Review Article

Lassa fever Epidemiology, Ribavirin pharmacology and Mechanisms against Arenaviruses and for Novel Application in the future

Abstract

Lassa fever is an acute viral hemorrhagic fever illness caused by Lassa virus which is zoonotics, or animal-borne. It is a member of the arena virus family, is a single-stranded RNA virus. The antiviral abilities of ribavirin have been explained by five different mechanisms. These include both direct (interference with RNA capping, polymerase inhibition, lethal mutagenesis) and indirect mechanisms (Inhibition of inosine monophosphate dehydrogenase and immunomodulatory effects). Ribavirin was the first synthetic nucleoside to display broad-spectrum antiviral activity and is one of the few antiviral medications currently being used in clinical settings that is effective against Lassa fever and other hemorrhagic diseases such as Ebola. Ribavirin has a long half-life resulting in more than 4 weeks to reach steady state ribavirin concentrations when administered orally in healthy individuals. This review aims at providing an overview of Lassa fever epidemiology, pharmacology and multiple modes of action of ribavirin as well as pointing to possible novel future uses.

Keywords: **Arenaviruses, Epidemiology, Lassa fever, Mechanisms, Pharmacology, Ribavirin**

Introduction

Lassa fever was originated in West Africa Nigeria (Lassa village Borno State Nigeria) and first described in the 1950s, although the virus was not isolated until 1969. The illness was discovered when three missionary nurses (Laura Wine a 65 year old female American missionary nurse, Charlotte Shaw and Dr. Jeanette Troupe) died in 1969 (1,2). The virus was isolated at Yale arbovirus research laboratory and was named Lassa Virus. The virus is named after the town Lassa Borno State of Nigeria where the first index cases occurred. The first case from Mali was discovered in a traveler who lived in southern Mali in 2009; the first cases from Ghana were discovered in late 2011. Burkina Faso and Côte d'Ivoire have reported isolated cases, and Togo and Benin have serological evidence of Lassa virus infection. An estimated 100,000 to 300,000 people in west Africa contract the Lassa virus each year; there are also about 5,000 fatalities (3). The potential for the spread of this extremely dangerous and contagious infection is illustrated by the entry of passengers on commercial aircraft into Germany, the Netherlands, the United

Kingdom, and the United States (4, 5, 6, 7).Lassa fever is known to affect 10%–16% of hospital admissions in some areas of Sierra Leone and Liberia each year, which highlights the disease's significant effects on the local populace.Ribavirin was the first synthetic nucleoside to display broad-spectrum antiviral activity and is one of the few antiviral medications currently being used in clinical settings that is effective against Lassa fever and other hemorrhagic diseases such as Ebola virus, Crimean-Congo, chronic hepatitis E virus infection, and other life-threatening viral infections (8, 9, 10). The mechanism of action of ribavirin has been controversial. Depending on the particular virus being examined, a variety of unique mechanisms have been proposed. For ribavirin, five main modes of action have been proposed. Indirect mechanism are Inosine monophosphate dehydrogenase (IMPDH) inhibition, which reduces cellular guanosine triphosphate (GTP) pools, and immunomodulatory effects that sustain a T-helper type 1 (antiviral) immune response. Direct mechanisms include inhibition of RNA capping activity, directly inhibiting viral polymerases, and increase mutation frequency through the error catastrophe caused by the integration of ribavirin into newly synthesized genomes(11). This article aim to review the lassa fever epidemiology, Ribavirin pharmacology and mechanisms of actions against Arenaviruses and for novel application in the future

Epidemiology of Lassa fever

Lassa fever (LF) belongs to a group of diseases called the viral hemorrhagic fevers (VHFs). Lassa fever is an acute viral hemorrhagic fever (HF) illness caused by Lassa virus which is zoonotics, or animal-borne (a member of the arena virus family, is a single-stranded RNA virus). Lassa cases increases to 0.5M new infections and 10,000 deaths per year estimated across West Africa, 80% of people infected will have no or mild symptoms and the incubation period is 2-21 days which affects all age groups and both sexes. The geographic distribution of LF, It occurs typically in west Africa Benin, Côte d'Ivoire, Ghana, Guinea, Liberia, Mali, Nigeria, Sierra Leone and Togo. The illness is estimated to affect 300,000 people annually, resulting in approximately 5,000 deaths in West Africa and Endemic in Nigeria - One case is an outbreak. The reservoir for Lassa virus (LASV) is a rodent ''multimammate rat" from the Muridae family known as *Mastomysnatalensis*(12). Other rodent species – Hylomyscuspamfi, Mastomyserythroleucus, M. huberti and even Rattus rattus and Mus musculus may also host LASV and the rodent species which carry the virus are found throughout West Africa. The Peri-domestic, average lifespan of 2 years, breed around the year (16-20 litters per pregnancy), they are Infected at birth and become life long asymptomatic carriers of Lassa virus and they Shed virus in the urine and faeces and direct contact with these materials, can lead to infection (13).Mastomys rodents can be found in vast numbers in the savannas and forests of west, central, and east Africa. They reproduce regularly and have a lot of young. Mastomys also easily colonize human dwellings and places where food is kept. All of these elements help the Lassa virus move from infected rodents to people rather well. Mastomys characteristics are granivorous, omnivorus& carnivorous, It can bite humans at the least provocation, Mastomys gets easily frightened and Minor stress could kill it. Mastomys can cohabit easily with other species, It is a good traveler and a prostitute that Loves peace and quiet, they Shares homes and

scavenge on leftover human food items or poorly stored food, direct contact transmission is common with man and other rodents easily (14).

Transmission of Lassa fever

Transmission of Lassa virus to humans occurs most commonly through ingestion, contact or inhalation. The followings are routes of transmission of lassa fever;

1. Exposure to excreta/urine of infected Mastomys via ingestion of contaminated food or water or indirectly through eating from unwashed utensils.
2. Direct contact with the blood, urine, faeces, or other bodily secretions of patients through broken skin or mucus membranes
3. Inhalation of virus ridden particles
4. Sexual transmission of Lassa virus has been reported
5. In a health care setting, spread can occur through contaminated medical equipment and reused needles, accidental needle stick injuries ,Contact with soiled beddings, Contact with body fluids of patients
6. It is possible to transport Lassa fever from an endemic area to a non-endemic one during the incubation period

About 80 – 90 % of humans are infected through rodent to human exposure, Food or household items contaminated by rats urine or faeces and Handling of rats Fig 1. Human to human transmission are Body fluids or secretion (Blood, tissue, secretion) of LV infected persons (15). Lassa virus cannot be transferred through inadvertent touch, including skin-to-skin contact without the exchange of bodily fluids. Person to person transmission is also common in Healthcare workers HCWs (nosocomial transmission) and caregivers, where the appropriate PPE is either unavailable or not applied. Reused needles and other contaminated medical supplies are potential carriers of the Lassa virus. And can be sexual transmitted Fig 2.

Mastomys rodents can infect people when they are caught and processed for food, and they are occasionally eaten. A human may come into contact with the virus if they breathe microscopic airborne particles contaminated with infected rodent excretions (fig 1). Cleaning tasks like sweeping may result in this aerosol or airborne transmission. There are other ways for people to become infected except through direct contact with sick rats (16).

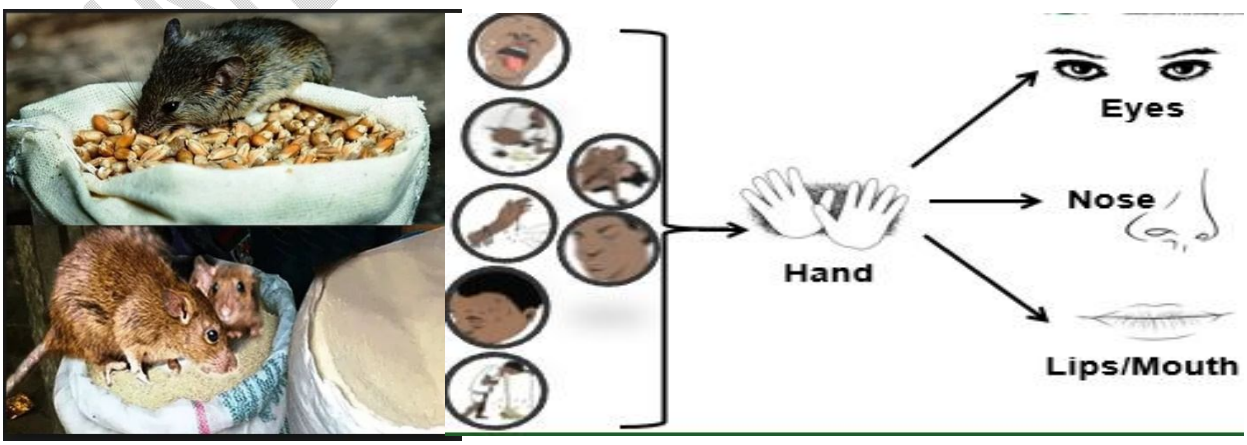

Fig 1: Rodent to Human route Fig 2: Human to Human transmission

Signs and Symptoms

Symptom onset typically occurs within 6 – 21 days after the patient comes into contact with the virus. Approximately 80% of the infection exhibit mild symptoms and are undiagnosed.LF is most commonly characterised by the presence/history of mild symptoms include slight fever, headache, general malaise, weakness, vomiting, nausea, and abdominal pain. The other 20% of infected patients will present with more severe, life-threatening forms of illness including hemorrhaging (in gums, eyes, or nose, as examples), seizure, acute kidney injury, shock, respiratory distress, repeated vomiting, facial swelling, pain in the chest, abdomen and back and breathing difficulty (12). There have also been reports of neurological issues like encephalitis, hearing loss, and tremors (17). Due to multi-organ failure, death could hApen two weeks after the onset of symptoms.

