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Efflux Modulator-Loaded Nanostructured Lipid Carriers Loaded With Combinatorial Anti-HIV Agents: Design And Fabrication

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Abstract : Antiretroviral treatment is not efficient in removing HIV-1 from viral reservoirs because of the drugs' difficulties in being absorbed by the body and the considerable adverse effects they cause. For the purpose of enhancing its efficiency against HIV-1, a nano-structured lipid carrier that contains etravirine (ETR) and darunavir ethanolate (DRVE) and is embellished with D-α-tocopheryl polyethylene glycol succinate (TPGS) was conceptualized, improved, and discussed. The effectiveness of the random method was evaluated by the use of in silico experiments, docking scores, and free binding energies. According to research conducted on ED (ETR and DRVE) in TMZ-b1 cell lines, the best ratio of the two agents, which was discovered to be 1:6.5, gave findings that were contradictory. Therefore, the combination of the two drugs results in an increase in the effectiveness of the medications against the enzymes in question. An enhanced variant of the combo, known as ED-TPGS-NLCs, was developed by the use of CCRD in conjunction with an alternate emulsification method. Subsequently, it was analysed using transmission electron microscopy (TEM), globule size, polydispersity index (PDI), and percentage trapping efficiency. The increased lipid nano system was shown to greatly improve and extend the release of drugs, according to tests that were carried out in vitro using the Korsmeyer-Peppas dynamics model. Additionally, in-vitro testing was conducted on Wistar rats to investigate intestinal depth and absorption, in addition to pharmacokinetics.

Keywords : HIV, TEM, Pharmacokinetics, In-vitro, etravirine.

1. INTRODUCTION

Over the course of the last four decades, acquired immune deficiency syndrome (AIDS) has emerged as a significant health and business concern all over the world. Despite the fact that HIV is something that affects people all over the globe, more than 67 percent of the world's HIV-positive population lives in countries that are located in sub-Saharan Africa. In view of the fact that antiretroviral drugs are now more readily available, the United Nations Program on HIV/AIDS (UNAIDS) has reported that there has been a marginal reduction in the number of newly diagnosed cases. The United Nations Population Fund (UNAIDS) has reported that there has been a decrease in the number of new HIV diagnoses (38.4 million) as well as the number of deaths that have been caused by AIDS. As a consequence of this, it is possible that advancements will be achieved toward Sustainable Development Goal 3.3 in the fight against AIDS. This goal stipulates that the illness should be eliminated from public health systems by the year 2030 (UNAIDS, 2023; WHO, 2022). HIV is largely responsible for targeting and destroying key cells of the immune system, despite the fact that it stores itself in a number of organs and tissues, such as the central nervous system, lymph nodes, blood, spleen, lungs, and CD4+ lymphocytes, dendritic cells, and macrophages. When ARDs in HIV-reservoir zones cannot be accessed, it is very challenging to completely eradicate HIV-1 from the host body. Furthermore, this circumstance raises the probability that the virus may mutate into a strain that is resistant to medicine (Bagasra, 2006).

Darunavir ethanolate (DRVE) is an example of a protease inhibitor (PI)-based first-line antiviral drug (ARD) that has a high level of resistance to both mutant and wild-type HIV strains. DRVE is able to prevent the virus from infecting new cells because it inhibits the maturation process of HIV. According to Elkateb et al. (2020), the key disadvantages of DRVE are its low water solubility, first-pass metabolism, p-gp efflux pump, enhanced CYP450 family degradation, and high lipophilia. It is common practice to combine oral DRVE with a low dosage of Ritonavir (RTV) in order to boost the drug's bioavailability, which may vary anywhere from 37 percent to 85 percent. This is due to the fact that RTV inhibits the export of p-gp (Ronaldson et al., 2008). It is recommended that patients living with HIV take 600 mg orally twice day in conjunction with 100 mg of RTV. The liver changes DRVE into molecules that are 90 percent less effective than DRVE (EMA, 2024). This transformation takes place as a consequence of CYP enzymes, namely

CYP3A4. Because RTV inhibits CYP3A4, which in turn slows down the metabolism of DRVE, the combination of DRVE and RTV is the most important thing happening. The concentration of pure medicine (DRVE) in the blood and plasma is increased as a result of this circumstance. It is estimated that around 80% of the DRVE is removed in the stool when it is delivered with RTV, whereas 42% of it stays unaltered. According to Meyer et al. (2005), the EC50 value of DRVE ranges from 1 to 5 nanomolar, and its final elimination half-life is 15 hours. This information was obtained from specific HIV-1 sites. The research conducted by McKeage et al. (2009) indicates that DRVE has the potential to result in a wide range of unfavorable side effects, such as skin rashes, gastrointestinal problems, paresthesia in the mouth, and difficulty with glucose tolerance. Our research team was able to effectively design a DRVE-loaded formulation that is both safe and effective (Muheem et al., 202^). This was accomplished by lowering the dosage of DRVE and the viral load in the lymphatic system, increasing the drug's bioavailability throughout the body, and limiting the number of side effects that were connected with the dose.

The anti-retroviral medicine known as etravirine (ETR), which is a non-nucleotide transcriptase inhibitor of the second generation, is one of the available options. BCS class IV includes a medicine that is available. Individuals of all ages often make use of it in combination with other antiviral chemicals in the majority of instances. Not only does it have the term Intelligence, but it is also water resistant. According to Kakuda et al. (2011), the binding is more stable (about 99%), and there are more than five times as many plasma proteins that connect to it. For the purpose of determining the EC50 value of ETR, a wide variety of HIV-1 stain types were employed, ranging from 0.9 to 21.7 percent. According to Pereira et al. (2023), the fact that ETR has a half-life of forty hours indicates that it may be able to sustain effective inhibitory levels, which may result in long-term preventative efficacy. There were a lot of studies that were conducted on ETR, and among the safety issues that were brought up were skin reactions and liver damage. Over the course of the last ten years, several alternative construction strategies have been researched in order to overcome the physical challenges that are often connected with ETR. It takes a very long time to dissolve, and it is difficult to dissolve, which causes a delay in the bioavailability of the substance (Johnson et al., 2009). It is doable that two distinct cytochrome P450 enzymes might be responsible for the degradation of ETR. CYP2C19 and CYP3A4 are two of the enzymes that are coming under scrutiny. The undigested form of the main metabolites is more susceptible to being digested by reverse transcriptase. However, 93.7% of the medicine that is taken orally is removed in stool, as shown by mass balance tests (Kakuda et al., 2011). This is in contrast to the fact that only 1.2% of the medication is eliminated in urine. In addition, our research team developed a nanostructured lipid transport that was loaded with ETR in order to protect against metabolism (Muheem et al., 2024).

