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Electro-magnetic Pulser *p.5*

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Inhibition of Dengue and other Enveloped Viruses by Metadichol®, a Novel Nano Emulsion Lipid

Abstract

Background: Metadichol[1,2] is a Nano emulsion of long-chain alcohols found in many foods. It is commonly called Policosanol and is present in foods such as rice, sugar cane, wheat, peanuts[3]. Metadichol acts on Nuclear Vitamin D receptors (VDR)[2] that are present in cells throughout the body to stimulate the immune system and inhibit a variety of disease processes, including those resulting from viral infections[4].

Methods: We had two patients diagnosed with Dengue Fever in SE Asia who volunteered to be treated with Metadichol®. Based on the positive outcome, we then tested for antiviral activity of Metadichol® in Vero and MDCK cells infected with Dengue, Ebola, Marburg, Influenza A (H1N1), Chikungunya and Human Respiratory Syncytial viruses. In addition, we tested the efficacy of Metadichol® in preventing cell death caused by Adenovirus, Tacaribe Mammarena virus, Rift Valley Fever virus, SARS coronavirus, Japanese Encephalitis virus, West Nile virus, and Yellow Fever virus.

Findings: Metadichol rapidly helped the two dengue patients with declining platelets to fully recover from Dengue Fever within a few days. In the in vitro assays, Metadichol showed no cytotoxicity and strongly inhibited cell death caused by each of the viruses tested.

Interpretation: Metadichol is a safe and effective inhibitor of enveloped viruses in humans. Since it is known to bind to the vitamin D receptor (VDR)[2], its mechanism of action likely involves the competitive displacement of virus particles from VDR's on host cell membranes. Metabolism studies of long chain alcohol in fibroblasts suggest that very long chain fatty alcohols, fatty aldehydes, and fatty acids are reversibly interconverted in a fatty alcohol cycle[3]. Because it consists of natural components of common foods and has no known negative side effects, Metadichol has the potential to serve as a novel, broad-spectrum antiviral treatment for Dengue, Ebola, Zika, H1N1, SARS,MERS Chikungunya and other enveloped viruses.

Key words: Ebola, Dengue, Chikungunya, H1N1, Respiratory Viruses, Metadichol

Introduction

Dengue viral infection is one of the most important mosquito borne diseases in the world. It may be asymptomatic or may give rise to undifferentiated fever, dengue fever, dengue haemorrhagic fever (DHF), or dengue shock syndrome. Annually, 100 million cases of dengue fever and half a million cases of DHF occur worldwide. Ninety percent of DHF subjects are children less than 15 years of age. Currently, dengue is endemic in 112 countries in the world[5,6].

Most antiviral compounds block replication processes that are common to a variety of viruses and host cell types. Such compounds are potentially toxic, mutagenic, or teratogenic for the host and can induce drug-resistant mutant viral sub strains. Consequently, it is important to identify more effective new antiviral compounds that do not have deleterious side effects. Metadichol® is a Nano emulsion of long-chain alcohols, which are found in many foods including fruits, vegetables, and grains. Metabolism studies of long chain alcohol in fibroblasts suggest that very long chain fatty alcohols, fatty aldehydes, and fatty acids are reversibly interconverted in a fatty alcohol cycle[3]. Since the metabolites of long chain alcohols are interconverted, a single dosage even at low doses can theoretically have long lasting effects. Metadichol® acts on VDR (vitamin D receptor) that is present in all cells throughout the body to stimulate the immune system and inhibit a variety of disease processes, including those resulting from viral infections.

Presently, there is no drug for treating Dengue. Standard treatment is limited to electrolytic solutions, rest, measurement of body temperature, blood pressure, hematocrit, platelet count, and administration of antipyretics like paracetamol when the fever is too high. Recently a new vaccine has been introduced by Sanofi Aventis in Mexico.

Case study

Metadichol was seen to improve platelet counts in some cancer patients[2]. During the marketing phase of Metadichol in SE Asia, there were also multiple self-reported cases of dengue patients who had fully recovered from dengue fever within a few days of taking Metadichol. Based on that observation, we tested Metadichol in vitro on dengue and other viruses and found that Metadichol completely inhibited all the viruses tested. We also tested Metadichol on two volunteer patients in SE Asia who were diagnosed with dengue fever. Since Metadichol is a safe nutritional supplement with no toxicity, 5 mg of Metadichol was administered orally four times daily for 6 days to the two dengue patients (one female 34 years old and one male 35 years old). Patients were seen by a doctor daily and blood samples were drawn. With no effective treatment for dengue fever, both patients were not given any prescription drugs. In both cases, administration of Metadichol helped to reverse the platelet declining trend and improve the platelet and WBC counts to normal level within a few days. No side effects were reported by the 2 dengue patients. The results are shown in Figures 1a, 1b, 2a and 2b.