The most complication of Lassa fever is hearing loss. About one-third of infections result in deafness of varying degrees, and in many cases, hearing loss is permanent. According to what is known, the severity of the disease has little bearing on this complication; deafness can occur in both mild and severe cases. About 15%–20% of people with Lassa fever who are hospitalized pass away from the illness. Only 1% of Lassa virus infections cause death, nevertheless. In hospitalised cases, the CFR is at 24-27% overall and 34% for pregnant women (Third-trimester pregnant women have very high mortality rates). Clinical diagnosis of Lassa fever is often difficult since the symptoms are so numerous and ambiguous. Lassa fever is also linked to sporadic outbreaks (occasional or seasonal), during which the case-fatality rate in hospitalized patients might exceed 50% (18).

Risk of Exposure

People who reside in or travel to endemic areas, such as Sierra Leone, Liberia, Guinea, and Nigeria, and have exposed to multimammate rats, are most at risk of contracting the Lassa virus. Other west African nations with Mastomys rodents may also have exposure risk. As long as precautions are taken and the right sterilizing techniques are applied, hospital employees are not at significant risk for infection (14).

Diagnosis and Treatment

Enzyme-linked immunosorbent serologic assays (ELISA), which may detect IgM and IgG antibodies as well as Lassa antigen, are most frequently used to diagnose lassa fever. In the early stages of an illness, reverse transcription-polymerase chain reaction (RT-PCR) can be employed. The virus itself can be cultivated in 7–10 days, however this operation needs to be carried out in a high containment environment using best practices. Using formalin-fixed tissue samples and immunohistochemistry, a postmortem diagnosis can be made (19).

To date, no treatment has been specifically developed and none is registered for LF and there is no vaccine available yet for LF (20). Ribavirin (in conjunction with supportive care) is currently the only available treatment that has been recommended for LF (21, 22, 23). Patients with LF have been successfully treated with the antiviral medication ribavirin. It has been demonstrated

that in the early course of the illness to be more effective. Supportive care for patients should also include the management of any other aggravating infections, as well as the preservation of an optimal fluid and electrolyte balance, oxygenation, and blood pressure (19).

Drivers and Prevention of Lassa fever

The drivers of LFis poor Environmental sanitation such as bush burning, poor housing standards, improper food storage techniques, hunting of rodents, cultural practices promoting and contact with infected corpse. The prevention and control of Lassa fever may be approached from the agent, environment, and host. The agent rodent can be control by trapping - As much as possible avoid trapping to minimize contact with secretions. Sanitation- Avoid bush burning, good housekeeping, cover burrows. Rodenticides – Use rodenticides that kill silently and Fumigation. It is not advised to use these rodents as food. Rodent populations can be reduced by trapping inside and around dwellings, however comprehensive control of this rodent reservoir is problematic due to the widespread distribution of Mastomys across Africa. Environmental control are done by the followings Building/ housing patterns – roofs, pipes, floor, nets, rat proof, good housekeeping, Waste disposal methods – waste bin with cover, regular disposal, Avoid bush burning, Water source/purification – Properly constructed wells, boiling/ chlorination of water, Food hygiene – food should be properly cooked and preserved, store raw food in containers, wash fruits and vegetables before eating, do not eat food bitten by unknown animals, wash and store utensils in containers, wash can drinks and sachet water, Store food in a well-covered containers and Starve Mastomys of food source, it will migrate away fig 3. Host control includes Personal hygiene, hand hygiene, health education, rapid diagnosis and treatment, use of PPEs by health workers, reporting cases to appropriate personnel, right attitudes and practices towards sick individuals and seeking health care assistance from appropriate channels.

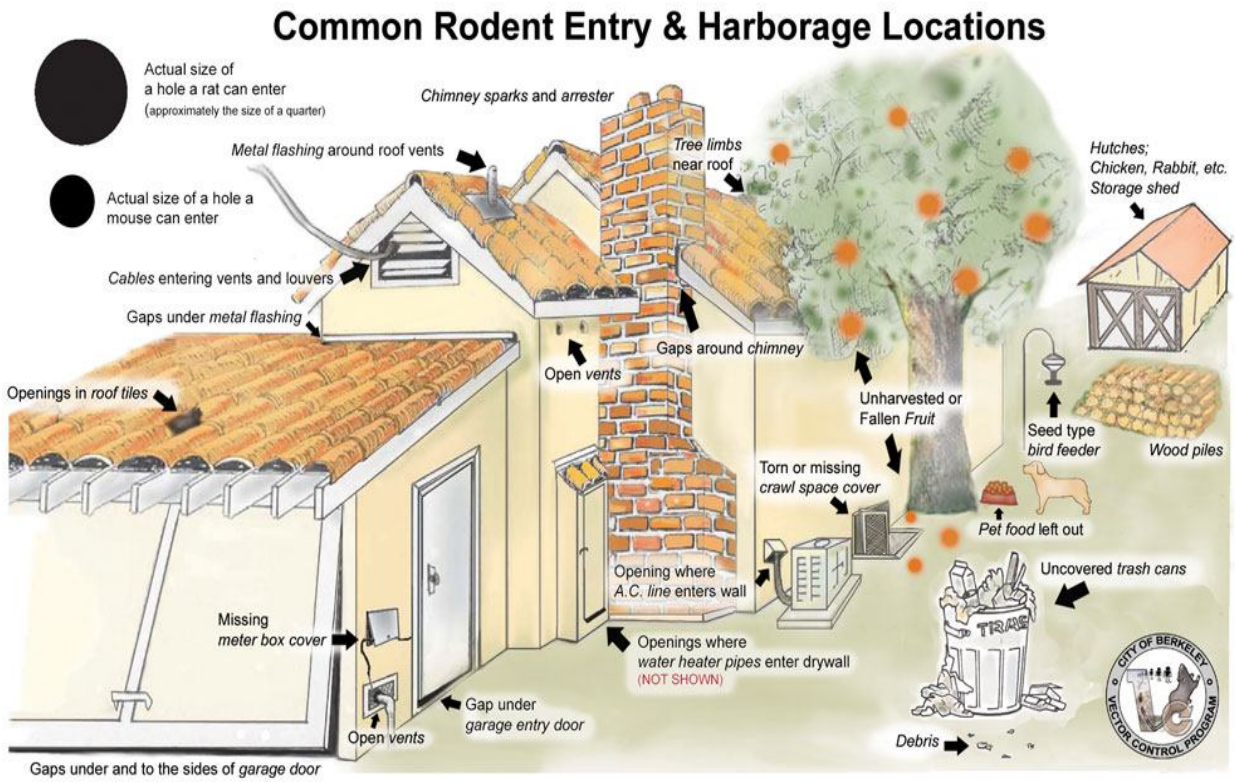

Fig 3: Rodent entry

Avoiding contact with Mastomys rat, particularly in the areas where outbreaks occur, can stop the primary transmission of the Lassa virus from its host to human. Rodents are deterred from homes by storing food in rodent-proof containers and keeping the house clean.

Precautions against contact with patient secretions should be taken when caring for patients with Lassa fever in order to prevent further disease transmission by person-to-person contact or nosocomial routes (called VHF isolation precautions or barrier nursing methods). The precaution includes the use of infection control measures, such as complete equipment sterilization, the wearing of protective clothing, such as masks, gloves, gowns, and goggles, and the isolation of infected patients from contact with uncovered individuals until the disease has run its course. Host control involves Personal hygiene, hand hygiene, health education, rapid diagnosis and treatment, use of PPEs by health workers, reporting cases to appropriate personnel, right attitudes and practices towards sick individuals and Seeking health care assistance from appropriate channels

Lassa fever can also be controlled and prevented by educating people of high-risk locations and how to reduce rat populations in their homes.Other difficulties include creating quicker diagnostic tests and expanding the accessibility of the only available medicinal therapy, ribavirin. There is currently research being done to provide a vaccine for Lassa fever.