Both the docking score and the free binding energy of the two drugs indicate that it is possible for them to effectively block the reproduction of HIV either in conjunction with one another or alone. When attempting to evaluate the effectiveness of the combinatorial technique, it is usual practice to carry out tests in a computer simulation. For the purpose of preventing the reproduction of HIV, it is recommended to make use of both ETR and DRVE (ED). It's possible that the synergistic effects of some HIV subtypes might make treatment more effective against certain subtypes. There is a possibility that the antiretroviral load may be reduced with the use of ETR in combination with DRVE. In a number of clinical studies, 800/100 mg of DRVE/RTV was combined with 400 mg of ETR. Although there were no changes seen in the pharmacokinetics of DRVE, the Cmax value of ETR was demonstrated to decrease as a result of RTV activating CYP2C families, which is a well-known ETR metabolizer (Gazzola et al., 2014; Pereira et al., 2023). Because of this, it is possible that solutions based on nanotechnology might be used to get around these problems. In order to put a stop to the breakdown of ETR and DRVE, it is required to design formulations that include ED and that hinder the p-glycoprotein efflux system for DRVE by employing solutol and TPGS via the lymphatic channel. This will result in the drug having a higher bioavailability, the virus will be stored at a lower level, and the patient will have fewer unpleasant effects as a result of the therapeutic dose. It is thus possible that combining nanotechnology-based techniques with p-gp inhibitors might be a viable solution to the problems that have been discussed above.

In order to avoid these challenges, nano-based drug delivery systems are among the most frequent options. This is due to the fact that these systems are able to pass through biological barriers and release the helpful molecules at the exact time that the patient needs them. Micelles, lipidic nanocarriers, polymeric nanoparticles, and nanosuspensions are some of the possible approaches that might be used for the transportation of drugs that have a low solubility (Jindal et al., 2017; Economidou et al., 2018). One possible disadvantage of these nanocarriers is that they can be manufactured in large quantities (Cirri et al., 2012). To add insult to injury, other nanocarriers, such as SLNs, perform poorly when it comes to delivering medications in comparison to NLC formulation. According to Jaiswal et al. (2016), hydrophobic drugs often leak out of these containers from time to time as they are being stored.

Within a non-structured matrix consisting of solids and liquid lipids, the medication is shielded from enzyme breakdown, used for delayed release, biocompatibility, lymphatic targeting, non-toxicity, and decrease of storage loss. According to Shevalkar et al.'s 2019 research, the medication is so powerful that it is able to target HIV viral sites while simultaneously avoiding any adverse effects. According to Tsai et al. (2012), all NLCs have a common denominator, which has to do with the fact that they lower the particle size and the particle density index (PDI). NLCs have been produced by a majority of researchers in order to facilitate the delivery of antiviral drugs to the lymphatic system. These medications include lopinavir, ritonavir, and atazanavir (Alex et al., 2011; Ahmed et al., 2017; Gurumukhi et al., 2022). Oral administration of NLCs allows them to circumvent the breakdown process that occurs in the liver and instead go via the lymphatic system to the site where HIV is stored, making them a popular option. NLC is thus your best option if you wish to provide

ED by oral administration. It has been reported by Ganesan et al. (2017) and Hobson et al. (2018) that non-labile compounds (NLCs) are often covered with a surfactant layer that serves to stabilize the combination at the colloidal level.

An further problem with DRVE is that it is not effective as a p-gp substrate. This is a challenge that it faces. When compared to a conventional DRVE treatment, TPGS in NLCs have the ability to considerably improve oral absorption, which might be a solution to this problem (Tan et al., 2017). It has been shown that TPGS is superior to other p-gp inhibitors, such as tween, solutol, and pluronic, when it comes to improving DRVE absorption across the gastrointestinal barrier. The TPGS has the potential to operate as a binding agent in addition to its ability to inhibit p-gp. Pham et al. (2017), as shown in Figure 1.1, came to the conclusion that TPGS is an efficient p-gp inhibitor. This conclusion was reached by the scientific community.

