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Ivermectin Interactions with Alcohol & Ivermectin Liposomes:

For Better Bioavailability in Treatments of COVID-19

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Abstract. This documentary research focused on the Ivermectin Interactions with alcohol & ivermectin liposomes for better bioavailability in treatments of COVID-19. It is reported in this study that plasma ivermectin concentrations are significantly higher when co-administered with alcoholic water than with water, without side effects. The solutions of ivermectin showed to have approximately twice the systemic availability as either of the solid forms. In a debatable discussion it is not recommended by several researchers because there might be an association with GABA receptors and the effect of alcohol in the central nervous system. The important improvement of ivermectin activity loading in liposomes with ions compositions creates a promising starting point for a future development of these nanocarriers which could be the possible solution to the COVID-19 treatment. This might be a controversial topic of research for people who antagonize the consumption of alcohol, but it should serve as a motivation for further research.

Keywords. Ivermectin, alcohol, liposomes, bioavailability, COVID-19.

Introduction

Regarding the pharmacokinetics and interactions of ivermectin in humans, González Canga et al (2008) have explained that ivermectin is a semisynthetic derivative of avermectin B1 and consists of an 80:20 mixture of the equipotent homologous 22,23 dehydro B1a and B1b. This antiparasitic agent, developed by Merck & Co., (Now Patent Free) is frequently used in veterinary medicine, due to its broad spectrum of activity, high efficacy and wide margin of safety. Presently, ivermectin is approved for use in humans in several countries to treat onchocerciasis, lymphatic filariasis, strongiloidiasis and/or scabies.

Ivermectin is exceptionally potent, with effective dosages levels that are unusually low. In the treatment of onchocerciasis, the optimal dose of ivermectin is 150 µg/kg, but the frequency of administration is still controversial, ranging from 150 µg/kg once to three times yearly. The optimal duration of treatment has not been established). It is effective in most patients with scabies after a single oral dose of 200 µg/kg, but often the regimen involves two or three repeated doses, separated by interval of 1 or 2 weeks. Due to the extended use of this compound in humans, the knowledge of ivermectin

pharmacokinetic behavior becomes essential. Nevertheless, little is known about the kinetics and interactions of ivermectin in humans compared to animals (González Canga et al, 2008).

It has been shown that in healthy subjects that received ivermectin as oral solution, tablets or capsules, the solution had approximately twice the systemic availability as either of the solid forms (tablets and capsules showed similar systemic availability). It is necessary to highlight that the oral solution was given as an ethanolic solution. This may affect bioavailability and could explain why the solution resulted in a twice as high availability as tablets and capsules (Edwards, 1988). In addition, Due to the high lipid solubility of ivermectin, this compound is widely distributed within the body (González Canga et al, 2008).

This documentary research focused on the ivermectin Interactions with alcohol & ivermectin liposomes for better bioavailability in treatments of COVID-19. This might be a controversial topic of research for people who antagonize the consumption of alcohol, but it should serve as motivation for further research given the potential of improving bioavailability of ivermectin for the treatment of COVID-19.

Ivermectin in the Prophylaxis and Treatment of COVID-19

In a comprehensive systematic review, Heidary and Gharebaghi (2020) found out that ivermectin proposes many potentials effects to treat a range of diseases, with its antimicrobial, antiviral, and anti-cancer properties as a wonder drug. It is highly effective against many microorganisms including viruses. Ivermectin plays a role in several biological mechanisms, therefore it could serve as a potential candidate in the treatment of a wide range of viruses including COVID-19 as well as other types of positive-sense single-stranded RNA viruses.

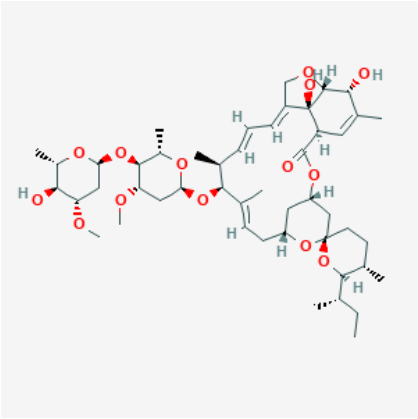
In March 2020, the Front Line COVID-19 Critical Care Alliance (FLCCC) was created and led by Professor Paul E. Marik to continuously review the rapidly emerging basic science, translational, and clinical data to develop a treatment protocol for COVID-19. The FLCCC recently discovered that ivermectin, an anti-parasitic medicine, has highly potent anti-viral and anti-inflammatory properties against SARS-CoV-2 and COVID-19. This conclusion is based on the increasing study results reporting effectiveness, not only within in-vitro and animal models, but in numerous clinical trials from around the world. Repeated, consistent, large magnitude improvements in clinical outcomes are reported when ivermectin is both as a prophylactic agent and in all phases of the disease from multiple, large,

randomized and observational controlled trials. Further, data showing impacts on population wide health outcomes have resulted from multiple large “natural experiments” that appear to have occurred when various regional health ministries and governmental authorities within South American countries initiated “ivermectin distribution” campaigns to their citizen populations in the hopes the drug would prove effective. The tight, reproducible, temporally associated decreases in case counts and case fatality rates in each of those regions compared to nearby regions without such campaigns, suggest that ivermectin may prove to be a global solution to the pandemic (Kory et al, 2020).