Lassa fever case definition

Case definition is the list of criteria that public health professionals use to decide whether to include a person's illness as a case in an outbreak investigation—that is, if a person is regarded as being directly affected by an outbreak. Lassa fever case definition can be a suspected case, confirmed case and probable case

Suspected Case

Any person presenting with one or more of the following: malaise, fever, headache, sore throat, cough, nausea, vomiting, diarrhoea, myalgia, chest pain, hearing loss and either:

a. History of contact with excreta or urine of rodents
b. History of contact with a probable or confirmed Lassa fever case within a period of 21 days of onset of symptoms
c. Any person with inexplicable bleeding/hemorrhagia

Confirmed Case

Any suspected case with laboratory confirmation test

Probable Case

Any suspected case who died/absconded without collection of specimen for laboratory testing

Ribavirin Metabolism and cellular effects

Clinical dosages of ribavirin are given as the nucleoside. The cellular enzyme that produces ribavirin monophosphate is adenosine kinase (RMP). Only small levels of the phosphorylated forms of ribavirin are accumulated by cells with insufficient adenosine kinase activity (24,25). The di- and triphosphorylated nucleotides are produced by further phosphorylating RMP (25, 26). After exposure to cultured cells, phosphorylation occurs quickly, with half-maximal levels of metabolites being reached in a few of hours. Ribavirin triphosphate (RTP) is typically the main metabolite, although the relative concentrations of the mono-, di-, and triphosphorylated forms vary by cell type (25).

When given ribavirin, GTP pools are lowered by almost twofold while cellular CTP and UTP are simultaneously elevated (26, 27). This alteration in nucleotide pools is brought about by RMP's capacity to operate as an IMPDH inhibitor (see following section). It is possible that ribavirin diphosphate is not a substrate for cellular ribonucleotide reductase because deoxynucleotide forms of ribavirin have not been found inside cells. Deoxynucleotide pools in cells, however, are typically significantly smaller than ribonucleotide pools. As a result, undetected very low amounts of ribavirin deoxynucleotides may exist. Comparing Vero 76 cells to 3T3 cells, Smee and colleagues found that ribavirin metabolites were reduced, with almost 13-fold less RMP accumulating in these cells (28). Although the nucleotides are much more stable in erythrocytes, the half-life of ribavirin metabolites in cultured fibroblasts and lymphoblasts is rather brief (25). Reversible hemolytic anemia, a side effect of clinical ribavirin therapy, is brought on by this buildup of ribavirin in erythrocytes (29).

Ribavirin has a significant impact on treated cells. The production of DNA, RNA, and proteins is decreased in exposed cells as a result of this cytostatic substance (30). Ribavirin has not been found in cellular RNA or DNA, despite the fact that ribavirin triphosphate accumulates to large quantities in treated cells (30). However, this might be as a result of a low integration rate that falls below the limit of detection for experiments using cells.

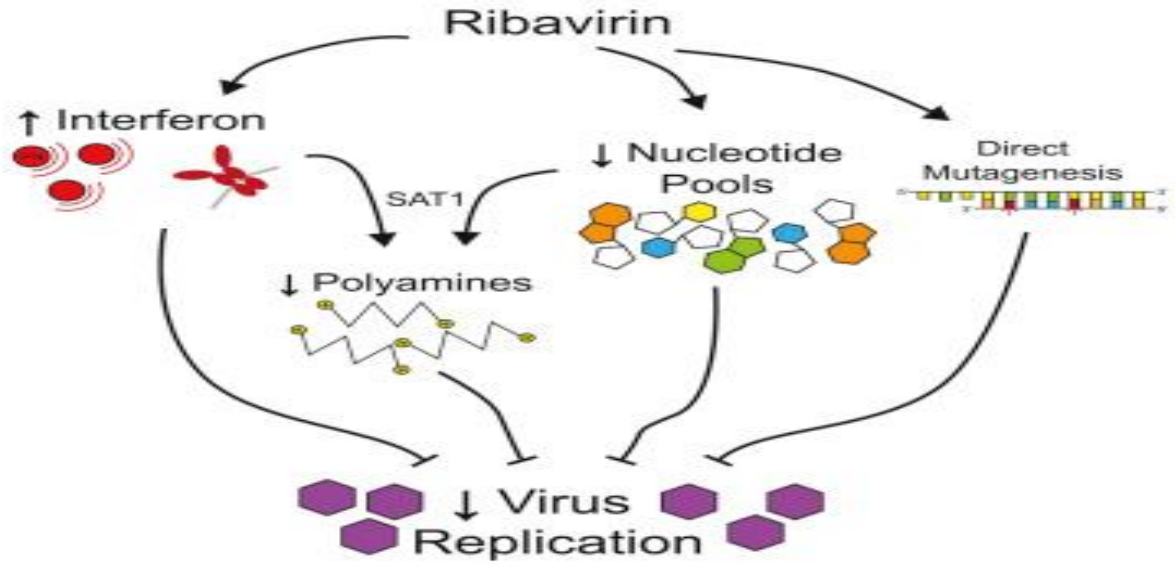

Fig 4: Ribavirin Metabolism

Arenaviruse

The negative-stranded RNA genome of arenaviruses is bi-segmented and they are enveloped viruses. Ambisense coding is used in each of the two genomic segments, L (7.3 kb, large) and S (3.5 kb, tiny), to drive the synthesis of two polypeptides that face each other (IGR). The site 1 protease (S1P) performs post-translational processing on the mature virion surface glycoproteins GP1 and GP2, which together with the 58-amino acid stable signal peptide (SSP) form the GP complex that mediates virus receptor recognition and cell entry. The S RNA encodes the viral nucleic acid (NP) and the glycoprotein precursor (GPC), which is co-translationally cleaved by the signal peptida (31).

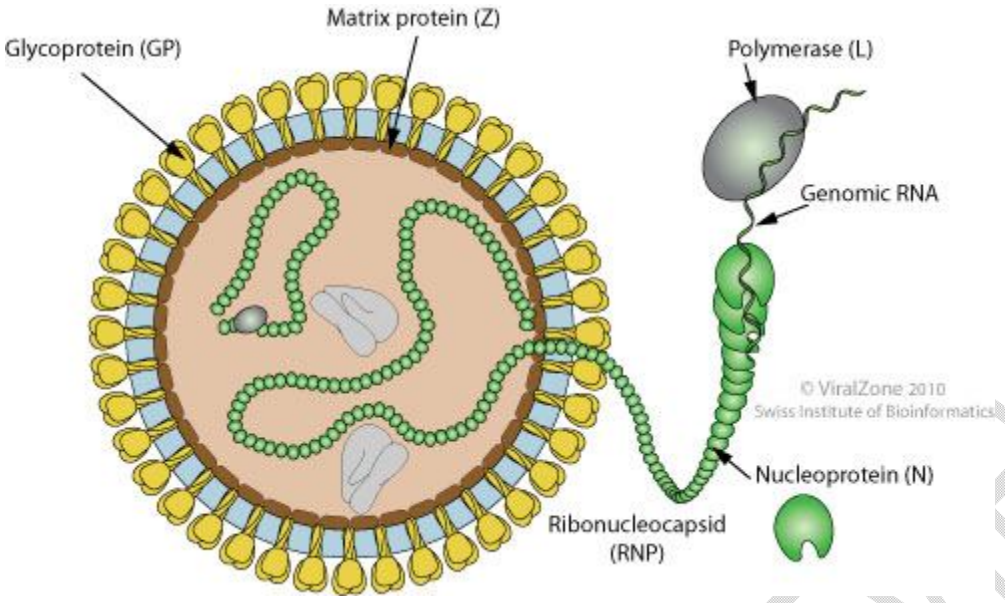

Fig 5: Structure of Arenavirus

The viral envelope glycoproteins' function and trafficking have both been connected to the SSP in a variety of ways (32, 33, 34). The apex of the spike, away from the membrane, is home to GP-1, which mediates virus interaction with host cell surface receptors. Ionic interactions with the N-terminus of the transmembrane GP-2 keep GP-1 in place (Fig 6).The arenavirus RING finger protein Z, a structural element of the virion, has been demonstrated to be the arenavirus equivalent of the M protein, which is present in many other NS enveloped RNA viruses and is essential for the production and release of mature infectious virions from cells (35, 36). The tiny RING finger protein Z, which performs the duties of a true matrix protein, and the viral RNA dependent RNA polymerase (L protein) are both encoded by the L RNA (37).

The 3′-termini of arenavirus genomes show a high degree of sequence conservation, and like other viruses with segmented negative strand (sNS) RNA genomes, their L and S genome segments show 5′- and 3′- complimentary sequences, which are projected to form panhandle structures. Between isolates and strains of the same arenavirus species, the S and L IGR sequences change significantly in terms of both sequence and predicted folded structure, yet they are both highly conserved.Thus, Z mRNA transcription does not start during the initial transcriptional rounds of infection but rather after the L anti-genomic RNP species have been generated. Z expression levels are controlled, and variations between early and late stages of the virus life cycle may be due to the diverse functions Z performs at various stages of infection.By directly interacting with the L protein early on in the virus' life cycle, Z can facilitate its role as a regulator of viral RNA replication and gene transcription (38); however, increased Z expression levels later on in the arenavirus life cycle will favor virus assembly and budding (36, 37).