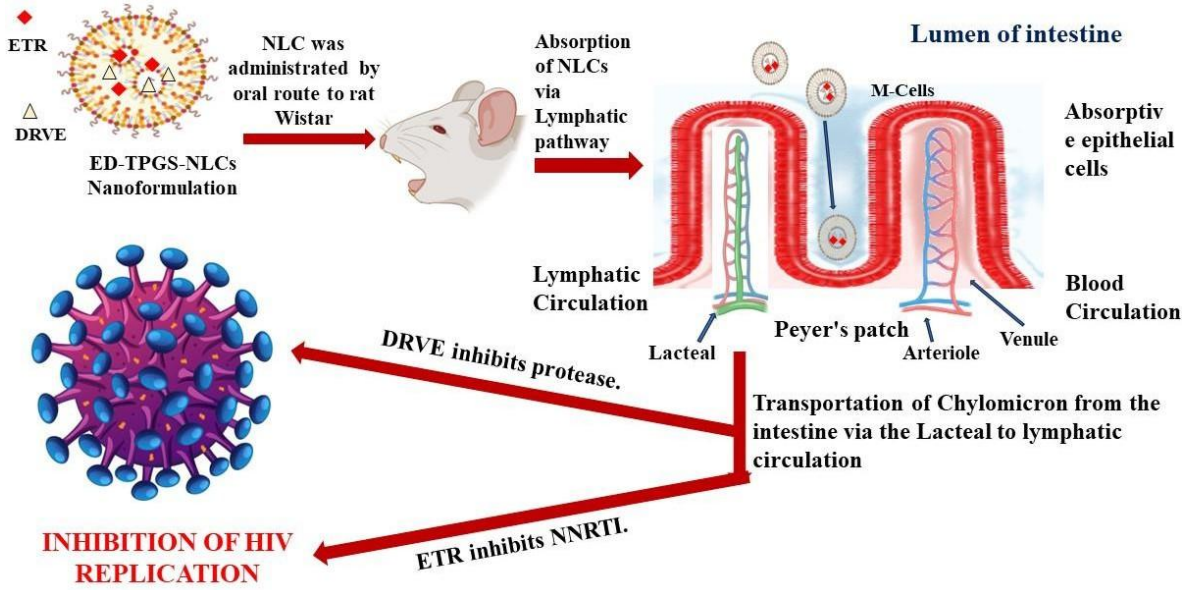

Figure 1.1 Inhibiting HIV replication with oral administration of ED-TPGS-NLCs.

Nanocarriers are difficult to produce due to the fact that modifications to the manufacturing process may have a considerable influence on the specific properties of the nanocarriers. These characteristics include PDI, globule size, zeta potential, and shape. In addition, it is difficult to produce consistent results because of recurrent issues, especially when it comes to the preparation of the NLC combination. The concept of quality by design (QbD) was developed in order to ensure compliance with the demanding standards and criteria that are required for the certification of dual-drug formulations. There is a statistical method known as quality-by-design (QbD) that may be used in order to guarantee that nanoformulations are of a satisfactory standard. According to the principles of quality by design, the only way to ensure the quality of a product is to determine the factors that contribute to the inconsistency of the product. After the hit-and-trail method had been utilized to determine whether key independent pieces were present, the strategy was implemented (Luiz et al., 2021; Garg et al., 2017). The use of a statistical method known as central composite rotatable design (CCRD) is an essential component in the process of accomplishing the desired purpose. According to Bonde et al. (2019), the quality by design (QbD) tool makes it simpler to recognize, comprehend, and exercise control over the independent factors that have an effect on the variable that is being studied to be dependent. The use of QbD methods ensures improved variable control, consistent performance, and the production of high-quality items. It has been stated by Kincl et al. (2005) that the QbD-based approach includes the creation of a "quality goal product profile" that provides an overview of the characteristics of the product.

Both the HIV-replicating protease and the NNRTI enzymes are shown in Figure 1.1. The findings of the study suggest that ETR and DRVE (ED) may work together to limit the activity of these enzymes. The present literature was

extensively analyzed, and the results showed that ED was required in order to improve treatment effectiveness in HIV strains, increase bioavailability, avoid drug degradation during lymphatic absorption, and stop the p-gp efflux mechanism by the use of nanostructured lipid carriers (NLCs). Utilizing ETR in combination with DRVE is a revolutionary approach that has been created in order to achieve the highest possible level of effectiveness in HIV therapy. In order to validate the effectiveness of the combinatorial method, research was carried out on experimental cell lines as well as on computer systems. Experiments including zeta potential, chemical interaction, surface shape, drug release, diffusion, anti-HIV, and pharmacokinetics were carried out by the researchers in order to ascertain the composition and function of the ED-TPGS-NLCs that were predicted. According to the findings of these research, the drugs enhanced the oral bioavailability of the ED medication by inhibiting the p-gp export mechanism. Additionally, the medications decreased the unpleasant effects that were linked with higher doses. To this day, nanoformulations that combine ETR and DRVE have not shown any effectiveness in the prevention of HIV, the control of p-gp release, or the preservation of ED metabolites

2. Literature Review

Nachman and associates (2015) reported the 48-week When it comes to the treatment of HIV type 1 in children, how successful and safe is the oral suspension of Raltegravir? 3. Dosing could be calculated in an acceptable manner since the goal PK values (AUC0-12hr and C12hr) were fulfilled for all of the groups. Up to the 48th week, ten of the participants had experienced adverse events of Grade 3 or above. There were two medications that were taken into consideration for the study. However, there were no deaths that were related with the drugs, despite the fact that one person had immunological reconstitution syndrome and another participant withdrew owing to a skin rash. At the end of the 48th week, 87.5% of the patients in Cohorts IV and V were clear of the virus, and 45.5% of those people had HIV RNA levels that were lower than 20 copies per milliliter. At the 48th week, there was a rise of 7.3% in CD4 cells and 5.27.6 cells per millimeter squared. According to Nachman et al. (2015), oral administration of RAL tablets at a dose of 6 mg/kg twice day resulted in a positive response from both the organism's immune system and the virus.

Sandkovsky and associates (2012) The best dosage of raltegravir and etravirine for a patient with a serious HIV infection that is resistant to many drugs was reviewed for a patient who was carrying a gastrostomy tube infection. The results of the experiments showed that the presence of a gastrostomy tube did not have any impact on the absorption of raltegravir, etravirine, emtricitabine, or tenofovir when administered. In the event that oral administration of the medicine is not an option, more study should be carried out on the pharmacokinetics, safety, tolerance, and antiviral response of raltegravir, etravirine, and emtricitabine-tenofovir (Sandkovsky et al., 2012).

Leonard and associates (2022) Oral treatment for HIV that is administered over a prolonged period of time using a combination of cabotegravir and rilpivirine is innovative. One dose is administered once per month. Patients whose viruses have been suppressed may now use a monthly injectable medicine that combines cabotegravir and rilpivirine with antiretroviral characteristics. This treatment was recently approved for usage. The findings of Leonard et al. (2022) suggest that this is beneficial for those who are interested in a regimen that has a longer duration of action or who struggle to adhere to a daily oral regimen.