Chemical and Physical Properties of Ivermectin

Ivermectin is an orally bioavailable macrocyclic lactone derived from Streptomyces avermitilis, with antiparasitic and potential anti-viral activities. Upon administration, ivermectin exerts its anthelmintic effect through binding and activating glutamate-gated chloride channels (GluCls) expressed on nematode neurons and pharyngeal muscle cells. This causes increased permeability of chloride ions, causing a state of hyperpolarization and results in the paralysis and death of the parasite. Ivermectin may exerts its antiviral effect, including its potential activity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), by binding to the importin (IMP) alpha/beta1 heterodimer, which is responsible for the nuclear import of viral proteins such as the integrase (IN) protein. This inhibits nuclear import of host and viral proteins and may inhibit viral replication (NIH-PubChem, 2020).

Figure 1. Chemical Structure of Ivermectin (NIH-PubChem, 2020).



Molecular Formula: C48H74O14 / **Molecular Weight:** 875.1 g/mol

Ivermectin is an anti-infective agent with activity against several parasitic nematodes and scabies and is the treatment of choice for onchocerciasis (river blindness). It is typically given as one or two oral doses. Ivermectin therapy has been associated with minor, self-limiting serum aminotransferase elevations and very rare instances of clinically liver injury (NIH-PubChem, 2020).

Alcohol Consumption During Ivermectin (IVM) Treatment

Shu, Onwujekwe and Okonkwo (2000), researchers from the Department of Pharmacology and Therapeutics (College of Medicine) at the University of Nigeria, reported the results of study on the effects of alcoholic beverages on the availability of ivermectin. It has been established that ivermectin is relatively safe, with the incidence of side effects decreasing markedly after the first year of ingestion (Pacqui et al, 1991; Njoo, Stilma and Lelij. 1992).

It was encountered in Oji River, South Eastern Nigeria, by Shu, Onwujekwe and Okonkwo (2000), an indiscriminate ingestion of alcohol beverages. Alcohol restriction during mass drug administration (MDA) of ivermectin is normally resisted by the population. The restriction is for all drugs affecting the central nervous system because of the association of the drug with gamma-aminobutyric acid (GABA) receptors. However, some member of the community reported cases of “potent” effects of ivermectin after ingestion of the local alcoholic beverage, palm wine.

In their research, Shu, Onwujekwe and Okonkwo (2000), reported their results on the co-administration of an alcoholic beverage and ivermectin. For that purpose, they investigated ivermectin concentration on plasma. They concluded that during the period of study, plasma ivermectin concentrations were significantly higher when co-administered with alcoholic water than with water. They did not find side effects in either study or control subjects. However, ivermectin is toxic to beagle dogs, premature infants, adult epileptics and those with other central nervous system disorders.

In a similar experiment carried out previously by Edwards et al (1988), they showed that ivermectin in alcoholic solution was shown to have approximately twice the systemic availability as tablets, capsules and oral solutions. They administered 12-mg doses of ivermectin to 12 healthy volunteers in the form of tablets, capsules, and alcoholic oral solution. The solutions showed to have approximately twice the systemic availability as either of the solid forms. It was evidenced both by the maximum concentrations

of drug attained in plasma and by the corresponding areas under the plasma concentration vs time curves. However, the two solid formulations showed similar systemic availability.

In another research, Takougang et al (2008) carried out an investigation to determine if alcohol consumption increases the risk of severe adverse events to ivermectin treatment. They conducted a case-control study designed to assess the level of association between alcohol consumption and the occurrence of severe adverse reaction (SAE) following ivermectin consumption. Thirty-six (36) cases of SAE occurred in the health districts of Bankim, Nanga Eboko, Obala, Okola and Sa'a. Case and control (43) individuals were submitted to a questionnaire related to their alcohol consumption 24 before and 24 to 48 h following ivermectin intake. An in-depth interview of siblings and local health worker was conducted to assess alcohol consumption around Mectizan intake. The degree of alcohol use was assessed using the level of serum transaminases and the alcohol use disorder identification test (AUDIT). The alcoholic beverages of the study communities were conventional such as beer, whisky, or locally made. Locally produced beverages included "arki" ("Odontol", "Hah", ...) and palm wine. The bark, sap or fruit of plants adjuvant are known to contain alkaloids and tannins which are potent neurotropic substances. The likelihood of developing SAE among cases and controls did not differ significantly with history of consumption of alcoholic beverages. Nor did it differ for other indicators of chronic alcohol consumption.