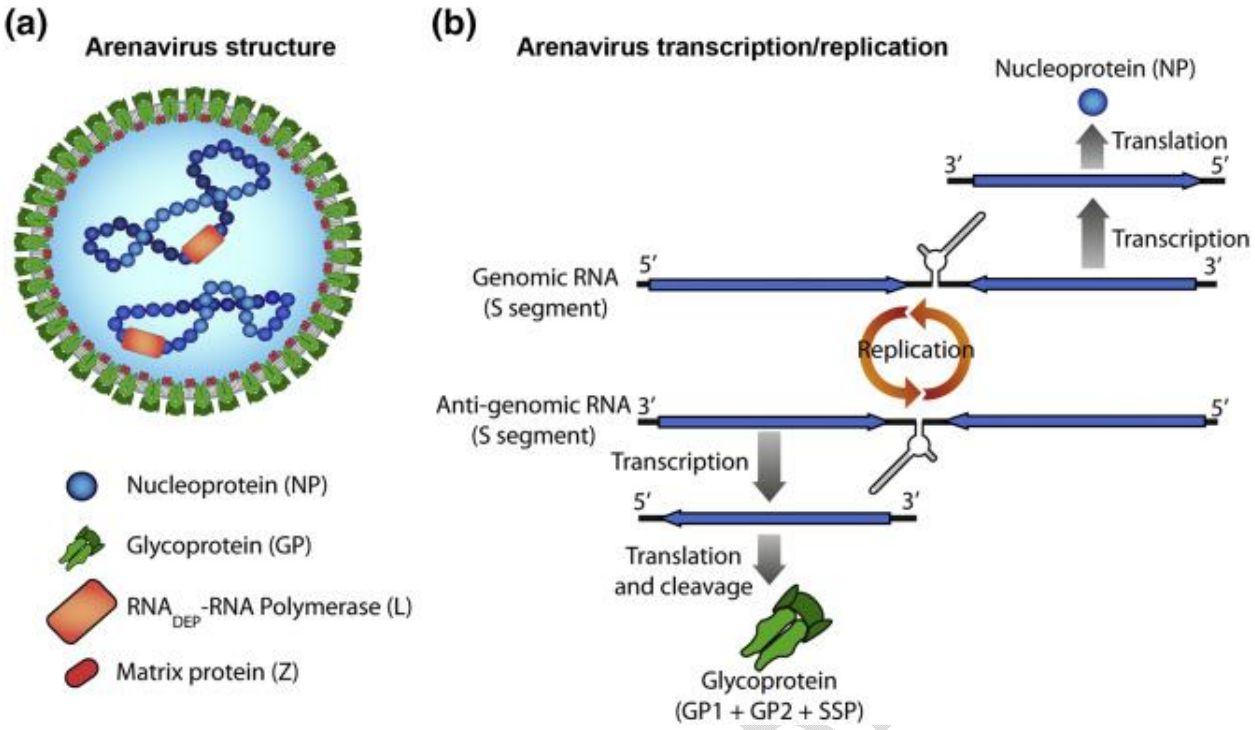

Fig 6: Arenavirus Transcription

Arenavirus life cycle

Arenavirus Virus enter the host cell-surface receptor (alpha-dystroglycan alpha-DG), a flexible receptor for extracellular matrix proteins (39). Initial virus infection occurs when the host inhales aerosolized virus particles. Due to the mode of infection and the virus' presence in macrophages at the beginning of infection, it is anticipated that alveolar macrophages are the first cell types to become infected (40). Although pulmonary epithelium from sick humans and animals has been found to contain arenaviruses and their antigen, it is unknown whether these cells are the virus's primary targets (41, 42).The virus can then spread to different tissues as a result of the first infected macrophages moving to the draining lymph node (40, 43). Old World and New World viruses not only have different cellular receptors, but also diverse entry points into the cell. Old World viruses, including LASV and LCMV as well as Clade C viruses, bind to the relevant cellular receptor to begin the entry process. The basement membrane frequently contains an extracellular matrix protein called -dystroglycan, which is used by New World viruses to enter the cell (39, 44). Recently, it was shown that LUJV mediates entrance via using the neurophin (NRP)-2 receptor (45).

When LP and NP successfully acquire the 5′ caps from host mRNA, replication can begin. These caps—NP and LP ORFs on the S and L segments, respectively—prime the start of early gene transcription. Arenavirus RNA cannot be immediately translated in its genomic form since it is negative sense RNA. This requires the production of 5′-3′ nascent RNA-dependent RNA transcription. This first transcribed region contains the NP and LP ORFs, which causes their initial creation as early products (46). Intergenic hairpin structures seen in both the L and S segment IGR are used to end transcription (47).

In order to promote continued transcription, replication, and finally progeny virus assembly, translated NP attaches to viral RNA. The "stolen" host mRNA cap may fit in the N terminal domain of NP, according to crystallographic study of the protein, permitting further transcription (48).The Lassa virus uses the "Ambisense" replication mechanism, which is extremely quick and exhibits replication of temporal control (49). The negative sense gene's initial stage of transcription results in enough viral proteins being deposited for the subsequent stage of replication. L and NP proteins are subsequently translated from the mRNA. From the positive sense gene, copies of the viral complementary RNA (vcRNA) are produced. While mRNA is being created from it, templates of RNA copies yield negative-sense offspring. Later on, the Z and GP proteins are created by translating the mRNAs that were created from the vcRNA. The host immune system's detection is delayed as a result of the temporal controls' enhancement of the final protein production spike (50).

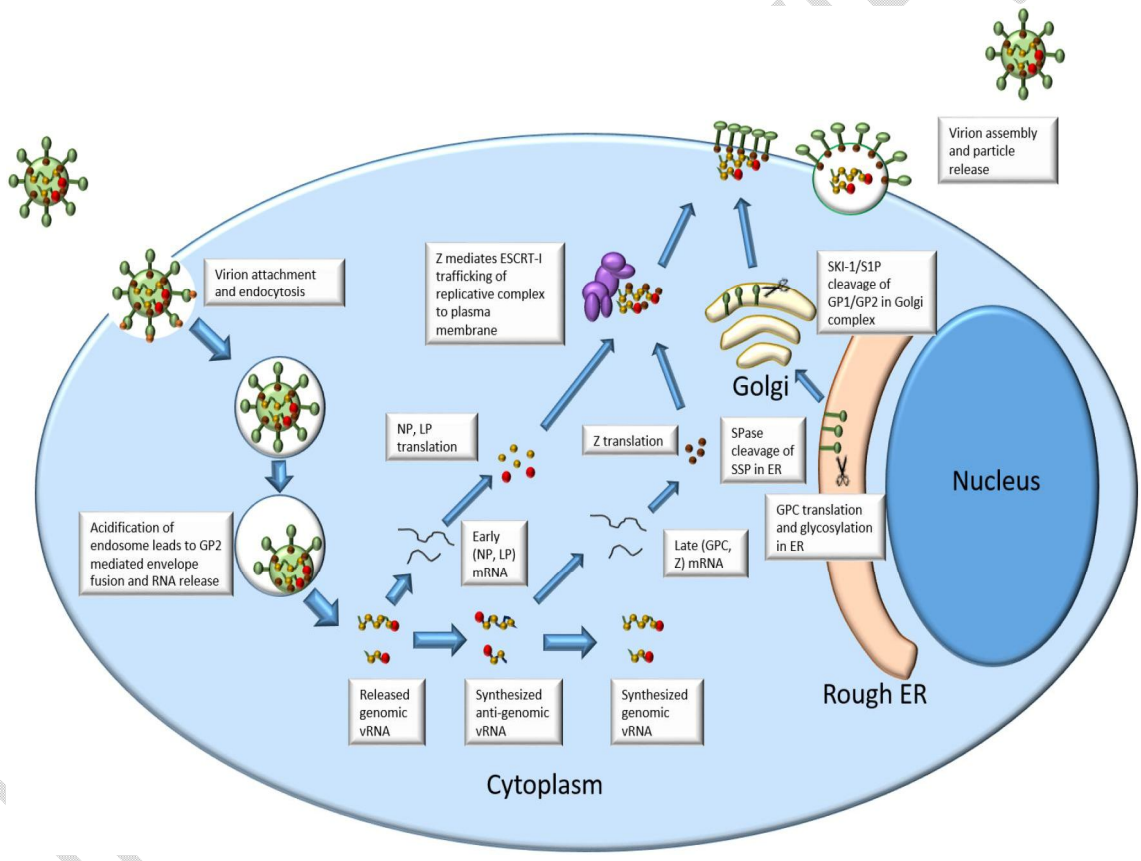

Fig 7: Arenavirus life cycle

Ribavirin mechanism of action

Ribavirin is a guanosine analog with broad-spectrum action against numerous RNA and DNA viruses. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3- carboxamide), also known as Virazole,

is a synthetic purine nucleoside analogue that was created for the first time by Sidwell and colleagues in 1972 (Fig 7) (51, 52). The nucleoside analogue ribavirin exhibits antiviral action in vitro and in vivo against a wide variety of viruses. Ribavirin was the first synthetic nucleoside to display broad-spectrum antiviral activity and is one of the few antiviral medications currently being used in clinical settings that is effective against Lassa fever and other hemorrhagic diseases such as Ebola virus, Crimean-Congo, chronic hepatitis E virus infection, and other life-threatening viral infections (8, 9, 10).

