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REVIEW

Beneficial Efficacy of Aloe Vera to Viral Infections: Case Reports of Kampo Medicine With Aloe Vera Juice

Akira Yagi¹, PhD; Megumi Hasegawa²; Suzuka Ataka³, MD, PhD

1 Editor in-Chief of Journal of Gastroenterology and Hepatology Research; Professor Emeritus, Fukuyama University, Hiroshima, Japan;

2 Pharmacist, Kampo Pharmacy Grace-Meg-Salon, Toshima-ku, Tokyo, Japan;

3 Department of Neurology, Osaka City University, Medical School, Part time Lecturer; The director of Med Cell Clinic, Osaka, Kita-ku, Umeda, Osaka, Japan.

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Correspondence to: Akira Yagi, 2-10-1 Hanagaura-ku, Kasuyamachi, Kasuya-gun, Fukuoka-ken, Japan 811-2310.

Email: 0131akirayagi@gmail.com

Telephone: +81-92-938-2717

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ABSTRACT

We discussed evident effects of aloe-emodin and aloe polysaccharide on the therapy of viral infections and epigenetic factor butyrate that is involved in latency and reactivation in viral infection. Furthermore we presented eight case reports of *Kampo* medicine with Aloe vera juice (AVJ) to virus infections. *Kampo* medicine with AVJ-successive ingestion demonstrates to be suitably personalized medicine for virus infections and improves gut-microbial ecosystem in host. The potential *Kampo* medicine with AVJ may provide fresh insights as an

anti-virus medicine for homeostatic regulation in host.

Key words: Antiviral infection; Aloe-emodin; Epigenetic butyrate; case reports; *Kampo* medicine; Aloe vera juice ingestion

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INTRODUCTION

Viruses are obligated to intercellular parasites, relying to a major extent on the host cell for replication. A combination of a lytic infection characterized by the release of new progeny virus particles and a latent phase where the virus is “quiescent” (a state in which the virus is not replicating) falls under the umbrella of a persistent infection. Reactivation may be provided by a combination of external and/or internal cellular stimuli. Understanding this mechanism is essential in developing future therapeutic agents against viral infection and subsequent disease.

In our previous paper we described the importance of butyrate and host-miRNA microbiota interaction in regulation of gut immunity, and demonstrated butyrate is capable of facilitating induced-pluripotent stem cell germination, suggesting an important step for the establishment of pluripotency⁽¹⁾.

Aloin (barbaloin) is inactive as a laxative but activated to aloe-emodin anthrone to transform to a strong purgative component by a human intestinal anaerobe *Eubacterium* sp. International Aloe Science Council has established the quantity standard of < 10 ppm of aloin showing carcinogenic activity in aloe vera juice products for safe oral consumption.

We will describe beneficial efficacy of aloe-emodin from aloe vera extract and butyrate fermented by aloe vera endophytic bacteria to virus infection. And eight case reports on administration of *Kampo* medicine with aloe vera juice to virus infection are discussed.

ANTI-VIRAL ACTIVITY OF ALOE VERA ANTHRAQUINONES; ALOE-EMODIN, ALOIN (BARBALOIN; C-GLYCOSIDE OF ALOE-EMODIN ANTHRONE), AND EMODIN

Inactivation of enveloped viruses by anthraquinones

Several ingredients in *Aloe vera* have been shown to be effective antiviral agent. Sydiskis *et al*^[2] reported that anthraquinones extracted from Aloe vera leaf with hot glycerin are responsible for exhibiting the antiviral properties. This action may be direct due to aloe-emodin, de-glycosylated aloin, and indirectly due to stimulation of immune system. It prevents virus adsorption and replication. Aloe-emodin is reported to be virucidal to Herpes simplex virus (HSV) type 1 and 2, Varicella zoster virus, Pseudorabies virus, and Influenza virus. It directly affects both DNA- and RNA-containing enveloped viruses but has no effect on naked (un-enveloped) viruses. Parvez *et al*^[3] demonstrated that aloe-emodin appears as the most promising anti-herpes B natural drug with CYP3A4 (cytochrome p450) activity property towards its enhanced therapeutic efficacy. Li *et al*^[4] speculated the mechanism behind aloe-emodin based inactivation of influenza virus. Aloe-emodin appears to have interfered with the casein kinase 2, TLR4, NF- κ b, galectin-3, STAT1 and IFN- γ pathways. One or more these pathways could be instrumental in inhibition of virus attachment, virus penetration, mRNA synthesis, and virus release, leading to inhibition of influenza virus.