In similar studies, it has been concluded that while there is an increased bioavailability of ivermectin following the co-administration of ivermectin with ethanol it is unlikely that the level of Ivermectin reached is enough to cause SAE. (Edwards et al., 1988; Cerkvenik and Grabnar, 2002).

Ivermectin and Alcohol Use Disorders (AUDs)

Asatryan et al (2014) investigated ivermectin (IVM) and other members of the avermectin family as new pharmaco-therapeutics to prevent and/or treat alcohol use disorders (AUDs). They hypothesized that structural modifications that enhance IVM's effects on key receptors and/or increase its brain concentration should improve its anti-alcohol efficacy. Available evidence indicates that IVM can reduce alcohol intake. Given that IVM is already approved for use in humans, IVM has the potential for rapid repurposing as a novel treatment for AUDs. The anti-alcohol actions of IVM likely reflect its ability to modulate one or more ligand-gated ion channels in the brain.

Taken together, the findings of Asatryan et al (2014) suggest that chemical structure and effects on receptor function play key roles in the ability of avermectins to reduce ethanol intake and that these factors are more important than brain penetration alone.

Yardley et al (2014) from the renowned University of Southern California, proposed that ivermectin, a drug used by millions of humans for treatment of parasites can be repositioned as a novel pharmacotherapy to treat and/or prevent excess alcohol consumption and abuse. Further support for the repositioning of IVM is drawn from a number of studies showing that IVM significantly reduces ethanol intake and preference in mice as determined across several validated alcohol drinking paradigms. This work found that IVM doses ranging from 1.25 to 10.0 mg/kg can be safely administered and can significantly reduce alcohol intake using a 24-h access model that mimics “social” or non-intoxicating levels of alcohol drinking.

It was also found by Yardley et al (2014) that acute administration of IVM can significantly reduce higher levels of alcohol drinking using the intermittent limited-access model, which mimics binge-like drinking. Importantly, in humans, young adults who participate in binge or heavy drinking are more likely to progress to alcohol abuse or dependence than age-matched counterparts. Further, individuals participating in binge-like drinking behavior and/or drinking to intoxication is associated with significant increases in vehicle accidents, injuries, date rape and other types of violence, pregnancy, and blackouts. Their findings that IVM significantly reduces binge-like drinking in mice further supports the development of IVM as a new pharmacotherapeutic agent for treatment and/or prevention of AUDs.

The approved dosing and administration regimen for IVM is based on acute use of the drug in human subjects. However, chronic administration would be anticipated in patients for treatment of AUDs. Several pieces of information support the safety of the chronic administration of IVM. First, doses up to 10 times that of the recommended dosage (i.e., 2.0 mg/kg/day) have been safely tested in human clinical trials. Second, in rodents, doses less than 10 mg/kg IVM do not cause detectable CNS depression, and is more than 2.5 fold lower than the LD50 (25–50 mg/kg). Third, allometric scaling identified a dose of 3.1 mg/kg/day IVM in mice that corresponds to an oral dose (30 mg or approximately 0.5 mg/kg) already shown to be safe in humans. Fourth, a case-control study reported that there were no significant increases in severe adverse events (SAEs) for patients that had self-reported consuming alcoholic beverages at the time of IVM administration. Collectively, these findings point to IVM as an

attractive agent for the treatment of AUDs, with good margin of safety and tolerability (Yardley et al, 2014).

Importantly, single doses of up to 120 mg, a dose 4 times greater than the dose tested in the current study, have been safely administered to human subjects with no reported adverse experience for other indications (Guzzo et al, 2002 in Yardley et al, 2014). In this same study, a 30 mg dose in humans (equivalent to a 3.1 mg/kg dose in mice), was administered three times in the first week (days 1, 4, and 7) followed by a washout period of 1 week and then a single dose of IVM (30 mg). Of these patients receiving IVM, 33.3% (n=5) had one or more adverse experience compared to 35.3% of the placebo group (n=6). There was no apparent evidence of CNS toxicity associated with IVM administration in this study at either dose measured assessed using quantitative pupillometry. This suggests that IVM has a good therapeutic index as a treatment for AUDs since the minimum effective dose necessary to decrease alcohol intake is far lower than the lethal dose 50 (LD50) of IVM which is approximately 25–50 mg/kg in Mice (Merck; Sharp and Dohme, 1988 in Yardley et al, 2014).