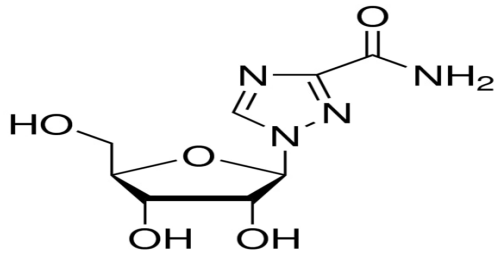

Fig 8: Structure of Ribavirin

The mechanism of action of ribavirin has been controversial for more than 30 years. Depending on the particular virus being examined, a variety of unique mechanisms have been proposed. For ribavirin, five main modes of action have been proposed. Indirect mechanism are Inosine monophosphate dehydrogenase (IMPDH) inhibition, which reduces cellular guanosine triphosphate (GTP) pools, and immunomodulatory effects that sustain a T-helper type 1 (antiviral) immune response. Direct mechanisms include inhibition of RNA capping activity, directly inhibiting viral polymerases, and increase mutation frequency through the error catastrophe caused by the integration of ribavirin into newly synthesized genomes (11).

Ribavirin is an Inhibitor of Inosine Monophosphate Dehydrogenase

The enzyme IMPDH catalyzes the first step in the de novo cellular production of guanine nucleotides (Figure 9). In this NAD-dependent process, inosine monophosphate (IMP) is changed into xanthosine monophosphate (XMP). The enzyme GMP synthase can then aminate XMP to produce guanosine monophosphate (GMP). GMP is subsequently transformed into guanine metabolites like GTP and dGTP, which are necessary as building blocks for the production of RNA and DNA, respectively. In addition, GTP is crucial for energy storage, intracellular signaling, ribosome translation, and glycoprotein production.

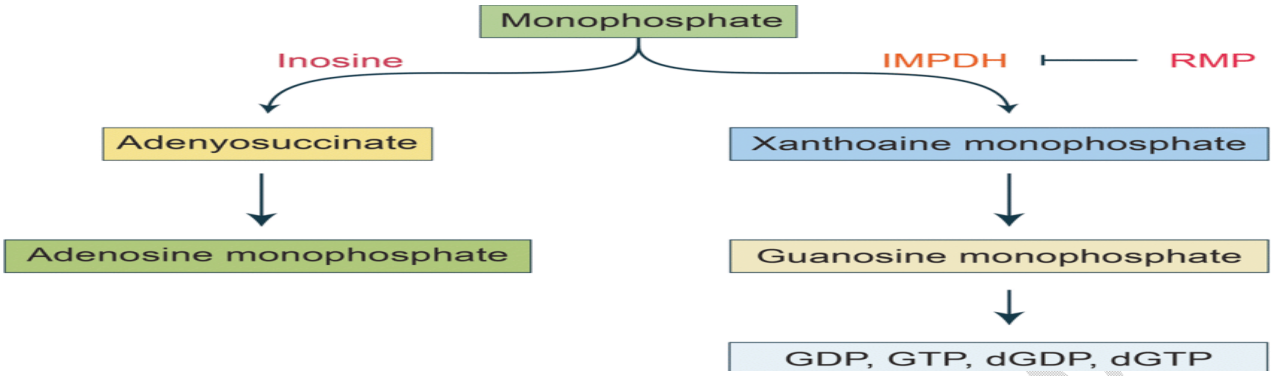

Figure 9:Guanine nucleotide biosynthesis's de novo pathway is shown schematically. The first committed step is the conversion to XMP. Ribavirin monophosphate inhibited this step. RMP, or ribavirin monophosphate, and IMPDH, or inosine monophosphate dehydrogenase

RMP is a strong competitive inhibitor of IMPDH because of its structural resemblance to GMP (53). RMP has a Ki of 650 nM and 390 nM, for inhibiting the type I and type II isoforms respectively of human IMPDH (54). It has been determined the X-ray crystal structure of RMP in association with the human IMPDH type II core domain (55). This structure showed that RMP, an excellent replica of the natural substrate IMP, binds to IMPDH's active site substrate pocket. One potential mechanism for the antiviral effects of ribavirin is the inhibition of IMPDH.It has been shown that ribavirin-treated cells exhibit a roughly two-fold reduction in GTP pools. Translation, transcription, RNA replication, and dGTP are all required for these processes. Therefore, it was proposed that decreasing the amount of accessible GTP would hinder these crucial viral life cycle events, which would account for ribavirin's antiviral capabilities. Ribavirin's broad-spectrum efficacy may possibly be explained by IMPDH inhibition, which is a non-specific cellular impact. Utilizing a flavivirus (yellow fever virus) and paramyxoviruses (human parainfluenza virus 3, respiratory syncytial virus), ribavirin, 5-ethynyl1-beta-D-ribofuranosylimidazole-4-carboxamide (EICAR), and mycophenolic acid (MPA) have been compared for their antiviral activity (56). The 50-monophosphate of EICAR, which is a structural counterpart of ribavirin, is also a powerful inhibitor of IMPDH (57).IMPDH is inhibited by MPA uncompetitively without the necessity for metabolic activation (58).

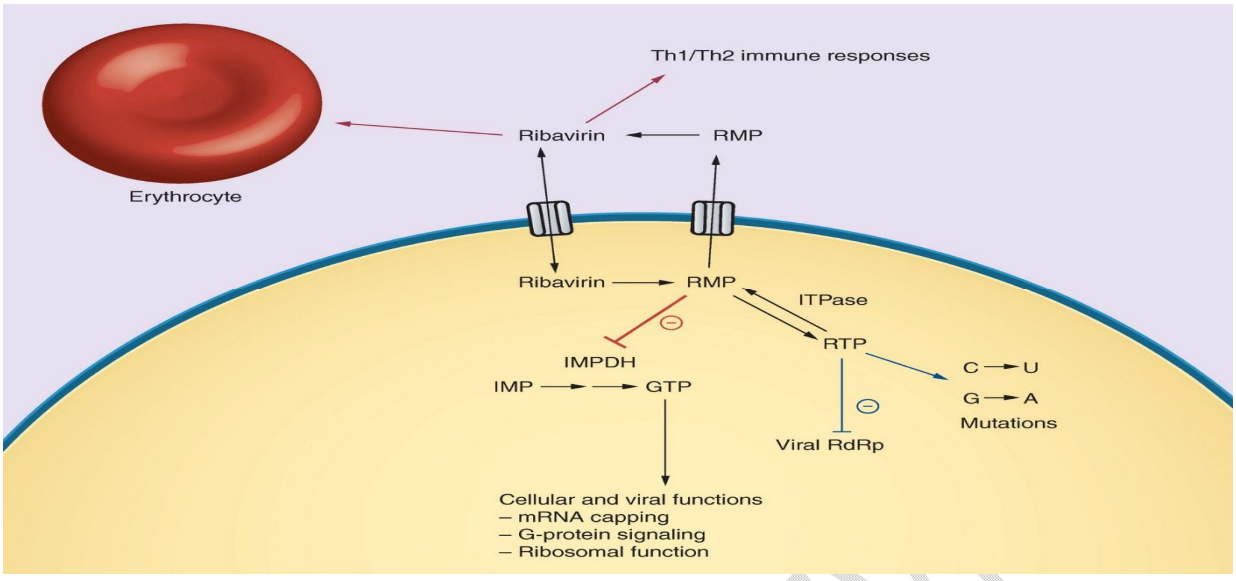

Figure 10. Modes of action of ribavirin, ribavirin monophosphate and ribavirin triphosphate on cellular functions (red) and viral functions (blue).