Anthraquinone derivatives, including barbaloin, aloe-emodin and emodin have been shown to inhibit several viruses *in vitro*^[2]. However, it is unlikely that systemic antiviral effects would follow from the ingestion of these compounds, due to their low bioavailability. Borges-Argaez *et al*^[5] investigated that aloesaponarin-1 and -2, isolated from Aloe vera roots, and six derivatives were obtained by methylation, acetylation and O-glycosyl reactions starting from aloesaponarin-1. They highlighted the tetra-O-acetyl- β -D-glucopyranosyl substituent at C3-position of aloesaponarin-1^[6] might be an effective against the influenza A H1N1 virus based on antiviral assays.

Zika virus (ZIKV) has been associated with serious health conditions, and an intense search to discover different ways to prevent and treat ZIKV is underway. Batista *et al*^[7] found that emodin and berberine trigger a virucidal effect on ZIKV. When the virus was expressed to 160 μ M of berberine, a reduction of 77.6% in the infectivity was observed, when emodin was used 40 μ M, this reduction was approximately 83.3%. Dynamic light scattering data showed that both compounds significantly reduce the hydrodynamic radius of virus particle in solution.

Anti-severe acute respiratory syndrome (SARS) coronavirus 3C-like protease effects of aloe-emodin and emodin

The 3C-like protease (3CLpro) of SARS-coronavirus mediates the proteolytic processing of replicase polypeptides 1a and 1ab into functional proteins, becoming an important target for the drug development. Lin *et al*^[8] investigated several plant-derived phenolic compounds for anti-SARS-CoV 3CLpro effects using cell-free and cell-based cleavage assays. Two phenolic compounds aloe-emodin and hesperidin dose-dependently inhibited cleavage activity of the 3CLpro, in which the IC₅₀ was 366mM for aloe-emodin and 8.3mM for hesperetin (aglycone of hesperidin) in the cell-based assay.

The antiviral activity of aloe-emodin against Japanese encephalitis virus (JEV) and enterovirus 71 (EV71) was evaluated by Lin *et al*^[9] using dose- and time-dependent plaque reduction assays in HL-CZ

cells and TE-671 cells. The 50% inhibitory concentration of aloe-emodin ranged from 0.50 μ g/mL for JEV and from 0.14 μ g/mL to 0.52 μ g/mL for EV71. Aloe-emodin showed clearly potent virus inhibitory abilities and achieved high therapeutic indices, in particular for HL-CZ cells.

Drugs that inhibit the ion channel formed by the 3a protein can be expected to inhibit virus release, and would be a source for the development of novel therapeutic agents. Several acute respiratory syndromes (SARS) are an emerging infectious disease caused by a novel coronavirus (SARS-CoV). SARS-CoV spike protein, a type 1 membrane-bound protein, is essential for the viral attachment to the host cell receptor angiotensin-converting enzyme 2(ACE2). Emodin significantly blocked the S protein and ACE2 interaction in a dose-dependent manner. It also inhibited the infectivity of S protein-pseudotyped retrovirus to Vero E6 cells. These findings suggested that emodin may be considered as a potential lead therapeutic agent in the treatment of SARS^[10].

Schwarz *et al*^[11] demonstrated that emodin can inhibit the 3a ion channel of coronavirus SARS-CoV and HCoV-OC43 as well as virus release from HCoV-OC43 with a K_{1/2} value of about 20 μ M. The authors suggested that viral ion channel may be a good target for the development of antiviral agents.