Works Cite As ETR and DRVE Combination Therapies

Larson and associates (2016) We recruited HIV-positive persons between the ages of 9 and 24 who were receiving optimum background treatment (for example, 800/100 mg QD of Darunavir/Ritonavir alone, or 200 mg BID or 400 mg QD of Etravirine) to take part in the study. Our evaluation of the effectiveness of each method was carried out with the use of protocol-defined objective drug dose ranges that were obtained from adult data. Noncompartmental analysis was performed on a whole blood sample collected over the course of 24 hours in order to ascertain the PK parameters. In order to verify the program's safety and effectiveness for older children, adolescents, and young adults, further testing of the program that is administered once daily is required, as shown by the data. Larson et al. (2016) state that another alternative is to take 400 mg of ETR once day or 200 mg twice daily with DRV/r. This is an alternative that may be considered.

Belkhir and associates (2016) There is a genetic variation in CYP3A5 that has been demonstrated to be related with lower DRV plasma trough concentrations in CYP3A5 expression. This genetic variant has been proven to have an effect on the interaction between Darunavir (DRV) and Etravirine (ETR). These findings suggest that the activation of CYP3A5 by ETR is contingent upon the expression of CYP3A5. It is possible that infra-therapeutic plasma, also known as DRV, will have a more significant effect on these particular people. Based on this discovery, a genotype-based pharmacological interaction has been discovered. This interaction, when taken with other drugs that are metabolized by CYP3A5, has the potential to provide catastrophic results. Because of this, it can be of utmost significance. Before recommending CYP3A5 pre-emptive genotyping for the simultaneous administration of DRV and ETR, it is necessary to conduct additional research in order to validate this association and investigate its therapeutic consequences. This is especially true in the African population, which has a higher frequency of CYP3A5 genes (Belkhir et al., 2016).

Ebers and associates (2017) data from a retrospective cohort study that included adult patients who were treated at an HIV facility for this group between the years 2008 and 2013 were included in the analysis. Patients who had previously had substantial treatment were amenable to the combination of raltegravir, darunavir/ritonavir, and etravirine. These patients were from a facility in the United States that was located outside of metropolitan areas. Those individuals who remained steadfast were successful in halting the spread of the virus. Given the recent revisions to the first-line pharmacological criteria for HIV medication, it is possible that future prospective studies may give a more accurate depiction of the treatment's intended use (Ebers et al., 2017).

Research on the efficacy of nanoformulations in the treatment of HIV/AIDS

Nasiri and associates (2019) The different ways that may be used to penetrate the blood-brain barrier were studied by scientists. The improved NLCs that were used as IDV carriers were the outcome of a two-stage procedure that started with a screening that was done "one factor at a time" and culminated with a full multiobjective optimization. The most noteworthy aspect of the in vivo experiment is that the concentration of NLCs and TR-NLCs in the brain was much greater than it was in the free medicine given to the participants. According to Nasiri et al.'s 2019 research, the brain clearance rates of NLC and TR-NLC were 6.5 and 32.75 times higher, respectively, as compared to free medications correspondingly.

Rojekar and associated (2021) These nanostructured lipid carriers containing etravirine were improved by the addition of nano-selenium. The creation of the dual-loaded nanocarrier system was accomplished by the use of the double emulsion fluid evaporation method. In the next step, the design of testing technique was used in order to evaluate

and improve it. The delivery of the nano-selenium-containing dual-loaded nanocarrier system resulted in a considerable increase in the levels of glutathione, superoxide dismutase, and catalase in the animals. At this point, it became clear that the lipidic nanoparticle that included nano-selenium had the potential to eliminate harm. An increase in the accumulation of the dual-loaded nanocarrier in distant HIV storage organs including the lymph node, ovary, and brain occurred at the same time as an improvement in the pharmacokinetic characteristics of the material in vivo. According to the results, a dual-loaded product has the potential to improve the antioxidant balance of cells while simultaneously targeting the HIV1 infection, which might potentially lead to more effective long-term HIV treatment (Rojekar et al., 2021).

Efavirenz (Efa) and enfuvirtide (Enf) co-loaded polymer-lipid hybrid nanoparticles (PLN) were created by Surve and colleagues (2020) with the intention of enhancing the efficiency of medicine transport into cells. It was created to particularly target macrophage cells and T-cells, which are two hiding places for the human immunodeficiency virus. These PLN were built to target these cells. In this study, a near-infrared dye was administered by an intravenous injection of PLN, which is an alternative to Efa-Enf PLN treatment. Based on the results of in vivo biodistribution experiments, it was determined that the dye was no longer distributed evenly after two hours. The presence of this condition was shown in a variety of organs, including the brain, spleen, liver, lymph nodes, thymus, lungs, FRT, heart, and kidneys. The depot created a slow-release medicine that lasted until day 5 in the sites where the infection spread (lymph nodes and FRT), stored (liver and spleen), and was the most difficult to reach (brain). This medication was administered at the injection site. This was a result of the subcutaneous treatment that was delivered after three days, which resulted in an uneven distribution of the drug throughout the body. In addition to this, it offers a significant instance of the potential of virtual replacement PLN for the prediction of medication levels in particular tissues. In the year 2020, Surve and his employees

Latronico and associates (2021) Researchers studied the feasibility of synthetic biodegradable polymeric nanoparticles (NPs) loaded with DRV and bright, nontoxic carbon dots (C-Dots) passing an artificial blood-brain barrier orally in order to reduce MMP-9 activity in vitro. This was done in order to achieve the desired aim of inhibiting MMP-9 activity. MMP-9 is one of the components that has been thought to be related with the beginning of neurological illnesses that are connected with HIV. Nanoformulations that were based on biodegradable poly(lactic-co-glycolic) acid (PLGA) displayed a number of desired qualities. These properties included a tiny average hydrodynamic size (less than 150 nm), good colloidal stability in water, high drug encapsulation efficiency, and visible light emission. When researchers combined DRV with PLGA nanoparticles, they discovered that the combination had a more significant influence on lowering the levels of matrix metalloproteinase-9 (MMP-9) expression. Furthermore, they discovered that this effect was even more evident than when DRV was employed on its own. DRV nanovectors of this kind may be effective in the treatment of HANDs, according to these studies, which give encouraging evidence (Latronico et al., 2021).