On the GTP pools of Vero and HeLa cell, the impacts of these three IMPDH inhibitors were examined. Antiviral activity as determined by viral RNA synthesis and antiviral impact as measured by reduction in CPE were found to be linearly correlated for all three drugs, indicating that IMPDH inhibition is the main mechanism of antiviral activity for all three drugs. The measured GTP pool reduction in this instance, however, was far greater than what other researchers had previously found.The antiviral effect of ribavirin, however, has not always been fully explained by IMPDH inhibition alone. It was discovered that the reduction in intracellular GTP pools was saturated at a rather low dose of ribavirin (25 mM) in a study of the antiviral action of ribavirin against influenza virus (59). Higher quantities of ribavirin, albeit they did not further reduce cellular GTP pools, had a more powerful antiviral effect. Ribavirin's powerful antiviral action was not followed by significant declines in translation or RNA synthesis, according to studies with the poliovirus (PV) (60).

Additionally, not every IMPDH inhibitor possesses antiviral action (60, 61). Therefore, in most instances, IMPDH inhibition may not be the main mechanism of antiviral action. However, ribavirin's antiviral efficacy may be significantly boosted by the suppression of IMPDH. Ribavirin can be more effective as a polymerase inhibitor, capping inhibitor, or deadly mutagen by lowering levels of competitive GTP. In order to boost the effectiveness of other antiviral nucleosides used to treat HIV (62, 63, 64), hepatitis B virus, and herpes simplex virus type 1 (65), ribavirin has also been employed as an IMPDH inhibitor. Yet, ribavirin has also demonstrated antagonism in combination with some nucleoside drugs (66). However, ribavirin has also shown antagonistic effects when used with several nucleoside medications (67).

Ribavirin is an immunomodulatory agent

Another indirect antiviral method through which ribavirin may function is by boosting the host T-cell response. This result is based on findings that ribavirin can lower serum levels of alanine aminotransferase (ALT), a marker of liver damage, in HCV-infected patients without significantly lowering levels of circulating HCV RNA as assessed by PCR (68). It has been proposed that ribavirin works in combination therapy by preserving the response to interferon therapy. The type 2 to type 1 phenotypic flip in T-helper cells is hypothesized to be induced by ribavirin (69).Cellular immunity and the expression of IL-2, gamma-interferon, and tumour necrosis factor-alpha are linked to the T-helper type 1 response (70). The T-helper 2 response, which is linked to the expression of IL-4, IL-5, and IL10, increases humoural immunity. An inadequate host immune response may play a significant role in persistent infections, according to some research. Chronic illness brought on by HCV infection has been linked to a T-helper 2 response (71). It has been demonstrated that ribavirin can alter the expression of cytokines in human T-cells (72).In vitro, ribavirin at low concentrations (5–10 mM) stimulated a Type-1 response and inhibited a Type-2 response in CD4 and CD8 human T-cells. This theory is supported by data for the L-enantiomer of ribavirin (ICN 17261, also known as levovirin). In a mouse model, this substance was equally effective at eliciting Type-1 cytokine responses and lowering serum ALT levels (73). Levovirin did not, however, show direct antiviral efficacy in vitro against ribavirin-sensitive viruses.This molecule should not be able to be converted to the phosphorylated metabolites, which are required for the direct antiviral actions of ribavirin, as stereochemical differences probably prevent intracellular phosphorylation (74). Failure of levovirin clinical studies in HCV patients suggests that the in vitro immunomodulation observed is insufficient to induce clinical effects (75). Ribavirin boosts the immunological response, but its exact method of action is unknown. It's interesting to note that new findings seem to indicate that ribvavirin monotherapy may in fact have an antiviral effect (76).

Additionally, PCR-based approaches for viral load monitoring may ignore antiviral processes that result in the creation of non-infectious genomes. The approximate proportion of immunomodulatory effects to ribavirin antiviral activity has been determined using mathematical modeling. Modeling has been done to determine how interferon and ribavirin therapy affects viral load decline (77). For this model, it was presumpted that ribavirin could lessen the specific infectivity of newly formed virions (via lethal mutagenesis, see below). The model anticipated that ribavirin has little effect on viral load decline in patients with high interferon effectiveness.However, ribavirin should have a significant role in lowering viral load when interferon efficiency is poor. This model was able to resolve disparate evidence about the efficacy of ribavirin by posing the possibility that a patient's response to interferon may be crucial. Additionally, this model disregarded ribavirin's potential immunomodulatory effects. This model offered excellent fits to the viral load data when used with clinical data from 17 patients. Patients with strong interferon effectiveness accepted a model with little effect from ribavirin.

The observed loss rate of infected cells following combination therapy, however, was lower for individuals with low interferon efficiency than that anticipated by interferon alone, indicating that ribavirin is likely to have an additional antiviral effect. The model's design assumptions, however, might be contested. First, there is insufficient evidence to support a decrease in HCV infectivity during clinical ribavirin therapy.Furthermore, although this is not generally acknowledged, the authors presume that ribavirin has a progressive impact that peaks at 28 days and reaches a plateau. Finally, a comparison of our predictions to larger samples of clinical data might be instructive. According to their findings, the side effects of combination therapy can be minimized by only administering ribavirin therapy to individuals who will benefit from it and avoiding it in those who will receive minimal benefit.

Ribavirin is an inhibitor of RNA capping

Most cellular RNAs and some viral RNAs have a 7-methylguanosine cap structure at the 50-end that is crucial for the stability and translation of the RNA. Ribavirin has the ability to interact with the enzymes that 'cap' viral genomic RNAs and cellular mRNAs since it is a guanosine nucleotide analogue. Typically, three enzymatic processes (Figure 11) are used to synthesize the cap structure: An RNA triphosphatase converts the RNA's 50 triphosphate to a diphosphate; a unique 50-to-50 linkage is used by an RNA guanylyltransferase to add GMP to this 50 terminus; and a (guanine-7-) methyltransferase adds a methyl group from S-adenosylmethionine to the terminal guanosine at the N-7 position (reviewed in (77)).

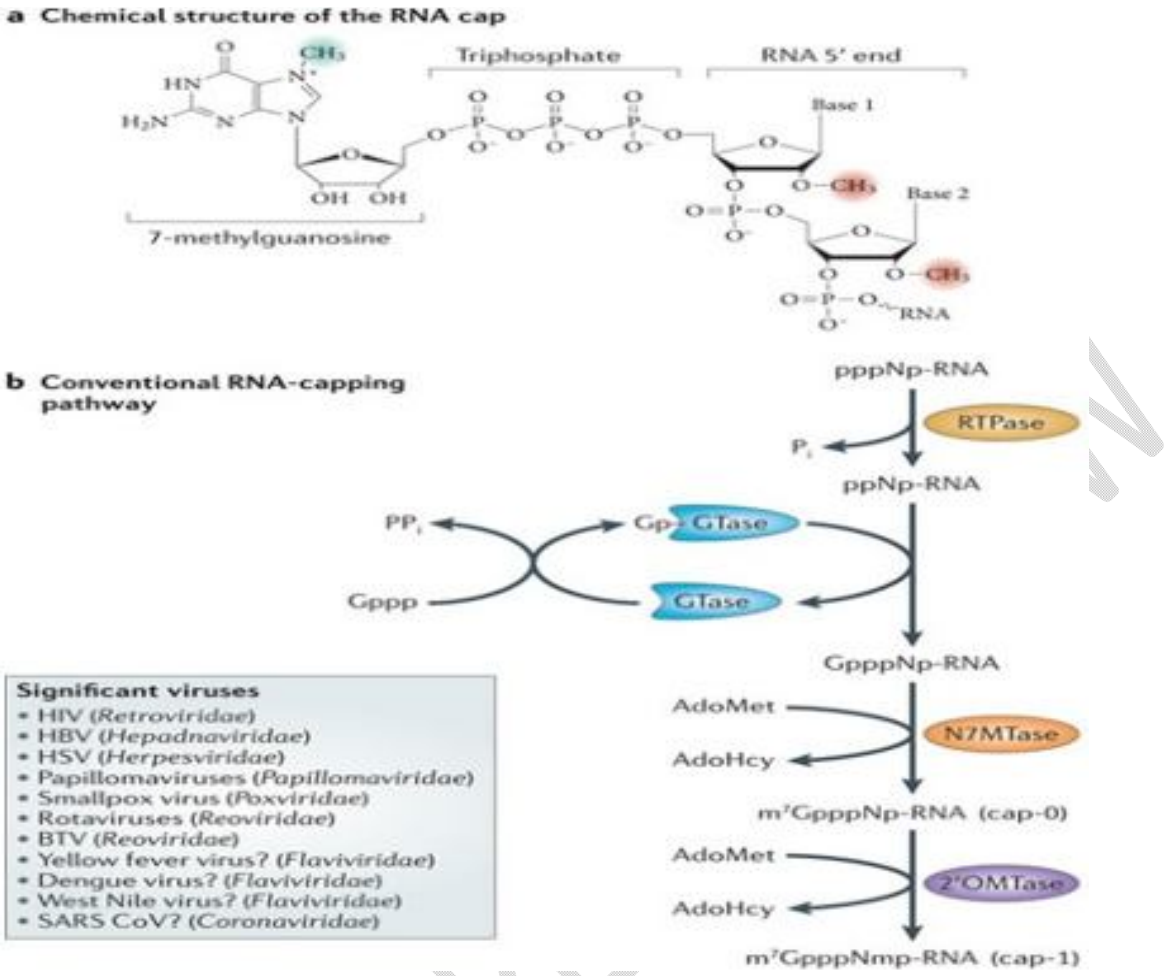

Figure 11: Three enzymatic processes result in the RNA's 50 7-methylguanosine cap structure. The 50 end of the RNA is first cut by an RNA triphosphatase, releasing a diphosphate. An RNA guanylyltransferase then links GMP to the end of the RNA via a 50–50 linkage. Finally, an RNA methyltransferase uses S-adenosylmethionine to methylate the terminal guanosine at the N-7 position. Nucleotides positioned thirty nucleotides away from the cap's phosphate bridge may undergo further methylation.