The potential for repositioning IVM as an anti-alcohol therapy is further supported by previous investigations that reported that IVM at doses needed to produce the anti-alcohol effects in C57BL6 mice did not induce overt signs of toxicity across a wide range of well validated behavioral paradigms for the assessment of sensory, motor and cognitive competence (Bortolato et al, 2013).

A pilot study of the safety and initial efficacy of ivermectin for the treatment of alcohol use disorder was carried out by Roche et al (2016). They explained that Ivermectin (IVM) is an antiparasitic agent that has been shown to reduce alcohol intake in mice, suggesting IVM as a potential treatment for alcohol use disorder (AUD). The safety profile of IVM administered in combination with an intoxicating dose of alcohol has to be characterized in humans. This pilot project sought to provide the first clinical evidence that IVM could be repositioned as an AUD pharmacotherapy by examining 1) the safety of combining IVM (30 mg oral) with an intoxicating dose of intravenous alcohol (0.08 g/dl) and 2) the effects of IVM on alcohol cue-induced craving and subjective response to alcohol. Eleven individuals with AUD participated in a randomized, placebo-controlled, crossover study in which they received the study medication, participated in a cue exposure paradigm followed by intravenous alcohol administration, and remained in an inpatient unit overnight for observation.

These results obtained by Roche et al (2016) suggest that IVM (30 mg oral) is safe in combination with an intoxicating dose of alcohol, but do not provide evidence that this dose of IVM is effective in reducing alcohol craving or its reinforcing effects. Given the preclinical data suggesting IVM is effective in reducing alcohol consumption in mice, additional studies testing larger samples and alternate dosing regimens are warranted to further characterize the potential efficacy of IVM as an AUD treatment.

In summary, Roche et al (2016) found that IVM (30 mg) was safe in combination with an intoxicating dose of alcohol but did not display efficacy in reducing alcohol craving or affecting subjective response to alcohol. They advise against interpreting the null initial efficacy results of this pilot study as an indication that IVM is not a promising AUD pharmacotherapy. Indeed, other members from IVM's class of drugs, such as abamectin, have also been shown to decrease alcohol intake and preference in mice (Asatryan et al., 2014). The study and its results as a whole are promising for several reasons: it's an excellent example of a translational study, it provides support for the safety of IVM, and it identified methodological changes that future studies should employ when testing this medication for AUD (e.g., higher dosage, additional measures). These strengths speak to the importance of using human laboratory studies to effectively translate preclinical findings and fostering working collaborations between preclinical and clinical scientists to facilitate the development of novel treatments for AUD. Given the paucity and limited efficacy of available pharmacotherapies, as well as IVM's strong preclinical findings and its safety and tolerability, IVM and other avermectins warrant investigation as potential AUD pharmacotherapies.

Nanoparticles for antiparasitic drug delivery

With the continuous development and innovation of nanomedicine, nanoparticles have been researched for antiparasitic drug delivery to improve their bioavailability, sustained release, and intracellular penetrability performances. Immobilization of antiparasitic drugs on or into nanoparticles is an effective way to improve efficacy and decrease the toxic side effects of drugs. As nanocarriers, liposomes have the advantages of targeting, controlling release and reducing toxicity. Liposomes can be targeted to specific tissues via controlling their self-specific properties or by attachment of specific ligands onto their surfaces (Sun et al, 2019).

Some liposomes could be prepared by combining some chemically and biologically inert synthetic polymers to produce long-circulating liposomes (Asthana et al., 2015), furtherly prolonging the drug

circulation time in vivo, thereby enhancing the effectiveness. For example, the duration of efficacy of liposomal avermectin was increased from 21 to 30 d. In all, liposomes have advantages of improved specific distribution, prolonged circulation, decreased toxicity, and fewer side effects of antiparasitic drugs.

For Sun et al (2014), nanocarriers hold the advantages of delivering their laden drugs into cells and organelles using smart cellular uptake and intracellular transport pathways. Recently, increasing research demonstrated that the physical and chemical properties of nanoparticle could influence their interaction with the cellular surface and the subsequent endosomal properties, thus mastering the cellular uptake and intracellular transport of nanoparticles and their payload release (Xie et al., 2014). The new understanding how the nanoparticles control their intracellular delivery via themselves properties is very important (Sun et al, 2014).