Research on p-gp efflux inhibitors and the HIV/AIDS epidemic

Shaik and associates (2007) In order to ascertain whether or not P-gp plays a substantial role in the regulation of the concentration of abacavir that is delivered to the central nervous system, researchers investigated the connection that exists between the two proteins. Both in vivo and in vitro testing methods were used in order to investigate this connection. According to the findings of a study that investigated the distribution of mdr1a(-/-) CF-1 in the brains of living mice, the area under the curve (AUC) obtained was twenty times higher than that obtained in wild type mice. The area under the curve (AUC) in the plasma of the mutant animals was almost twice as high as it should have been. We are able to conclude that the CNS medication targeting measure was effective if the area under the curve (AUC) between the brain and the plasma is more than 10. As a result of these discoveries, the first proof that P-gp can be found in both laboratory and biological settings as a substrate for nucleoside reverse transcriptase inhibitors has been uncovered. The central nervous system may not get a large dose of abacavir. According to the findings of the research carried out by Shaik and colleagues in 2007, the brains of P-gp-deficient mutant mice contain much higher levels of abacavir when compared to the brains of wild-type mice.

Schmitt and associates (2010) Researchers investigated how different dosages of ritonavir-boosted saquinavir affected the pharmacokinetics of digoxin when it was taken orally. Administer a therapeutic dose consisting of 1,000 milligrams of saquinavir and 100 milligrams of ritonavir twice day. A significant portion of this model P-gp substrate is removed from the body via the process of urine in the form of a drug that has not been altered. Digoxin exposure was enhanced when saquinavir/ritonavir was administered at a dosage of 1,000/100 mg twice day. This may have been because the medication suppressed P-gp. Because digoxin has a very small window of action, it is essential to take care while mixing these three drugs. There is a recommendation made by Schmitt et al. (2010) that persons who are taking digoxin should decrease their dose and carefully check the levels of digoxin in their blood.

Huisman and associates (2003) Research has shown that ritonavir is ineffectual as a P-glycoprotein inhibitor in living beings. This is owing to the fact that saquinavir is poorly absorbed by the body, and as a result, it is unable to reach the brain or the developing newborn. In these regions, increasing the dosage of medicine with higher P-gp inhibitors has the potential to be beneficial to the treatment process; nevertheless, doing so also involves the risk of problems. In the course of our research, we gave the mice ritonavir and saquinavir by oral administration, either with or without the potent P-glycoprotein inhibitor GF120918, depending on the circumstances. The accumulation of saquinavir in the brain occurred over the course of many days after the administration of several doses of GF-120918 (twice daily). Animals that were treated with vehicles had levels that were 10 times higher than non-treated animals. However, the

neurotoxicity of ritonavir did not become apparent until after the fourth and last dose was administered. Although clinical studies that attempt to limit the activity of P-glycoprotein might potentially increase HPI clearance, it is important to note that these trials would need to avoid the use of ritonavir and be closely monitored for any unanticipated adverse effects. (Huisman and colleagues, 2003).

3. OBJECTIVE AND RATIONALE

Objective Of The Study

It is possible for HIV to be present in a variety of organs and tissues throughout the human body. There are three basic categories of these cells: cellular (which includes macrophages, follicular dendritic cells, and CD4+ T cells), geographic (which includes the liver, kidneys, lymph nodes, GALT, and spleen), and distant (which includes the brain and bone marrow) (Blankson et al., 2002; Haase., 1999). According to Pierson et al. (2000) and Mbonye et al. (2007), viruses have the potential to dwell in the testicles as well as the urinary system of females. On the other hand, there are now antiviral drugs (ARTs) that are effective, and Highly Active Retroviral Therapy (HAART) is a treatment option for HIV infection. ARTs are not especially successful in removing HIV from viral pools. This is due to the fact that they are not well absorbed and thus have the potential to cause harm. They have a difficult time breaching the Blood-Brain Barrier (BBB), which is a barrier that hinders the diffusion of drugs into the Central Nervous System (CNS). This is because antiretroviral therapies (ARTs) do not possess the required physical features. In addition, the BBB is a barrier that prevents drugs from passing through it because it contains efflux transporters on its surface. Having stated that, research has shown that following the typical mode of administration (HAART treatment), antiretroviral therapies (ARTs) at sub-therapeutic concentration levels inside macrophages have a lowered efficiency (Puligujja et al., 2013). Given the circumstances, it could be a good idea to increase the dosage. Nevertheless, when these standard methods are used, raising the dose does not seem to result in a perceptible rise in concentration level. Having knowledge that the administration of antiretroviral therapy (ART) with the assistance of nanotechnology leads to increased medicine concentrations at the target area is a source of encouragement (Fotooh Abadi et al., 2020). Those nanoparticles that are smaller than 200 nm may be responsible for increasing the concentration of nanocarriers in distant organs such as the lymphatic system, genital organs, and the brain. Nanoparticles that are larger than 200 nm may be better able to target the kidney, liver, spleen, and lung. In order to totally eradicate HIV, antiretroviral treatment (ART) must be provided to all areas where the virus is stored concurrently. This is necessary in order to successfully eliminate HIV. Nano-drug delivery of very hydrophobic drugs (DRVE and ETR) is something that we provide in order to get around the constraints of the treatments that are now available (Varatharajan et al., 2009).

By targeting lymphatics, the use of dual drug-loaded non-label chemotherapy (NLC) that contains two antiretroviral medications, namely ETR and DRVE (ED), has the potential to enhance the outcomes of treatment for treating HIV infection. To summarize, the following are the key goals that the study aimed to accomplish:

- ✓ Provide an appropriate preparation technique and optimize it to prepare a stable, dual drug-loaded NLC of DRVE and ETR via oral delivery for HIV infection.
- ✓ Characterization of the optimized combinatorial lipid nanosystem of ED to improve the release profile and intestinal permeation.
- ✓ Eliminate the problems of p-gp inhibitor after oral administration.
- ✓ Evaluation of cell line studies of developed combinatorial lipid-nanosystem in HIV.
- ✓ Enhance the availability of ETR and DRVE in the plasma through the oral route.
- ✓ Use the lymphatic system to administer drugs orally, avoiding the first-pass metabolism.
- ✓ Establishment of the stability of optimized combinatorial lipid-nanosystem in terms of shelf-life.