In vitro and in vivo effect of aloin against influenza virus infection

Aloin is a potent anti-influenza compound that inhibits viral neuraminidase activity, even of the oseltamivir-resistant influenza virus (Tamiflu TM, influenza A: H1N1 pdm09). With suppression of the virus machinery, aloin boosts host immunity with augmented hemagglutinin-specific T cell response to the infection. In addition, in the context of compromised benefit with delayed oseltamivir treatment, adjuvant aloin treatment ameliorates the disease and improves survival. Taken together, aloin has the potential to be further evaluated for clinical application in human influenza^[12].

The interaction with model membranes of barbaloin and emodin

In order to explain barbaloin and emodin effects in biological membranes Alves *et al*^[13] investigated a higher affinity for phospholipid membranes. Emodin showed a higher affinity for phospholipid membranes than barbaloin did, and was more effective in weakening hydrophobic interaction between hydrocarbon chains in phospholipid bilayers. Membrane disruption by emodin or barbaloin showed specificity for the two major phospholipid present in bacterial membranes; phosphatidyl-ethanolamine and phosphatidyl-glycerol. In order to relate their strong effects on membranes to their biological activity, the capability of these compounds to inhibit the infectivity of the viral haemorrhagic septicaemia rhabdovirus, a negative RNA enveloped virus, or the growth of *Escherichia coli* was tested. Both anthraquinones showed antiviral activity but only emodin was a virucidal agent.

EPIGENETIC FACTOR BUTYRATE THAT IS INVOLVED IN LATENCY AND REACTIVATION IN VIRAL INFECTION

Neumann *et al*^[14] presented a uniform, rapid, and reliable method of *in vivo* herpes simplex virus (HSV-1) reactivation in mice that yields high reaction frequencies by using sodium butyrate, and demonstrated that the reactivating virus can be detected at the original site of infection. During HSV-1 latency in mouse dorsal root ganglia (DRG), chromatin associated with the latency-associated

transcript (LAT) region of the viral genome is hyper-acetylated at lysine 9 and 14 of histone [H3(K9, K14)], while lytic genes are hypo-acetylated. Explanted DRG exhibit a pattern of deacetylation of the LAT enhancer followed by acetylation of the intracellular protein (ICP0) promoter at early times post-explant. Neumann *et al*^[15] assessed the effect of sodium butyrate on the chromatin patterns latent and butyrate-treated mouse trigeminal ganglia via chromatin immune-precipitation. The authors detected deacetylation of acetyl H3 (K9, K14) of the latency-associated transcript (LAT) promoter and LAT enhancer regions as early as 0.5 h post-butyrate treatment, and this deacetylation corresponded to an increase in the acetylation of the lytic promoters ICP0 and ICP4 at 0.5 h and 1 h post-butyrate treatment, respectively.

Butyrate-producing bacteria live in the colon and are primarily utilized by colon cells as a major energy sources in human immunodeficiency virus (HIV)-induced subjects

In the mitochondria of colon cells, 70% to 90% of butyrate is oxidized into acetyl-CoA, which is subsequently processed through the TCA cycle to generate a large quantity of ATP. Histone deacetylase inhibitors are studied in HIV-infected adults as latency reversing agents as a way to eradicate the latent HIV reservoirs by Rasmussen *et al*^[16]. Reduction in mucosal butyrate from diminished colonic butyrate-producing bacteria may exacerbate pathobiont-driven gut T-cell activation and HIV replication, thereby contributing to HIV-associated mucosal pathogenesis.

In an earlier paper we discussed the mechanism of how gut microbiota is involved in the host inflammatory processes that influence human immunodeficiency virus replication and beneficial efficacy of butyrate to human immunodeficiency virus-induced subjects^[17].

REGULATION OF HERPESVIRUS REACTIVATION BY HOST MICRORNA

Recent studies have proposed the interesting perspective that viral gene expression is downregulated by host microRNA (miRNA), small non-coding RNAs well known as post-transcriptional gene regulators. Watanabe *et al*^[18] listed 62 possible human miRNA-targeted viruses from 6 families, most of which consisted of single-strand RNA viruses. These results suggested that miRNAs extensively mediate antiviral defenses in human. In mammalian system, miRNA are associated with numerous pathological and physiological pathways. MiRNAs are important in many viral infections, with different viral families expressing their own miRNAs, manipulating host miRNA expression or showing direct or indirect regulation by host or viral miRNAs.