Liposomes

Lasic and Papahadjopoulos (1998) defined liposomes as synthetic analogues of natural membranes. They are composed of polar lipids, which are molecules essential for the appearance of life on earth and its evolution. The special physico-chemical characteristics of polar lipids, such as their peculiar solubility, self-aggregation and membrane forming properties, as well as their phase behavior with their thermodynamic and kinetics effects, define the properties of liposomes. Their utility in biological systems derives from their biocompatibility, colloidal character, and encapsulating properties. As a model membrane system, liposomes have helped unravel the mechanism of many cell membrane functions. As a carrier system for drugs and other macromolecules, they hold great promise for tissue- and cell-specific delivery of a variety of pharmaceuticals and biotechnology products.

It has been explained by Çağdaş, Sezer and Bucak (2014) that when lipids are placed in contact with water, the unfavorable interactions of the hydrophobic segments of the molecule with the solvent result in the self-assembly of lipids, often in the form of liposomes. Liposomes consist of an aqueous core surrounded by a lipid bilayer, much like a membrane, separating the inner aqueous core from the bulk outside. Liposomes have been used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life or reducing toxicity. Drug distribution is then controlled primarily by properties of the carrier and no longer by physico-chemical characteristics of the drug substance only. The unique feature of liposomes is their ability to

compartmentalize and solubilize both hydrophilic and hydrophobic materials by nature. This unique feature coupled with biocompatibility and biodegradability make liposomes very attractive as drug delivery vehicles.

Liposomal formulations enhance the therapeutic efficiency of drugs in preclinical models and in humans compared to conventional formulations due to the alteration of biodistribution. Liposome binding drugs, into or onto their membranes, are expected to be transported without rapid degradation and minimum side effects to the recipient because generally liposomes are composed of biodegradable, biologically inert and non-immunogenic lipids. Moreover, they produce no pyrogenic or antigenic reactions and possess limited toxicity. Consequently, all these properties as well as the ease of surface modification to bear the targetable properties make liposomes more attractive candidates for use as drug-delivery vehicles than other drug carrying systems (Çağdaş, Sezer and Bucak, 2014).

Mechanism of Vesicle Formation

Liposomes are lipid vesicles, which are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self-close to form large, multilamellar vesicles (LMV) which prevents interaction of water with the hydrocarbon core of the bilayer at the edges. Once these particles have formed, reducing the size of the particle requires energy input in the form of sonic energy (sonication) or mechanical energy (extrusion) (Merck, 2020).

Preparation of Lipid for Hydration

Merck (2020) indicates that when preparing liposomes with mixed lipid composition, the lipids must first be dissolved and mixed in an organic solvent to assure a homogeneous mixture of lipids. Usually, this process is carried out using methanol or chloroform mixtures. The intent is to obtain a clear lipid solution for complete mixing of lipids. Typically, lipid solutions are prepared at 10-20mg lipid/ml organic solvent, although higher concentrations may be used if the lipid solubility and mixing are acceptable.

Sonication

Disruption of LMV suspensions using sonic energy (sonication) typically produces small, unilamellar vesicles (SUV) with diameters in the range of 15-50nm. The most common instrumentation for

preparation of sonicated particles are bath and probe tip sonicators (MERCK, 2020). In this method, lipids are directly solubilized in water upon application of high mechanical agitation, through the use of probe sonication. It is one of the simplest methods of liposome preparation. Sonication disrupts MLV suspensions by using sonic energy to produce SUVs with diameters in the range of 15-50 nm. There are two methods of sonication: bath sonication and probe sonication. The former method is used for large volumes of dilute lipids whereas the latter one is used for suspensions which require high energy, such as high concentration of lipid suspensions (Çağdaş, Sezer and Bucak, 2014).

Ethanol injection method

In this method an ethanol solution of lipids is injected rapidly into an excess saline or other aqueous medium. The injection force is usually sufficient to achieve complete mixing, so that ethanol is diluted in water, and lipids are dispersed evenly throughout the medium. This method yields a high proportion of SUVs. This method is extremely simple, and it has a very low risk of degradation for sensitive lipids (Çağdaş, Sezer and Bucak, 2014; Pons, Foradada and Estelrich, 1993). With respect to drug encapsulation, drugs dissolved in the same ethanol as the lipid are relatively well encapsulated (Pons, Foradada and Estelrich, 1993).

Ivermectin Liposomes

Mastrangelo et al. (2012) identified the widely used antihelminthic drug ivermectin as a molecule able to inhibit the NS3 helicase activity of several flaviviruses in vitro at sub micromolar concentrations. Most importantly, ivermectin proved to be a selective inhibitor of the replication of yellow fever virus and, although less efficiently, of other flaviviruses such as dengue virus, japanese encephalitis virus, and tick-borne encephalitis virus. Ivermectin is under clinical trials on humans, as a dengue virus therapeutic.