A number of human tumors have been linked to the oncogene eIF4E, which regulates translation initiation (78). For cap-dependent translation to begin, eIF4E contact with the 50 7-methylguanosine caps of cellular mRNAs is necessary. A subset of sensitive transcripts translate more frequently when eIF4E is overexpressed, and this can cause cancer. The capacity of ribavirin to imitate the 7-methylguanosine cap was studied by Kentsis and colleagues (79). It has been demonstrated that ribavirin triphosphate binds to eIF4E's 7-methylguanosine cap binding site with the same affinity as 7-methylguanosine. Additionally, it was demonstrated that ribavirin disrupted eIF4E nuclear bodies and prevented eIF4Eregulated mRNAs from being transported or translated.Furthermore, both in vitro and in vivo tumor development and eIF4E-mediated transformation were inhibited by low micromolar quantities of ribavirin. Beyond the cellular ramifications, these findings also point to a method of action against viruses like the Lassa fever

virus (80) and SARS coronavirus that exploit cellular eIF4E or 7-methylguanosine. RTP may not, however, obstruct the interaction between eIF4E and 7-methylguanosine, according to recent biochemical evidence (81). On the genomic RNA's 50 end, several RNA viruses use a 7-methylguanosine cap structure.

Virus-encoded enzymes are required to catalyze the capping event for viruses reproducing in the cytoplasm since eukaryotic mRNA capping takes place in the nucleus. Ribavirin has the potential to inhibit the enzymes involved in this pathway because it is a guanosine analogue. A Sindbis virus strain was discovered by Scheidel and Stollar and shown resistance to both ribavirin and the noncompetitive IMPDH inhibitor MPA (82). The cross-resistance suggests that ribavirin's ability to combat this virus is at least partially a result of a reduction in GTP pools. Further analysis revealed that nsP1, the virus-encoded enzyme that regulates guanylyltransferase activity, was the site of resistance (83). This finding suggested that ribavirin inhibits the capping of RNA genomes, possibly by interfering with the guanylyltransferase or methyltransferase activities (both of which are thought to be encoded by nsP1) or by being incorporated as a cap analogue, which may affect RNA translation, even though direct biochemical evidence was not obtained. According to some theories, the capping response is what causes ribavirin to work so well against the vaccinia virus (84).

It has been discovered that RTP is a powerful inhibitor of the guanylyltransferase of the vaccinia virus mRNA, indicating that ribavirin works by creating mRNAs that are incapable of being translated. The N-terminal portion of the vaccinia virus D1 protein, which exhibits both triphosphatase and guanylyltransferase activity, was the subject of an investigation by Bougie and Bisallion (85). It was discovered that RTP is a substrate for this enzyme, and it was shown that a covalent RTP-enzyme complex can form. The biochemical transfer of RMP to RNA was also demonstrated. Compared to uncapped RNAs, ribavirin-capped RNAs were more resistant to destruction.However, the interaction of the 7-methyl group with eIF4E makes the usual cap structure required for translation. Ribavin was unable to add a 7-methyl group by the vaccinia virus capping machinery, which prevented ribavin-capped RNAs from being translated effectively. Although ribavirin was a poor GTP competitor in a guanylyltransferase experiment, it may have a stronger effect in vivo when used to inhibit IMPDH. The NS5MTaseDV (NS5 RNA 20 -O-methyltransferase domain) of the Dengue virus was studied by Benarroch and colleagues in their study (86).

With an IC50 of 100 mM, RTP reduced the activity of RNA 20 -Omethyltransferase in vitro. RTP was also demonstrated to compete with NS5MTaseDV for the GTP-binding site, with an apparent Kd in the vicinity of 50 mM. RTP was found at the GTP-binding site of the enzyme, but with an unexpected orientation, according to an X-ray crystal structure of RTP bound to the protein. RTP imitates GTP in this instance by rotating the NH2 group of ribavirin so that it is superimposed on the NH2 group at the 2-position of GTP. In contrast to earlier theories that claimed ribavirin resembled the 1- and 6-position of guanosine, this orientation is distinct (for

instance, the crystal structure of ribavirin complexed with IMPDH or models of base-pairing with cytidine).

The data covered above lead to the conclusion that ribavirin may interact with the RNA capping mechanism to exert its antiviral effect. The action may result from ribavirin being included as the 50 cap, rendering the molecule non-functional for translation, or from suppression of the enzymes responsible for attaching a 7-methylguanosine cap to viral RNA. This method might be effective against viruses that use caps on their genomes or transcripts. Since many viruses that are sensitive to ribavirin do not use a cap structure during infection, it is unable to explain the broadspectrum activity of the drug.

Ribavirin is a Polymerase inhibitor

RTP is ribavirin's main intracellular metabolite. This nucleotide may interact with viral polymerases to prevent the production of nucleic acids. RTP buildup within cells may enable effective competition with vital GTP or ATP pools. In vitro RTP suppression of influenza virus RNA polymerase was established by Eriksson and colleagues (87). Ribavirin and RMP didn't exhibit any inhibitory activity. RTP was demonstrated to function as a competitive inhibitor for both ATP and GTP. Additionally, it has been proposed that RTP specifically prevents reovirus RNA synthesis.An in vitro experiment for inhibition appeared to be unaffected by the concentration of the natural nucleotides used, indicating that no competitive mechanism was at work. According to the authors, RTP could bind nearby the active site and alter conformation. When ribavirin was added in the template, the HCV polymerase's in vitro elongation activity was similarly shown to be decreased (88). Vesicular stomatitis virus has also been linked to polymerase inhibition (89). As an HIV-1 reverse transcriptase inhibitor, ribavirin has also been researched. Both RTP and RDP prevented HIV-RT from elongating in an in vitro extension experiment, whereas RDP did so with an approximately 40% larger amount of inhibition. No sign of a chain termination was found. Noteworthy is the fact that this is a measure of DNA synthesis inhibition by a ribonucleotide.

Ribavirin is a lethal mutagen of RNA virus genomes

It was hypothesized that the activity of ribavirin might be due to the nucleotide's incorporation into RNA by cellular or viral RNA polymerases when it was discovered that RTP was the predominant intracellular metabolite of ribavirin. Early studies utilizing radiolabelled ribavirin, however, were unable to find any appreciable incorporation of ribavirin into RNA molecules. Ribavirin incorporation into RNA during virus replication was demonstrated in 2000 by Crotty and colleagues (90).The rate of incorporation of ribavirin triphosphate by the poliovirus RNA-dependent RNA polymerase was evaluated using an in vitro primer-extension test (RdRp). The rate at which ribavirin was incorporated was about equivalent to that of an erroneous nucleotide, which indicates that only one or two molecules should have been incorporated on average per

RNA genome with a 7500-nucleotide length. The finding that ribavirin could be equally effectively templated by uridine or cytidine was of greater significance.

Furthermore, the inclusion of either CMP or UMP may be controlled by the presence of ribavirin in the RNA template. Ribavirin can therefore imitate either of the natural purines in ambiguous basepairing (guanosine normally base-pairs with cytidine while adenosine normally base-pairs with thymidine (or uracil)). The rotation of the pseudobase's carboxamide moiety, which creates hydrogen bond acceptor/donor sites good for interaction with either of the pyrimidine bases, is likely the cause of this confusedbasepairing capacity (Figure 12).