Rationale Of The Study

- **Justification for choosing HIV**

Over the course of the last four decades, acquired immune deficiency syndrome (AIDS) has become a serious problem for both the health and the economy of worldwide communities. The virus may be present all over the world, but it is certainly having a disproportionate effect on countries located in sub-Saharan Africa. As a result of the availability of antiretroviral drugs (ARDs), the United Nations Program on HIV/AIDS (UNAIDS) reports that there has been a marginal reduction in the number of new infections. According to the World Health Organization (2023) and the United NationsAIDS Global AIDS Update (2023), the eradication of HIV/AIDS as a public health concern by the year 2030 would be achieved with the help of 38.4 million new cases of HIV/AIDS throughout the world. This will contribute to the attainment of Sustainable Development Goal 3.3. Human immunodeficiency virus (HIV) is a virus that may damage a broad range of organs and tissues in the body. Some of these organs and tissues include the lymph nodes, blood, spleen, lungs, and genital tract. In addition to CD4+ lymphocytes and dendritic cells, macrophages are also among the cells that have the potential to build reservoirs of HIV. On account of the fact that ARDs are unable to penetrate HIV-reservoir zones, it is very difficult to completely eradicate the virus from the body of the host population. According to Bagasra (2006) and Muheem et al. (2021), this results in an increase in the probability that the virus would acquire resistance to medical therapy. Due to the fact that its prevalence presents a significant threat to public health in countries such as India, where fair skin is common, academics have chosen to concentrate their attention on it.

- **Etravirine (ETR) is chosen as an antiretroviral drug.**

Etanercept (ETR), which belongs to the BCS class IV category of medications, is an inhibitor of non-nucleotide transcriptases of the second generation. The administration of this medicine, along with other antiviral treatments, is often carried out on both children and adults. Not only does it have the term Intelligence, but it is also water resistant. According to Kakuda et al. (2011), the binding is more stable (about 99%), and there are more than five times as many plasma proteins that connect to it. For the purpose of determining the EC50 value of ETR, a wide variety of HIV-1 stain types were employed, ranging from 0.9 to 21.7 percent. According to Pereira et al. (2023), the fact that ETR has a half-life of forty hours indicates that it may be able to sustain effective inhibitory levels, which may result in long-term preventative efficacy. There were a lot of studies that were conducted on ETR, and among the safety issues that were brought up were skin reactions and liver damage. Over the course of the last ten years, several alternative construction strategies have been researched in order to overcome the physical challenges that are often connected with ETR. It takes a very long time to dissolve, and it is difficult to dissolve, which causes a delay in the bioavailability of the substance (Johnson et al., 2009). It is doable that two distinct cytochrome P450 enzymes might be responsible for the degradation of ETR. CYP2C19 and CYP3A4 are two of the enzymes that are coming under scrutiny. The undigested form of the main metabolites is more susceptible to being digested by reverse transcriptase. However, 93.7% of the medicine that is taken orally is removed in stool, as shown by mass balance tests (Kakuda et al., 2011). This is in contrast to the fact that only 1.2% of the medication is eliminated in urine. In addition, our research team came up with a nanostructured lipid transport system that included ETR in order to stop the metabolic process (Muheem et al., 2024).

- **Darunavir Ethanolate (DRVE) is the antiretroviral medication of choice.**

Darunavir ethanolate (DRVE) is an example of a protease inhibitor (PI)-based first-line antiviral drug (ARD) that has a high level of resistance to both mutant and wild-type HIV strains. DRVE is able to block the development of HIV, which means that new cells are unable to acquire it. According to Elkateb et al. (2020), the key disadvantages of DRVE are its low water solubility, first-pass metabolism, p-gp efflux pump, enhanced CYP450 family degradation, and high lipophilia. It is common practice to combine oral DRVE with a low dosage of Ritonavir (RTV) in order to boost the drug's bioavailability, which may vary anywhere from 37 percent to 85 percent. This is due to the fact that RTV inhibits the export of p-gp (Ronaldson et al., 2008). It is recommended that patients living with HIV take 600 mg orally twice day in conjunction with 100 mg of RTV. The liver changes DRVE into molecules that are 90 percent less effective than DRVE (EMA, 2024). This transformation takes place as a consequence of CYP enzymes, namely CYP3A4. Because RTV inhibits CYP3A4, which in turn slows down the metabolism of DRVE, the combination of DRVE and RTV is the most important thing happening. The concentration of pure medicine (DRVE) in the blood and plasma is increased as a result of this circumstance. It is estimated that around 80% of the DRVE is removed in the stool when it is delivered with RTV, whereas 42% of it stays unaltered. According to Meyer et al. (2005), the EC50 value of DRVE ranges from 1 to 5 nanomolar, and its final elimination half-life is 15 hours. This information was obtained from specific HIV-1 sites. The research conducted by McKeage et al. (2009) indicates that DRVE has the potential to result in a wide range of unfavorable side effects, such as skin rashes, gastrointestinal problems, paresthesia in the mouth, and difficulty with glucose tolerance. Our research team focused on three primary areas in order to develop a DRVE-TPGS-loaded solution that is not only safe but also effective (Muheem et al., 2024). These areas were improved systemic absorption, reduced dose-related side effects, and viral reservoirs. The viral load in the lymphatic system as well as the dose of DRVE were both increased as a consequence of this condition.

