is a natural saturated bicyclic monoterpenoid that is extracted from various species of *Eucalyptus* (e.g., *Eucalyptus globulus* Labill., *Eucalyptus polybractea* R.T. Baker and *Eucalyptus smithii* R.T. Baker, Fam. Myrtaceae). The essential oil of eucalyptus contains not less than 70% of eucalyptol as reported in Ph. Eur. 10th ed. Pure eucalyptol is a clear liquid at room temperature, and the melting point is 1.5°C with a flash point of 49°C (Prasanthi et al., 2012).

Fig. 2: Eucalyptol.

Eucalyptus essential oil appears in almost all European national pharmacopoeias and is traditionally used as a mucolytic, as an antiseptic and to treat asthma, fever, flu, bronchitis and whooping cough. EMA indicates its traditional use of this essential oil as a treatment for the cough associated with a cold. In Germany, 1,8-cineole is a licensed medicinal product formulated in gut soluble capsules containing 100 mg/capsule and is indicated for acute and chronic bronchitis, sinusitis, and respiratory infections.

In vitro studies have underlined the effect of eucalyptol as a bronchodilator (credo), in enhancing the activity of mucociliary cells with a corresponding effect on clearance and in decreasing mucus production (Galan et al., 2020). *In vitro* investigation has shown up to a 92% inhibition of the release of pro-inflammatory cytokines tumour necrosis factor- α (TNF- α) and interleukin-1-beta (IL-1b) by lipopolysaccharide (LPS)-stimulated monocytes treated with 1.5 μ g/ml of eucalyptol (Juergens et al., 2003). Another study has reported 0.6 mg/ μ L eucalyptol extract to significantly inhibit the NF- κ B p65 gene promoter in LPS-stimulated human cell lines resulting in a decrease in inflammation compared to that of the control (Greiner et al., 2013). An interesting experiment was conducted analysing human blood of asthmatic patients and healthy subjects, both pre-treated with 200 mg eucalyptol thrice daily for 3 days, *ex vivo*. Up to 40.1% inhibition of LTB4 and PGE2 from monocytes from asthmatic patients (n=10) and up to 57.9% inhibition of those from healthy subjects (n=12) have been reported *ex vivo* (Juergens et al 1998).

A placebo-controlled clinical trial analysing 242 patients with acute bronchitis has measured the effect of 200 mg eucalyptol thrice daily for 10 day on the bronchitis endpoint "Bronchitis-Sum-Score" at 4 and 10 days. The group treated with eucalyptol showed a significant reduction in the score compared to that of the placebo group (3.55 vs 2.91) on day 4, but no significant differences were reported on day 10 (Fischer et al., 2013).

A double-blind placebo-controlled trial has considered glucocorticosteroid reduction endpoints for 32 asthmatic patients given 200 mg of eucalyptol, or placebo, thrice daily for twelve weeks. The results demonstrated a significant reduction of glucocorticosteroid medication in the treatment group, even when the use of salbutamol was doubled in this group, compared to the baseline condition, but the score of dyspnoea was significantly greater in the placebo group (Juergens et al., 2003).

The same dosage of eucalyptol, but taken for 6 months, has been used as an adjunctive therapy in a more recent multicentre placebo-controlled double-blind trial considering 247 asthmatic patients already under medication. The authors reported a significant improvement in lung function in the treatment group compared to the placebo group, by reducing dyspnoea demonstrated by primary end-points, such as forced expiratory volume 1 second, asthma symptoms and quality of life (Worth et al., 2012). The same authors conducted a similar trial on 242 smoking or former smoking patients with moderate/severe chronic obstructive pulmonary disease (COPD) with concomitant use of standard therapy. The group treated with eucalyptol showed a significant reduction in the frequency, duration and severity of COPD exacerbations compared

with those of the control group. Secondary end-points, lung function, dyspnoea and quality of life, improved without significance (Worth et al. 2009). Data from a COPD experimental animal model exposed to cigarette smoke have highlighted normal lung parenchyma and significantly less leukocyte infiltration by 40-50% in mice treated with 3 mg/kg and 10 mg/kg eucalyptol compared to placebo. In the same study, the eucalyptol group showed a significant 60% reduction in myeloperoxidase activity and 50% and 40% decreases in IL-1 β and interleukin-6 (IL-6) expression, respectively; TNF- α levels were reduced by 80% in the higher dosage group. In addition to the anti-inflammatory effect, a predictable disinfectant effect of reduced bacterial colonies was also measured in this experimental COPD model of mice treated with 260 mg of eucalyptol per day (Yu et al., 2017).