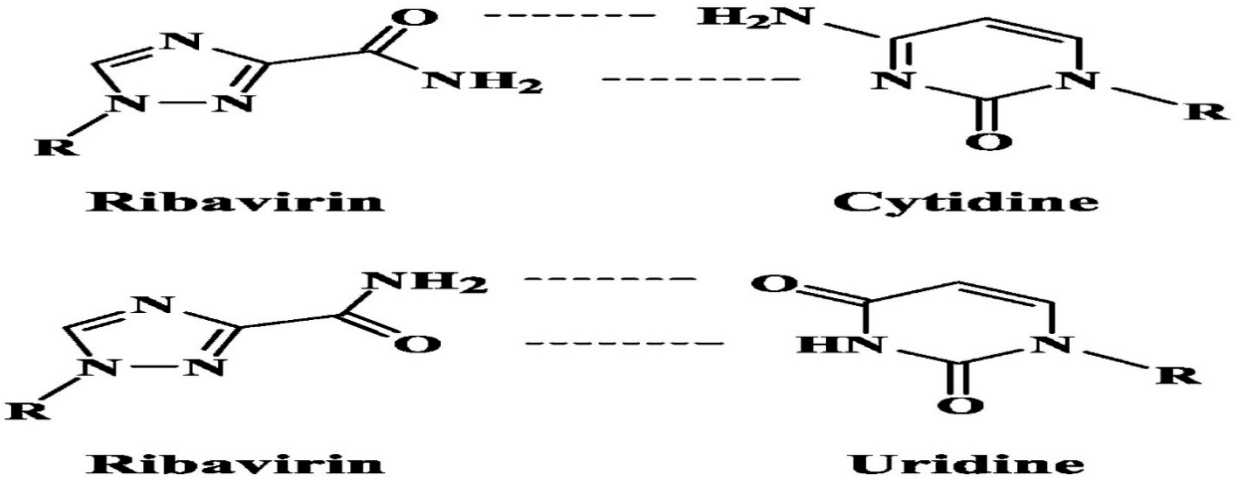

Figure 12. A purine mimic with unclear hydrogen bonds is ribavirin. Two different basepairing orientations can be produced by rotating the pseudobase's carboxamide molecule, permitting inclusion opposite either of the naturally existing pyrimidines.

The possibility of ribavirin being mutagenic to RNAs that it is integrated into was highlighted by the fact that it can function as an ambiguous purine base analogue. Ribavirin should cause transition mutations, according to in vitro incorporation studies (A-to-G and C-to-U). Crotty and colleagues sequenced the capsid-coding areas of poliovirus cultured in the presence of various ribavirin concentrations to test this theory. A rise in mutations, particularly the anticipated transition mutations, was found through sequencing.The possibility of ribavirin being mutagenic to RNAs that it is integrated into was highlighted by the fact that it can function as an ambiguous purine base analogue. Ribavirin should cause transition mutations, according to in vitro incorporation studies (A-to-G and C-to-U). Crotty and colleagues sequenced the capsid-coding areas of poliovirus cultured in the presence of various ribavirin concentrations to test this theory. A rise in mutations, particularly the anticipated transition mutations, was found through sequencing.Such a population's genetic heterogeneity might help individuals adjust to environmental changes like tropism, immunological responses, or antiviral medication more

quickly. But according to the quasispecies idea, there is a cap on genome variability known as the error threshold, beyond which more mutations would be harmful to the population. Lethal mutagenesis is the name given to an antiviral tactic in which the population is compelled to exceed the error threshold (91).

Pharmacology of Ribavirin

According to pharmacokinetic investigations, ribavirin had a mean bioavailability of 52% 22% and a mean half-life of 37 14 h in healthy volunteers who received 150 mg of intravenous 13C3-ribavirin followed by a 400 mg oral dose after 1 h (preston). Since ribavirin has a high volume of distribution and an elimination that depends on renal function, it takes longer than 4 weeks for concentrations to stabilize (morello, bruchfeld, paroni). Ribavirin is activated after entering cells by intracellular phosphorylation into mono-, di-, and triphosphates. Studies conducted in vitro have demonstrated that polyphosphorylation permanently retains ribavirin in erythrocytes, causing intracellular levels to exceed 550 M as opposed to plasma values of 15 M (92).Plasma concentrations of ribavirin of approximately 8-12 mol/l (2-3 g/ml) are reached at steady state, which frequently occurs after more than 4 weeks of treatment in patients with normal renal function receiving weight-based ribavirin dosing for HCV infection, that is, 1 g (75 kg bodyweight) or 1.2 g daily (75 kg bodyweight) divided into two oral doses. Although the ideal ribavirin target trough concentration is yet unknown, it should be highlighted that toxicity skyrockets at plasma concentrations more than 15 mol/l (93, 94).

Despite this, far greater ribavirin dosages are advised for the treatment of several viral illnesses that pose a life-threatening threat, such as Lassa hemorrhagic fever (95).
Ribavirin is mostly excreted by the kidneys, and individuals with renal impairment have significantly changed ribavirin pharmacokinetics (96). Despite this, ribavirin has been used to treat a number of individuals who have continuing hemodialysis or renal failure. However, the beginning of therapy with a decreased dose, depending on the severity of kidney impairment (97), as well as ongoing monitoring of plasma ribavirin concentrations and hemoglobin levels, are prerequisites for the use of ribavirin in this situation.

Adverse effects of Ribavirin

Hemolytic anemia is the main side effect of ribavirin, with a mean drop in hemoglobin of roughly 20 g/l after treatment (97). According to some theories, the secondary cause of this hemolysis is oxidative stress brought on by the erythrocytes' decreased levels of adenosine triphosphate (ATP) (98, 99). Ribavirin is not recommended in patients who have a history of or are currently experiencing cardiac disease due to the hazards associated with anemia. Ribavirin is linked to anemia, a higher incidence of rash, itching, and coughing, as well as neuropsychiatric side effects such sleeplessness (97). Ribavirin is not recommended for use during pregnancy or

breast-feeding due to the potential teratogenicity shown in all animal models examined. Although preliminary analysis of the "Ribavirin Pregnancy Registry" established in 2003 has not been able to demonstrate any clear evidence for human teratogenicity for ribavirin, pregnancy avoidance is still advised for treated women for 4 months after ribavirin exposure and for female partners of treated men for 7 months (100).

Inosine monophosphate dehydrogenase is inhibited by RMP at lower doses (10 M), leading to GTP depletion with subsequent effects on mRNA capping, G-protein signaling, ribosomal activity, immunological modulation (e.g., favoring Th1 over Th2 responses), etc. Viral RdRp may be directly inhibited by RTP. RTP alters the viral genome at doses as high as 100 mM.ITPase is able to dephosphorylate RTP into RMP. Hemolytic anemia could result from ribavirin entering erythrocytes, which is possible. Ribavirin triphosphate is referred to as RTP rather than RMP.

Conclusion

There is growing evidence that the antiviral impact of ribavirin, a special guanosine analog that works against a wide variety of viruses, is the result of numerous mechanisms of action. However, these several ways can differ depending on the virus under study and the ribavirin doses attained. In the future, ribavirin may have new applications, particularly when it is desirable to target both viral and host cell factors. Given that ribavirin has a somewhat lengthy half-life and takes a significant amount of time to reach steady state when taken orally, chronic viral infections rather than acute viral infections may be the most suitable scenario.

Future novel prospect

Ribavirin will probably continue to be a possible treatment for newly emerging viral illnesses because of its exceptional wide antiviral action. In the next 5 to 10 years, it's also extremely feasible that new indications may emerge, particularly for viral infections where it makes sense to target both viral and host responses. Given the prolonged period required to reach steady state ribavirin concentrations when given orally, this may be most suitable in situations involving persistent viral infections where there are few alternative therapeutic options. Ribavirin is also being researched more and more as a cancer treatment option.

Executive summary

Ribavirin is a unique guanosine analog with broad-spectrum activity against many viruses including: Hepatitis C virus, Hepatitis E virus, Respiratory syncytial virus, Lassa virus, Crimean–Congo hemorrhagic fever virus, Poliovirus and Influenza virus.

Pharmacology: Ribavirin: Has a long half-life resulting in more than 4 weeks to reach steady state ribavirin concentrations when administered orally in healthy individuals. Double dosing for 1 initial week may result in faster achievement of steady state.Has a large distribution volume.Has an elimination that is dependent on renal function requiring dose reduction in

patients with renal impairment.Has adverse effects including hemolytic anemia, itching, rash, cough and insomnia.Ribavirin triphosphate is dephosphorylated to ribavirin monophosphate by inosine triphosphate pyrophosphatase. Reduced inosine triphosphate pyrophosphatase activity results in decreased anemia, lower plasma but higher intracellular ribavirin concentrations, and improved HCV therapeutic outcome.

Modes of action: Inhibition of inosine monophosphate dehydrogenase (IMPDH) resulting in intracellular GTP depletion occurs at lower ribavirin concentrations (10 μM ribavirin). Reduced intracellular GTP secondarily may result in: Inhibition of mRNA capping, Impact on host cell gene expression, inflammation and immunomodulation, Inhibition of viral RdRPs.Enhancement of viral mutagenesis, which occurs at higher ribavirin concentrations *in vitro* (≥100 μM), by means of the incorrect substitution of ribavirin triphosphate for GTP. Ribavirin triphosphate can form two hydrogen bonds with UTP or CTP with equal efficiency, resulting in an increase in G-to-A and C-to-U single nucleotide variations.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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