Cellular miRNA have been involved in the regulation of nearly all cellular pathways, and their degeneration has been associated with several diseases such as cancer. Given the importance of miRNA to cell homeostasis, it is no surprise that viruses have evolved to take advantage of this cellular pathway. Viruses have been reported to be able to encode and express functional viral miRNA that target both viral and cellular transcripts. Viruses have developed multiple mechanisms to avoid being targeted by cellular miRNAs. This complex interaction between host and viruses to control the miRNA pathway usually favors viral infection and persistence either by reducing immune detection, avoiding apoptosis, promoting cell growth, or promoting lytic or latent infection. One of the best examples of this virus-host-miRNA interplay emanates from members of the *Herpesviridae* family, namely the herpes simplex virus type 1 and type 2, human cytomegalovirus, human herpesvirus

8, and the Epstein-Barr virus. Piedade *et al*^[19] focused on the general functions of miRNAs and the interactions between herpesviruses, human hosts, and miRNAs and delved into the related mechanisms that contribute to infection and pathogenesis.

THE ROLE OF miRNAs IN VIRAL INFECTION

Roberts *et al*^[20] examined the current evidence for interplay between the miRNA pathway and viral infections in mammals. In mammalian systems miRNA are associated with numerous pathological and physiological pathways. MiRNA are important in many viral infections with different viral families, expressing their own miRNAs, manipulating host miRNA expression or showing direct or indirect regulation by host or viral miRNAs.

Marek's disease virus-1 (MDV) is an oncogenic avian herpes virus. Small RNA profiling studies have suggested that miRNAs are involved in viral latency. Sodium butyrate treatment is known to induce herpes virus reactivation. Hicks *et al*^[21] determined transcriptome and miRNome changes induced by sodium butyrate in 2 MDV-transformed cell lines, RP2 and CU115. The outcomes of the current study suggested that cellular DNA replication and damage responses, as well as cell cycle progression pathways, are some of the main players in MDV reactivation by sodium butyrate treatment. These pathways are targeted by a number of cellular and viral miRNAs, whose expression is reciprocally altered by sodium butyrate treatment.

ANTI-VIRAL ACTIVITY OF ALOE VERA POLYSACCHARIDES; ACEMANNAN

Reduction of Herpes simplex infection by Acemannan

Acemannan has a reducing effect in Herpes simplex infection as was shown in a study with two cultured target cell lines. Aloe lectins have the potential to directly inhibit the cytomegalovirus proliferation in cell culture, possibly by interfering with its protein synthesis^[22,23]. The clinical study suggested that aloe vera extract (0.5%) in a hydrophilic cream is more efficacious than placebo, thus supporting the benefits of aloe vera therapy as an alternative, reliable and effective treatment to cure the first episodes of genital herpes in men^[24]. Zandi *et al*^[25] investigated that the extract from aloe vera gel showed antiviral activity against herpes simplex type 2 (HSV-2) not only before attachment and entry of virus to the Vero cells but also on post attachment stages of virus replication. The IC₅₀ before attachment and entry of virus to the cells is 428µg/ml and the CC₅₀ value which is the cytotoxicity of the extract on post attachment stages of replication is 536µg/ml and the S1 value for inhibition of the post attachment stages of HSV-2 replication is 6.04. The herpes simplex virus type 1 genome establishes latency in sensory neurons, where it exists as a circular episome (a genetic determinant that can replicate autonomously in bacterial cytoplasm or as an integral part of the chromosome) associated with histones.

Reduction of Anti-Herpes simplex virus (HSV) infection: One of the most common and debilitating oral diseases.

Rezazadeh *et al*^[26] found that *Aloe vera* in prearranged concentrations was observed one hour after the Vero cell was infected with HSV-1. The findings showed significant inhibitory effect of 0.2-0.5% aloe vera gel extracts on HSV-growth in Vero cell lines. Therefore the gel could be a useful topical treatment for oral HSV-1 infections without any significant toxicity.