Croci et al (2016) studied liposomal systems as nanocarriers for Ivermectin as an antiviral treatment for flaviviruses. Ivermectin is the common name of 22,23-dihydroavermectin B1, a semisynthetic derivative of avermectin family. It is a potent endo- and ectoparasitic agent with a broad spectrum of activity; its clinical use has been greatly recognized up the top award in science, being the Nobel Prize in Medicine and Physiology 2015 awarded to William C. Campbell and Satoshi ¯Omura for their discovery of avermectin.

The pharmacokinetic behavior of avermectin depends upon the route of administration, the formulation used, the animal species, and pathophysiological status of the host. It is established that subcutaneous injection is the most efficient route for ivermectin administration in terms of drug bioavailability in sheep, cattle, and goats when compared with oral and topical administration. Pharmaceutical technology has been applied to develop different drug formulations and delivery systems to optimize the pharmacological availability of ivermectin. The most promising approach for improving formulation lies in innovative delivery systems using carriers with defined physicochemical properties, such as liposomes (Croci et al, 2016).

Although the efficacy of ivermectin has been established in humans against several parasite diseases, Croci et al (2016) explain that the antiviral properties of this compound are not yet exploited, mainly due to its complex chemical structure which cannot be easily chemically modified. The lack of appropriate formulations which could improve cellular internalization of ivermectin reduces the effectivity of the drug.

According to these considerations, Croci et al (2016) reported the development of engineered liposome formulations for possible clinical use of ivermectin. In particular, their study described the production and the characterization of liposomes with ivermectin followed by subsequent tests of their antiviral activity and cytotoxicity in different cell lines.

The liposomes were prepared and characterized by by Croci et al (2016) by means of an ethanol injection procedure. Ivermectin was solubilized in ethanol (final concentration 50mM) and different volumes were mixed with an ethanolic solution of PC 30–180 mM,plus cholesterol (10mM) or DDAB (5–10mM), in order to obtain the final required ivermectin concentrations. 500 μL of the resulting ethanolic solution was injected by a syringe pump (500 μL/min) into 4.5mL of double-distilled water under magnetic stirring (300 rpm). Liposome size analysis was performed with photon correlation spectroscopy (PCS), cryogenic transmission electronmicroscopy (cryo-TEM) analysis, and determination of encapsulation efficiency.

Croci et al (2016) demonstrated that ivermectin, when delivered through liposomes, reduced cytotoxicity up to 5 times. They can effectively inhibit dengue virus replication with EC50 values in the same range of ivermectin alone and even improve its activity in several formulations. The possibility of dissolving ivermectin into an aqueous solution thanks to the use of liposome as drug carriers is a new

step towards the solution of its pharmacokinetics problems, in particular its high cytotoxicity. This could also amplify the spectrum of ivermectin activities. The important improvement of ivermectin activity loading in liposomes with ions compositions creates a promising starting point for a future development of these nanocarriers which one day could be the possible solution to the lack of drugs against flaviviruses.

Ultrasonic Encapsulation of Ivermectin

Drugs such avermectin and ivermectin are often encapsulated into nano-structured drug carriers to improve absorption rate and bioavailability as well to obtain a sustained-release effect. Ultrasonic nano-emulsification is an efficacious and reliable technique to entrap bioactive medicinal compounds into solid-lipid nanoparticles, liposomes or cyclodextrin complexes (Hielscher, 2020).

Ivermectin, a member of the avermectin drug class, is an anti-parasitic drug used mostly to treat roundworm infections. It works by by binding to and activating glutamate-gated chloride channel receptors (GluClRs) of the parasites, thereby paralyzing and killing them. During the research for a cure against COVID-19, the disease caused by the novel coronavirus SARS-CoV-2, ivermectin came into the focus of pharma research as it killed the SARS-CoV-2 coronavirus within 40hr in “in vitro” tests. Aa an already FDA-approved drug, the pharmacology of ivermectin is well known and the drug would be readily available for fast implementation of COVID-19 mass treatment (Hielscher, 2020).

Nano-sized drug carriers such as solid-lipid nanoparticles (SLNs), liposomes and cyclodextrin complexes are known to improve drug delivery and bioavailability, to provide a slow / sustained drug release, and to protect the bioactive molecule against degradation. As ivermectin is incompatible with various common excipients, which requires an alternative formulation. Furthermore, ivermectin is prone to be degraded by light, oxygen and hydrolysis, so that encapsulation helps to protect and stabilise the drug compound. Solid-lipid nanoparticles, liposomes or cyclodextrin-based ivermectin formulations show good results offering protection against degradation as well as improved solubility and stability (Hielscher, 2020).