- **Choosing an ETR/DRVE (ED) combo to combat HIV infection**

Both the docking score and the free binding energy of the two drugs indicate that it is possible for them to effectively block the reproduction of HIV either in conjunction with one another or alone. When attempting to evaluate the effectiveness of the combinatorial technique, it is usual practice to carry out tests in a computer simulation. For the purpose of preventing the reproduction of HIV, it is recommended to make use of both ETR and DRVE (ED). It's possible that the synergistic effects of some HIV subtypes might make treatment more effective against certain subtypes. There is a possibility that the antiretroviral load may be reduced with the use of ETR in combination with DRVE. The combination of 800/100 mg of DRVE/Ritonavir (RTV) and 400 mg of ETR was tested in a number of clinical studies. The Cmax value of ETR was found to decrease owing to RTV activating CYP2C families, which is a well-known ETR metabolizer (Gazzola et al., 2014; Pereira et al., 2023). This was noticed despite the fact that there were no alterations reported in the pharmacokinetics of DRVE. In order to get over these problems, it is likely that solutions based on nanotechnology might be used. For the purpose of putting a stop to the deterioration of DRVE and ETR, nanoformulations that are loaded with ED are required. By using solutol and TPGS, it is possible to achieve this goal by inhibiting the p-glycoprotein export of DRVE. As a result, the bioavailability of the medicine will be increased, the viral stockpile will be reduced, and the possibility of dose-related side effects will be decreased altogether.

- **Argument in Favor of Oral Medications**

Oral administration is the most patient-friendly mode of medicine administration since it may eliminate some of the problems that are connected with other methods of medication delivery. As an example, the intravenous procedure is one that has the potential to cause the patient to experience pain, infection, and an increase in the costs associated with manufacturing and handling (Batista et al., 2018). When it comes to patients, the oral course is the best option since it is not only inconspicuous but also does not cause any pain, is inexpensive, can be reused, and is appropriate for patients of any age. The prolonged transit through the gastrointestinal tract also allows for the possibility of adjusting the release of the drug correctly, which might be of use in assessing the effectiveness of the

treatment. It is also possible to modify medications such that they only release in certain organs, such as the stomach, the colon, or particular regions of the small intestine (Sosnik et al., 2013). This may be done in order to change the distribution of the drug or to make it less mobile. Streamlining the manufacture of the drug might potentially boost the therapeutic advantages while simultaneously lowering the undesirable effects (Ali et al., 2015). This would be accomplished by making the medicine more bioavailable and making it easier to distribute. In 2015, Ali and a few others;

- **Principles Underlying Nanostructured Lipid Carrier**

After combining solid and liquid lipids, nanostructured lipid containers are produced as a consequence of the mixture. The most important impact that it has when it is taken orally is to increase the bioavailability of medicines that are either hydrophobic or lipophilic. The majority of hydrophobic drugs have poor absorption rates when they are taken orally because of irregularities in the digestive system. This is the case when the prescriptions are for oral consumption. These medications are not available for oral administration for a variety of reasons, which is why they are not easily accessible. These explanations include a significant amount of first-pass metabolism, the release of the drug via metabolizing enzymes, as well as chemical and enzymatic degradation (Khan et al., 2015). It is necessary to make use of NLCs in order to effectively traverse all of this because of the advantages they possess over other lipidic nanoformulations. In addition to having superior loading capacities, release profiles, and storage characteristics in their combinatorial grid structure, NLCs also feature an optimal ratio of liquid to solid lipids. This is because NLCs are composed of a combination of liquid and solid lipids.

There is a potential that the medication will dissolve more easily in NLCs. Additionally, the lipids that are present in these containers may inhibit P-gp efflux transporters, which would prevent the drug from exiting the cell. An further characteristic of NLCs is the presence of biphasic drug release rhythms, which means that the release rate does not vary once the first burst release occurs. Once the first release has been made, this is the situation. The only thing that is necessary for NLCs to function well is a very little quantity of surfactant. This is because stability, trapping efficiency, and release patterns are all factors that are taken into account. The publication that Karamat and his colleagues made in 2015.

On the other hand, intestine pancreatic enzymes are responsible for the hydrolysis of NLC lipids. During the process of formation, the molecules of bile salt may be found around the monoglycerides and fatty acids that are being produced. The process in question results in the formation of micelles, which then continue to move towards the periphery of the enterocyte brush. After leaving the micelles, enterocytes have the capacity to attach themselves to cells in the intestines in order to produce lipids. This occurs after the organisms have left the micelles. Chylomicrons are evacuated from the cell by a process known as exocytosis to be more specific. The formation of chylomicrons occurs as a result of the combination of cholesterol and phospholipids derived from the lipid components. Although the drug molecules were not initially broken down, chylomicrons entered the body via the lacteals because they were too large to pass through the blood arteries. This was the case despite the fact that the drug molecules hadn't been broken down. After it has been taken in by the lymphatic system, the capillary connections in the brain that are tight become more relaxed, which allows endocytosis to take place. For the purpose of avoiding the first metabolic phase, researchers (Poonia et al., 2016) have investigated the mechanism of lymphatic transfer that is involved in the NLC system. It is possible that these issues might be avoided via the use of a medication in NLC. Niaz et al. (2016) indicate that it has an effect that is long-lasting and that it reduces the quantity of administration as well as the frequency of administration. Additionally, they state that it prevents the need for administration.

4. METHODOLGY AND HYPOTHESIS

Methodology:

The methodology can be divided into the following key stages:

1. Design of Nanostructured Lipid Carriers (NLCs)
   - Selection of Lipid Components:

Choose appropriate solid and liquid lipids for the formulation of NLCs. For example, solid lipids such as stearic acid, cetyl palmitate, or glyceryl monostearate, and liquid lipids like oleic acid or isopropyl myristate.

The ratio of solid to liquid lipids will be optimized to produce stable and small-sized NLCs (typically between 50–200 nm in diameter).

   - Incorporation of Efflux Modulator:

The efflux modulator (such as verapamil, cyclosporine A, or PSC-833) will be incorporated into the NLCs to inhibit the activity of efflux pumps, thereby increasing intracellular drug accumulation and overcoming multidrug resistance.

The concentration of the efflux modulator will be optimized to avoid toxicity while maintaining effective efflux inhibition.