The *in vitro* antiviral activity of nebulized eucalyptol essential oil has been explored by Usachev *et al.* (2013). The authors analysed the effect of eucalyptus aerosol on two viral model systems of nebulized influenza virus A strain NWS/G70C (H11N9), simulating hazardous bioaerosols in indoor and outdoor environments, by a plaque assay technique.

The results underlined that 99% viral inactivation is achieved after 15 seconds of aerosolization and exposure for 5 minutes. The antiviral activity was exerted by both the aerosol form and vaporized phase. The antiviral effect was measured in an *in vitro* experimental model of Herpes simplex virus, and 1,8-cineole had an antiviral IC₅₀ equal to about a quarter of the maximum non-lethal dose measured in a cytotoxicity assay (Astani et al., 2010).

A small number of both *in vivo* and *in vitro* pharmacokinetic studies have considered oral administration. In a rabbit model given 200 mg/kg of eucalyptol, a peak plasma concentration was reached after 1 h (Bhowal et al 2005). The oxidative metabolic pathway of eucalyptol produces 2-hydroxy-1,8-cineole and 3-hydroxy-1,8-cineole, conjugated to glucuronide products. It has been reported that chronic administration of 800 mg/day does not imply accumulation (Juergenes et al., 2003). By extrapolation from data on other monoterpenes with similar chemical structures, it could be supposed that lung elimination of the unchanged form of eucalyptol could range from 1 to 10% in the expired air (Kohlert et al. 2000).

The EMA monograph on eucalyptus essential oil (eucalyptol content of at least 70%) indicates oral use of up to 200 mg of essential oil for a maximum of 5 times a day for adults and adolescents, while use in children under 30 months is contraindicated.

5. DISCUSSION

The novel SARS-CoV-2 infection is straining global health systems. The massive spread of the virus is requiring new response paradigms from the scientific community. The first step of the pharmacological strategy was to consider molecules already on the market for a reasoned use in the treatment of viral pneumonia COVID-19 (Li and De Clercq, 2020). As a consequence, most of the antiviral drugs currently registered in clinical trials are medicines with other indications, but which can potentially benefit patients affected by COVID-19.

Based on clinical experience from SARS and MERS, and considering the characteristic of this single-stranded RNA beta-coronavirus, a number of antiviral nucleoside analogue drugs, such as favilavir, which selectively inhibits viral RNA-dependent RNA polymerase, or ritonavir, a protease inhibitor used against the hepatitis C virus, are currently in trials. Other specific antiviral drugs are being analysed in order to find a specific pharmacological response to this pandemic (Li and de Clercq, 2020).

Alongside this approach, some non-specific pharmacological strategies with secondary mechanisms of action against the virus have been considered, such as the immune modulator chloroquine (Touret and de Lamballerie, 2020). This strategy has shown promising *in vivo* and *in vitro* data and some clinical evidence, the latest reported by Gao et al. (2020) describing the superiority of chloroquine over the control group in a Chinese clinical trial. Now, a number of clinical trials are starting in order to best delineate these first evidence (Touret and de Lamballerie, 2020). Others non-specific pharmacological approaches are currently under investigation including the use of inhaled medicines (table 1). For example, nitric oxide gas is a selec-

tive pulmonary vasodilator administered to COVID-19 patients as a rescue therapy for refractory hypoxemia due to acute respiratory distress syndrome (ARDS). Some evidence from *in vitro* and clinical data have pointed out that inhaled nitric oxide gas (iNO) has exerted nonspecific antiviral activity (ClinicalTrials.gov id: NCT04290871).

Here, the possibility of a nonspecific pharmacological effect focused on the pulmonary site of action has been discussed. In addition to pharmaceutical aerosols targeting drugs to the lower respiratory tract, here the possibility of exploiting the pulmonary elimination mechanism to concentrate molecules with non-specific action in the lungs is considered (fig. 3). The limitations of this strategy are defined by the particular characteristics that the candidate molecules must possess; on the other hand, the pulmonary elimination process favours a local concentration of the xenobiotic, which consequently can better reach a dose-dependent pharmacological effect. This peculiar pharmacokinetic aspect linked to the characteristics of a non-specific antiviral molecule may provide a therapeutic advantage to reach and treat this aggressive viral pneumonia.

Fig.3: Comparison between drug inhalation and exhalation.