The ultrasonically prepared ivermectin-SLNs showed a relatively high encapsulation efficiency (EE) with a narrow particle size distribution. The release study displayed slow and sustained release patterns for ivermectin-SLNs. Ultrasonically promoted formation of solid-lipid nanoparticles is a well-known

technique used to formulate pharmaceutical complexes with a high absorption rate and bioaccessibility, low cytotoxicity, and sustained drug release (Hielscher, 2020).

Guo et al. (2018) showed in their research that ultrasonic encapsulation of ivermectin in solid-lipid nanoparticles (SLNs) resulted in amorphous ivermectin particles within the SLNs and displayed prolonged release of the drug molecules from the SLNs without burst release due to high encapsulation efficiency (EE).

The preparation of ivermectin SLNs was performed by Guo et al. (2018) in order to the following protocol:

SLNs were prepared by hot homogenisation, followed by ultrasonication. Briefly, palmitic acid (0.5 g) was melted in a 30-ml glass vial by a magnetic stirrer at 75ºC. Ivermectin (0.09 g) was then dissolved in the melted lipid. The lipid phase was subsequently poured into 15mL of boiling aqueous solution with 1% PVA (w/v) under magnetic stirring at 300rpm for 10 min to form a coarse oil-in-water emulsion. After that, the emulsion was sonicated for 5 min, and then 15 mL of cold water (4ºC) was immediately poured into to obtain the ivermectin-SLNs. Control SLNs were prepared following the same protocol without the addition of ivermectin.

Liposomes are composed from one or more phospholipid bilayers. Combining chemically and biologically inert synthetic polymers to produce long-circulating liposomes allows for the preparation of liposomes with prolonged drug circulation time in vivo, thereby enhancing the effectiveness. For example, the duration of efficacy of liposomal avermectin was increased from 21 to 30 days in goats (Sun et al., 2014). Besides the formulation into solid-lipid nanoparticles, ivermectin has been successfully encapsulated into liposomes and cyclodextrin complexes.

Ivermectin is a mixture containing at least 90% 5-O-demethyl-22,23-dihydroavermectin and less than 10% 5-O-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)avermectin, generally referred to as 22,23-dihydroavermectin. Avermectins are disaccharides (ivermectin, doramectin) or monosaccharides (selamectin). Avermectins are a series of macrocyclic lactone derivatives with antiparasitic effects, mainly used to treat nematode infections. Insoluble in water, avermectin can be solubilized in methanol and 95% ethanol.

Ivermectin Liposome Production by Microfluidics

Carugo et al (2016) proposed microfluidic architectures for the mass production of liposomes with a view to potential industrial translation of this technology. They have presented a microfluidic hydrodynamic focusing (MHF) approach with the typical physical characteristics of microfluidic systems. It seems to represent the only viable microfluidic method for producing lipid-based nanoscale vesicular systems with potential for clinical application.

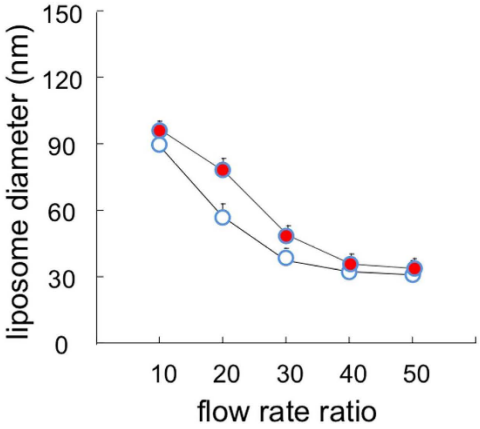
According to Carugo et al (2016), typically, a stream of lipid in alcohol solution is forced to flow in the central (or inner) channel of the device. The lipid stream is intersected and sheathed by two lateral (or coaxial) stream(s) of a water phase (typically distilled water or aqueous buffers). In this way, the lipid containing stream is hydrodynamically focused into a narrow sheet having a rectangular cross-section in the case of microchips with cross flow geometry, or a circular cross-section in the case of 3D annular coaxial chips. Notably, the size of the focused stream can be tuned by adjusting the volumetric flow rate ratio (FRR) between the lipid and water phase streams, and the total flow rate (TFR).

The formation of liposomes in MHF chips is governed by the diffusion of different molecular species (mainly alcohol and water, but also lipids) at the liquid interface between the solvent (alcohol) and non-solvent (water) phases. The alcohol in which the lipids are initially solubilized diffuses into the water (and concomitantly the water diffuses into the alcohol) until the alcohol concentration decreases to a critical level, below the solubility limit of the lipids. As such, the alcohol diffusion triggers the formation of liposomes by a mechanism described as “self-assembly”. Specifically, it is believed that the reciprocal diffusion of alcohol and water across the focused alcohol/water interface causes the lipid to precipitate, resulting in the formation of intermediate structures, in the form of oblate micelles, that subsequently form liposomes. MHF microfluidic techniques have been shown to produce uniformly dispersed liposomes and allow for direct control of liposome size via fine adjustments to either FRR or TFR (Carugo et al, 2016).