Ultrasound extraction extruded the choicest antiviral compounds,

aloin and aloe-emodin and led to exceptionally high antiviral activity recorded only in the cytotoxic solvent options such as methanol and ethanol but also in the non-cytotoxic solvent, water. This work is intended to arouse pharmaceutical interest in this direction for harnessing the rich reservoirs in *Aloe vera* through ultrasound assisted extraction^[27].

Sun *et al.*^[28] examined effect of aloe polysaccharide (APS) on chicken immunity indexes; 40 mg/mL of APS has excellent effect of immunity enhancement; APS can serve as efficient immunologic potentiator and adjuvant.

Influenza A virus (IAV) causes periodic outbreak and seriously threatens human health

Aloe polysaccharides from aloe vera leaf gel have evident effects on the therapy of virus infection. Sun *et al.*^[29] refined polysaccharides (APS) from aloe vera leaf gel. In vitro test revealed that APS could inhibit the replication of a H1N1 subtype influenza virus, and the most obvious inhibitory effect was observed in the viral adsorption period. Transmission electron microscopy indicated that APS directly interacted with influenza virus particles. Experiments on H1N1 virus infection in mice demonstrated that APS considerably ameliorated the clinical symptoms and the lung damage of the infected mice, and significantly reduced the virus loads and mortality. The direct interaction between APS and influenza A virus IAV is a likely mechanism of inhibiting viral infection.

The effect of acemannan (polysaccharides) in cats infected with feline immune-deficient virus

As an antiviral agent, acemannan (ACM) has demonstrated antiviral activity in vitro against human immune-deficiency virus, New castle disease virus and influenza virus. In addition, ACM has been shown to inhibit acquired immunodeficiency syndrome virus in vitro, and an injectable form has been found to be of benefit in feline immunodeficiency virus infected cats. ACM possess antiviral activity by modifying glycosylation of both virally infected cells and glycoprotein coats of viruses, thus inhibiting virus replication and infectivity^[30]. ACM was approved by United State Department Agriculture for use as a vaccine adjuvant in chickens, cats and dogs. Shahid *et al.*^[31] investigated the ameliorative effects of aloe vera gel aqueous extract on Newcastle disease virus in broilers.

Aloe vera and its components inhibiting influenza a virus-induced autophagy and replication

Choi *et al.*^[32] confirmed that aloe vera ethanol extract (AVE) treatment significantly reduces the viral replication of green fluorescent protein-labeled influenza a virus in Madin-Darby canine kidney (MDCK) cells. In addition, post-treatment with AVE inhibited viral matrix protein 1 (M1), matrix protein 2 (M2), and hemagglutinin (HA) mRNA synthesis and viral protein 1 (M1, M2, and HA) expressions. The authors demonstrated AVE inhibited autophagy induced by influenza a virus in MDCK cells, and also identified quercetin, catechin hydrate, and kaempferol as the active components of AVE. Furthermore, the authors found that post-treatment with quercetin, catechin hydrate, and kaempferol markedly inhibited M2 viral mRNA synthesis and M2 protein expression. A docking simulation suggested that the binding affinity of quercetin, catechin hydrate, and kaempferol for the M2 protein may be higher than that of known M2 protein inhibitors. Thus, the inhibition of autophagy induced by influenza virus may explain the antiviral activity of AVE against H1N1 or H3N2. AVE and its components may be potentially useful for the development of anti-influence agents.

Are miRNA important players in HIV infection?

Evidence is mounting that cellular miRNAs play important roles in viral replication, either usurped by virus to promote its replication or employed by the host to control viral infection by directly targeting the viral genome or by targeting cellular proteins required for productive virus replication.

Balsubramaniam *et al.*^[33] summarized the findings to date on the role of miRNA in HIV-1 biology.

The relation between HIV-1 infection and human miRNA expression profile has been previously investigated, and studies have shown that the virus can alter miRNA expression and vice versa.

Modai *et al.*^[34] found that human miRNA-186, 210 and 222 directly regulate the human gene Dicer 1, HRB and HIV-EP 2 thus may be filling key roles during HIV-1 replication and miRNA biogenesis. This finding may contribute to the development of new therapeutic strategies.