Looking for a molecule that meets the requirement to be eliminated via the lung, we may search among monoterpenes, whose chemical and pharmacological characterizations are well known. The 1,8-cineole eucalyptol is the main monoterpene of the essential oil of eucalyptus. The EMA community herbal monograph on eucalyptus essential oil has established a dose regimen of 200 mg orally up to 5 times/day, and its safety has been adequately assessed. It has been reported to have a large spectrum of antimicrobial effect against several type of bacteria, viruses and fungi, which is assumed to be a nonspecific pharmacological effect given its phenolic chemical characteristics. A specific *in vitro* investigation has highlighted the antiviral properties of an aerosolized solution of eucalyptol on nebulized viruses (Usachev *et al.*, 2013), confirming the antiviral properties already described (Astani *et al.*, 2010). Furthermore, its anti-inflammatory effect in the lung has been well characterized, and specific clinical trials of its traditional use have highlighted its efficacy and safety in asthmatic patients. All these pharmacological characteristics of this monoterpene could make it a candidate to test for the treatment of COVID-19, and its use as a prophylactic or when the viral load is particularly low is also hypothesized. A further advantage is given by the great flexibility of the molecule, which can be used both by inhalation and orally. Another point to consider is the great availability of different monoterpenes, of which 1,8 cineole can be considered a representative, with similar characteristics that can be taken into consideration in a more detailed scientific analysis and for subsequent use in therapy.

The use of naturally derived substances in this particular emergency situation is also reflected in recent clinical trials that focus on a possible role of traditional Chinese medicine (TCM) in support of standard therapy. Some Chinese guidelines have described the use of traditional herbal medicine for prophylactic purposes (Jin *et al.*, 2020). Furthermore, the recent precious gift made by the People's Republic of China to Italy as emergency support contained TCM products (Ansa, 2020).

6. CONCLUSION

A possible strategy of pharmacological research may focus on a targeted action in the lungs and renouncing specific molecular antiviral actions. This strategy takes into consideration the great variability that the virus could develop in the future. In light of the above and in consideration of the tight deadlines, it's clear that trials on small volatile molecules could lead to positive results. These results can be expected both from a prevention point of view and in slowing down and/or inhibiting the progression of the disease (against the virus) from the initial stages to the more severe phases (against the cytokine storm). This perspective is reinforced by the negligible side effects and, therefore, the positive risk/benefit ratio. A suggested approach could be to start with the already characterized volatile compounds from the points of view of safety and known effectiveness against viruses, for example, 1,8-cineole. In conclusion, a deeper clinical investigation could be reasonable.

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Conflict of interest

The author has declared that there is no conflict of interest.