Additional post processing steps are often required for obtaining homogeneous vesicle suspensions. These processes include: (a) vesicle extrusion through the pores of polycarbonate membranes, (b) treatment with ultrasound, or (c) repetitive freezing and thawing. In contrast, being characterised by laminar flow conditions and diffusive mass transfer, MHF has the ability-at least in theory-to produce liposomes with excellent control over size (Carugo et al, 2016).

The effective encapsulation of drugs by liposomes produced using MHF was investigated by Carugo et al (2016). Ivermectin was employed as a model drug since it has recently been shown to be a highly potent inhibitor of yellow fever virus replication and, although less efficiently, of several other flaviviruses. Figure 2 shows that this drug did not cause large modification of the liposome size produced by MHF microfluidics, as the liposome containing ivermectin were only marginally larger than the empty ones. Notably, the encapsulation efficiency of ivermectin in liposomes was extremely high, exceeding 95%. All together, the favorable characteristics of ivermectin loaded liposomes could be potentially ascribed to the physico-chemical properties of the drug molecule (i.e. size, charge, solubility etc.)

Figure 2. Effect of the encapsulation of 0.1mM ivermectin on the size of microfluidic produced liposomes (filled circles).



For comparison, the size of empty (water-filled) liposomes is also reported (open circles). Liposomes were produced by #chip1-MHF at TFR=37.50µl/min and FRR=30. Data are reported as the mean of three independent samples, measured in triplicate±S.D. **Reference: Carugo et al (2016)**

The concentration of ethanol and lipid significantly affected the dimensional characteristics of liposomes. For instance, upon increasing the lipid concentration a concomitant size increase of the liposome was observed. The ethanol content had the opposite effect; the progressive increase from 2% to 9% (v/v) caused a decrease in liposome size. In the effect of the concomitant variation of lipids and ethanol content it is reported that the two effects counterbalanced each other. The microfluidic experiments demonstrate that small and uniform liposomes can be produced using relatively high concentrations of lipids (Carugo et al, 2016).

Carugo et al (2016) concluded that microfluidic methods are potentially suitable for the preparation of ivermectin liposomal suspensions to be included in commercial, pharmaceutical formulations. Moreover, in order to minimize the content of toxic constituents they demonstrated that ethanol is a suitable solvent in microfluidic experiments, particularly in view of a potential translation of this technology into the industrial environment.

Conclusion

Information about the influence of foods in the pharmacokinetics of ivermectin is scarce. The knowledge of the influence of alcohol in ivermectin kinetic behavior may not be sufficient. Co-ingestion of alcoholic drinks, however, in a debatable discussion it is not recommended by several researchers because there might be an association with GABA receptors and the effect of alcohol in the central nervous system. In healthy volunteers administered ivermectin orally (150 µg/kg), plasma levels were significantly higher when co-administered with 750 ml of beer than with 750 ml of water; the plasma concentrations were significantly higher in patients who drank beer vs. those who drank water. Ivermectin (150 µg/kg) when administered to individuals with water or orange juice (750 ml). Orange juice decreased drug concentration in blood plasma and Cmax, possibly because fruit juices and constituents are potent inhibitors of certain drug transporters (González Canga et al, 2008)

Extensively motivated by the need to increase the stability and bioavailability of drugs, and to reduce their side effects by targeting to the site of action, research in new drug delivery vehicles has taken giant steps. Liposomes and their derivatives, so called new generation liposomes, present a vast area in this field where several advances have already been achieved, as explained by Çağdaşr, Sezer and Bucak (2014). Further research is required to overcome the limitations faced today in terms of prolonged stability, drug loading and active targeting, specially for ivermectin in the treatment of COVID-19.

Sun et al (2019) suggested that to implement the final application of nanoparticles, it is necessary to ensure that nanoparticles have the enhanced absorption, sustained release, target effects and intracellular delivery at adequate concentrations for an expected period to obtain satisfactory therapeutic results. In addition, nanoparticles should be safe, inexpensive, and easy and reproducible manufacture in large scale. Currently, most of the studies have reported that most nanoparticles are safe and even can reduce the side effects of drugs compared to traditional drug formulations.

The fine but rich challengeable aims lie in creating smart nanocarriers simultaneously possessing several functions to ensure satisfactory absorption, long-circulation time and targeting, and low or nontoxicity (Sun et al, 2019).

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