CASE REPORTS AND DISCUSSION

Case report 1: A woman about 20-years old infected with an early flu virus complains about joint pain and shows fever at 39.8 °C body temperature. She took *Kakkontokakikyosekko* with aloe vera juice (AVJ) for five days and well recovered.

Case report 2: A 30-years mother and 7-years son infected with an early flu virus complain about joint pain. The son was febrile at 39°C body temperature. They took *Kakkonto* with AVJ for 6 days and well recovered.

Case report 3: A woman about 40-years old infected with norovirus complains about nausea, vomiting, diarrhea and depression fatigue syndrome. She took *Kousosan* with AVJ for 10 days and well recovered.

Case report 4: A woman about 40-years old infected with severe flu symptom complains poor appetite, fever and depressed fatigue syndromes during 2 weeks. She took *Saikokeishito* with AVJ during 2 weeks, and well recovered.

Case report 5: About 18-years-female student infected with a flu virus complains sever cough and night sweat, depression and fatigue due to study. She took *Hochuikkito* with AVJ during 15 days, and recovered with well sleep and no cough.

Case report 6: A woman about 60-years old infected with flu virus complains lung dehydration with the symptoms of a dangerous complication of diarrhea and vomiting during one month. She took *Bakumondoto* with AVJ for 15 days, and she well recovered without cough.

Case report 7: A woman about 40-years old infected with flu virus complains heavy headache and joint pain and febrile at 38.5°C body temperature. She took *Kakukonto* and well cured joint pain but still loss of appetite and febrile at 38.0°C body temperature. Then she ingested AVJ 300ml/d for one week, and well recovered with good appetite.

Case report 8: A male about 50-years old infected with norovirus complains vomiting and diarrhea. He took *Kousosan* and well recovered in vomiting but still heavy diarrhea. Then he ingested AVJ 300ml/d for another day and well recovered.

Case 1-6 subjects with infected virus took *Kampo* medicine with successive ingestion of aloe vera juice (AVJ; aloe vera juice containing less than 10ppm aloin) 100-200ml/day during the time in virus infection. The subjects well recovered without any troubles.

In case reports 7 and 8 the subjects with infected virus were induced a remission with *Kampo* medicine, but still not fully ameliorated. After ingestion of AVJ 300 mL/day, they recovered fully. It was

clearly suggested that AVJ played a role of an adjuvant recovery from intestinal damages in the subjects with virus infection.

Kampo medicine is effective for the treatment of several infectious diseases such as viral hepatitis, influenza infection, methicillin-resistant *Staphylococcus aureus* and *Helicobacter pylori* infections, proved by basic and clinical studies. Some *Kampo* medicines show direct action against infected microbes, virus, and defensive action through immune-stimulation and immunomodulation.

In the present *Kampo* medical treatment it was possibly expected to show preventive effects in the patients with decreased immune action in transition to the serious stage of infectious disease. Furthermore each *Kampo* medicine with AVJ ingestion well improved gut-microbiome ecosystem in which gut microbiota help absorb the *Kampo* medicine, protected the subject against virus infection and trained immune system. It could be convinced that *Kampo* medicine works as a suitable personalized medicine to virus infection and AVJ may strongly support as an adjuvant for the subjects with virus infection.

SUMMARY

Aloe-emodin, de-glycosylated aloin, inactivated all of the viruses tested except adenovirus and rhinovirus^[2]. This prevents virus adsorption and replication, suggesting that aloe-emodin extracted from alo vera leaves is directly virucidal to enveloped viruses. Aloe polysaccharide could directly interact with H1N1 influenza virus particle to prevent virus particle adsorption.

In the present eight case reports beneficial roles of *Kampo* medicine with successive ingestion of AVJ to influenza virus and norovirus were clearly shown. *Kampo* medicine was demonstrated to be suitably personalized medicine to flu and novo virus infection and *Kampo* medicine with AVJ-successive ingestion markedly improved gut-microbiome ecosystem in host. Thus potential efficacious *Kampo* medicine with aloe-emodin, emodin, butyrate fermented and aloe-polysaccharide in AVJ may provide fresh insights as an anti-virus medicine for homeostatic regulation in host.

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