   - Combinatorial Anti-HIV Agents:

Selection of anti-HIV agents, such as nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), or integrase inhibitors (IIs). Combinatorial therapy (e.g., a combination of tenofovir and lamivudine, or a protease inhibitor and an integrase inhibitor) will be chosen based on their synergistic effect against HIV.

The anti-HIV agents will be encapsulated within the NLCs, and the encapsulation efficiency will be evaluated.

2. Preparation of NLCs
   - Hot Homogenization Method:

A hot homogenization method will be used for the preparation of NLCs. A lipid phase (containing the solid and liquid lipids, the efflux modulator, and the anti-HIV agents) will be prepared and then emulsified in an aqueous phase (containing surfactants like polysorbate 80 or poloxamer 188) under controlled heat and stirring conditions.

The mixture will be sonicated to reduce the particle size and form a stable nanostructured lipid carrier.

   - Characterization of NLCs:

Particle Size and Zeta Potential: The particle size distribution and surface charge (zeta potential) will be measured using dynamic light scattering (DLS) to evaluate the stability and colloidal properties of the NLCs.

Encapsulation Efficiency: The encapsulation efficiency of both the efflux modulator and anti-HIV agents will be determined by centrifugation, followed by analysis using high-performance liquid chromatography (HPLC).

Transmission Electron Microscopy (TEM): To visualize the morphology and structure of the NLCs, TEM will be employed.

3. In Vitro Release Studies
   - The release profile of the anti-HIV agents and efflux modulator from the NLCs will be assessed using dialysis bag diffusion methods.
   - The release kinetics will be studied in phosphate-buffered saline (PBS) at physiological pH and temperature, and samples will be collected at predetermined time points to evaluate the concentration of drugs released.
   - Release data will be analyzed using different models (e.g., zero-order, first-order, Higuchi, Korsmeyer-Peppas) to understand the drug release mechanism.
4. In Vitro Evaluation of Efflux Modulation
   - Cell Line Selection: Use multidrug-resistant human T lymphocyte (e.g., CEM-SS) or other appropriate HIV-infected cell lines expressing efflux pumps.
   - MTT Assay: Evaluate cell viability post-treatment with NLC formulations loaded with anti-HIV agents and efflux modulators.
   - Intracellular Drug Accumulation: Use fluorescence or HPLC to measure the intracellular concentration of anti-HIV agents in the presence and absence of efflux modulators.
   - Inhibition of Efflux Pump Activity: Measure the efflux pump activity using Rhodamine 123 or other fluorescent substrates, and quantify how the efflux modulator enhances the intracellular retention of anti-HIV agents.
5. In Vitro Antiviral Activity
   - Cell Culture: HIV-infected human T cells (e.g., Jurkat cells) or primary human CD4+ T cells will be cultured in the presence of the NLC formulations.
   - Antiviral Assay (e.g., TCID50, p24 ELISA): HIV replication will be measured by determining the p24 antigen concentration in culture supernatants. The effectiveness of the NLC formulations will be compared with free drugs.
   - Synergistic Effect Evaluation: The potential synergistic effect of the combination of anti-HIV agents will be evaluated using the combination index (CI) method, derived from the Chou-Talalay analysis.
6. In Vivo Evaluation
   - Animal Model Selection: An appropriate animal model (e.g., HIV-infected SCID mice or humanized mice) will be used for in vivo studies.
   - Pharmacokinetics: Evaluate the pharmacokinetics of the NLCs (drug plasma concentration over time) to assess the biodistribution and sustained release characteristics.
   - Antiviral Efficacy: Viral load will be measured before and after treatment to assess the antiviral efficacy of the formulations.
   - Toxicity Evaluation: Histopathological examination and clinical monitoring will be performed to evaluate the safety profile of the NLC formulations.
7. Data Analysis
   - All data will be analyzed using appropriate statistical methods (e.g., ANOVA, t-tests) to assess the significance of differences between groups.
   - The release data and in vitro antiviral activity data will be modeled to determine the optimal formulation.

Hypothesis:

The hypothesis for this study can be framed as follows:

"Efflux modulator-loaded nanostructured lipid carriers (NLCs) containing combinatorial anti-HIV agents will enhance the bioavailability and therapeutic efficacy of the anti-HIV agents by overcoming multidrug resistance (MDR) mechanisms associated with efflux pumps. This will result in improved intracellular drug accumulation and sustained antiviral activity, leading to enhanced HIV suppression and reduced viral load."

CONCLUSION

The ED-TPGS-NLCs were enhanced by the use of a modified emulsion-sonication process. Through the regulation and stabilization of p-gp, TPGS makes this process easier to accomplish. The CCRD-based reaction surface technique was used in order to achieve the desired improvement in composition. The ED-TPGS-NLCs demonstrated about four times the amount of ETR and three times the amount of DRVE penetration into the gut membrane when compared to the ED-S. Confocal microscopy demonstrated that ED-TPGS-NLC mixtures were superior to pure medicine solution in terms of their capacity to enhance intestinal permeability. The ED-TPGS-NLC formulations were slightly more successful in entering the intestines than the ED-loaded NLC formulations. This was due to the fact that TPGS inhibits p-gp, stabilizes the formulation, and provides an increase in permeability. In comparison to ED-S, the bioavailability of ETR and DRVE was shown to be enhanced by ED-TPGS-NLCs, according to the findings of a pharmacokinetic exploration. The results of the confocal depth and gut-intestinal penetration examinations were consistent with the pharmacokinetic parameters that were being studied. In light of the findings of this experiment, there is reason to be optimistic about the possibility that NLCs loaded with ED-TPGS might assist HIV patients in lowering their viral load and improving their oral absorption. Due to the enhanced bioavailability of the modified version, it may be able to lower the amount of ETR and DRVE, which may result in a reduction in the adverse effects that are associated with high doses. Taking into consideration the data, it would seem that the NLC kind of ED might be used in the development of more advanced HIV drugs. For the purpose of confirming these excellent findings and assisting in the introduction of this

combination into the medical sphere, we need more research and clinical studies. No research group has been able to generate an orally soluble NLC product that is loaded with ED-TPGS for the purpose of treating HIV.

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