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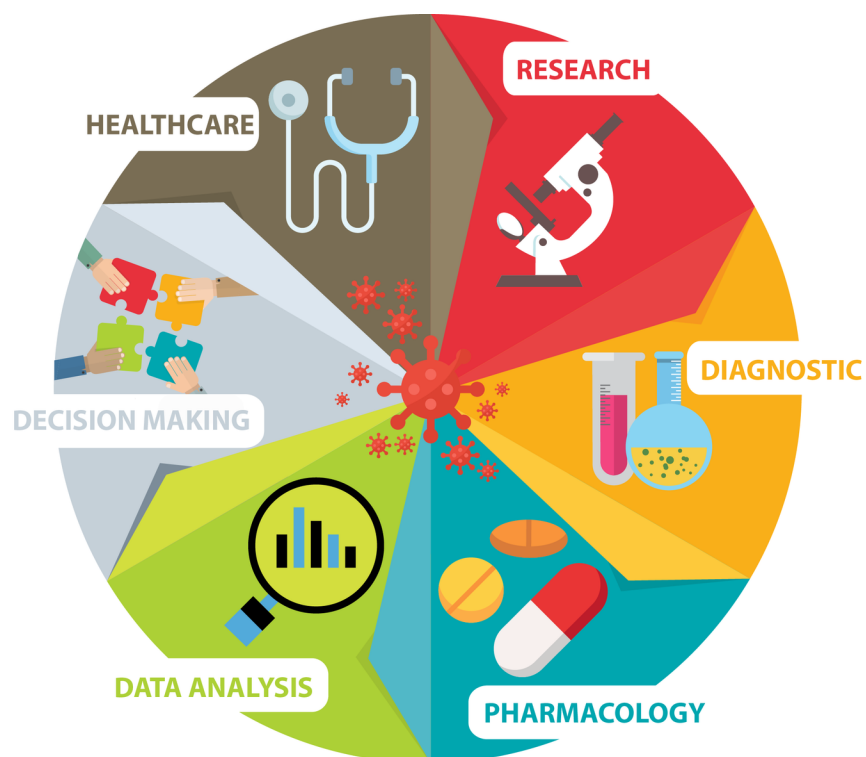
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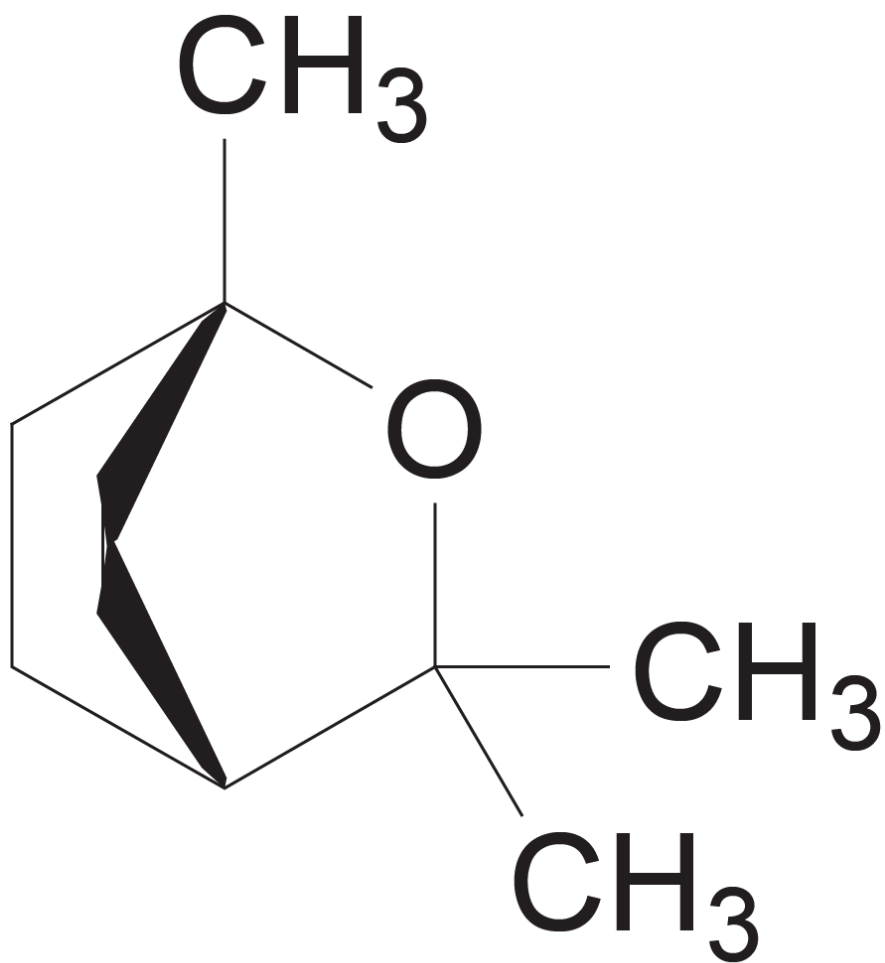
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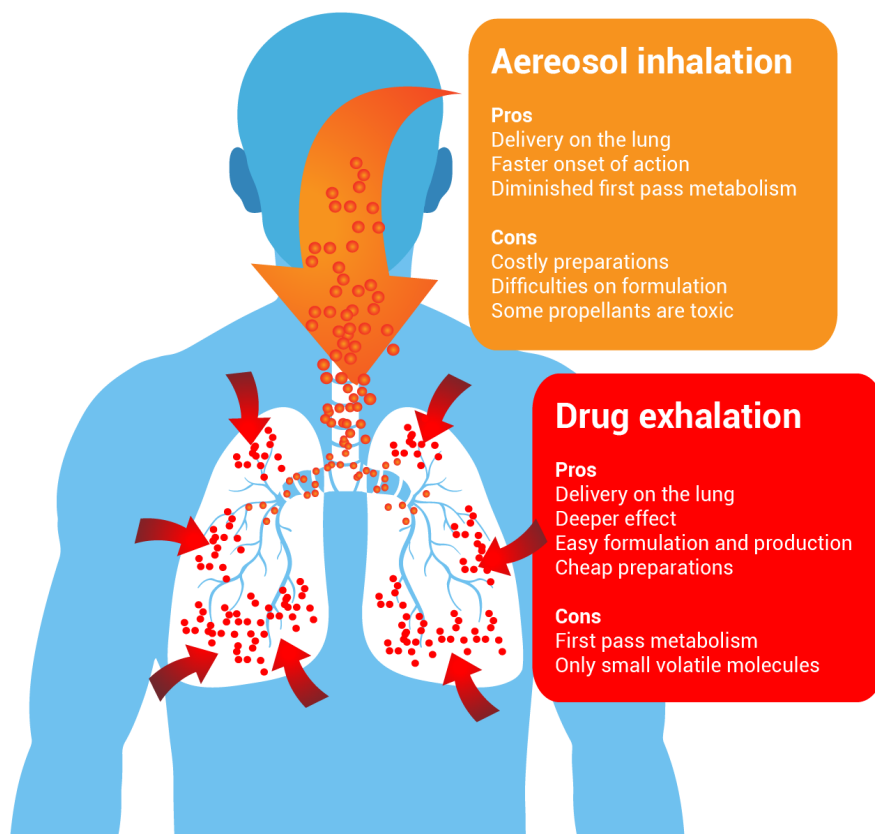
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