Results of in-vitro antiviral activity

The collection of all antiviral data was outsourced. Screening of BSL-2 viruses (CHIKV, influenza, DENV-2, and HRSV) was performed by IBT Bio services (Gaithersburg, MD, USA). Screening of filo viruses (Ebola, Marburg) was performed under BSL-4 conditions at US Army Medical Research and Materiel Command (USAMRIID), Fort Detrick, MD, USA. Adenovirus (A-549), Tacaribe Mammarena virus, Rift Valley Fever virus, SARS corona virus, Japanese Encephalitis virus, West Nile virus, and Yellow Fever virus testing was performed at Institute for Antiviral Research, Utah State University, Logan, Utah, USA.

The EC50 of Metadichol against Ebola (Mayinga) and Marburg virus (Musoke) was 8·75 µg/mL (2.2 nM) and 3·96 µg/mL(0.99nM) , respectively. The EC50 of Metadichol against DENV-2 (New Guinea C), CHIKV (181/25), and HRSV (A2) was 2·91 µg/mL(0.72nM), 3·54 µg/mL(0.88nM), and 0·41 µg/mL(0.10nM), respectively. (The EC50 of Metadichol against Influenza A (CA/07/09) was 5·44 µg/mL(1.26nM). For each of these viruses, replication was almost completely inhibited by Metadichol at concentrations above the EC50.

The EC50 of Oseltamivir against Influenza A (CA/07/09) was 11·0 µg/mL, and the inhibitory effect increased progressively with further increasing concentrations of Oseltamivir. The EC50 of Ribavirin against DENV-2 (New Guinea C) or HRSV (A2) was 332·3 µg/mL and 69·6 µM, respectively. The inhibitory effects of Ribavirin increased further at higher concentrations. The EC50 of 6-azauridine against CHIKV (181/25) was <0·1 µM, and the inhibitory effect of 6-azauridine declined at higher concentrations.

Metadichol at a concentration of 0·16 µg/mL(0.04 nM) reduced the cytopathic effects of Adenovirus, Rift Valley Fever virus, SARS coronavirus, Japanese Encephalitis virus, and West Nile virus by 100% and reduced the cytopathic effects of Yellow Fever virus by 96%. Higher concentrations of Metadichol reduced the cytopathic effects of each of those viruses to varying degrees (Table 1).

Discussion

Metadichol is a Nano emulsion of long-chain lipid alcohols (C-26, C-28 and C-30), which are commonly known as policosanols. It has a particle size of less than 60 nm. We have shown that it binds to the VDR as an inverse agonist[2]. It is the only known inverse agonist of VDR in medical literature.

Calcitriol (1,25-Dihydroxy Vitamin D) is the natural ligand for the VDR and acts as an agonist. Vitamin D is essential to the skeletal system[7] and recent evidence suggests that it also plays a major role in regulating the immune system, perhaps through the involvement in immune responses to viral infection[8,9]. Epidemiological studies suggest that vitamin D deficiency increases the risk for Dengue, influenza and other respiratory tract infections. Cell culture experiments support the hypothesis that vitamin D has direct antiviral effects, particularly against enveloped viruses. The antiviral mechanism of vitamin D may be due to the ability of vitamin D to up regulate the antimicrobial peptides LL-37 (cathelicidin) and human beta-defensin[10]. Human cathelicidin has been shown to affect several viruses including VV, RSV, influenza virus, HIV, HSV, DENV and Adenovirus via virus envelope disruption, and polymerase or protease inhibition[11].

Viruses have evolved strategies to exploit VDR and other receptors to regulate the expression of their genes and to optimize the cellular processes intrinsic to the viral life cycle. Persistent Epstein-Barr virus infection down regulates VDR >10 fold[11,12]. While the specific receptors targeted by viruses vary, they involve processes that directly or indirectly modulate receptor function. The specific receptor(s) targeted by a particular virus are likely to reflect the tissue tropism of the virus[13]. By binding to the VDR

(which is a key receptor for innate immunity and is present in all cells), Metadichol can displace viruses bound to it and block viral entry into host cells. The fact that Metadichol has inhibitory effects against many viruses suggests that viral binding to the VDR likely occurs and that Metadichol competitively disrupts this process. In addition to VDR binding, Metadichol shares cross-reactivity with other nuclear receptors[14], which may explain its activity against a wide range of viruses, bacteria and parasites [2].

Previous studies have demonstrated the antiviral activities of moderate-length saturated and unsaturated alcohols at mM concentrations[15]. Optimal antiviral activity is observed with saturated alcohols 10 to 12 carbons long; however, those compounds also exhibit cytotoxic and hemolytic effects. Less antiviral activity is observed with alcohols 14 to 18 carbons long; alcohols with longer chain lengths were not tested. Katz[16] showed that compositions of one or more aliphatic alcohols containing 27 to 32 carbons were suitable for intravenous or intramuscular injection into humans or mammals.

Conclusion

Metadichol is a product made from agricultural waste and is a renewable resource. It has the potential to serve as an antiviral molecule with a broad spectrum of activity, particularly given that its constituents (long-chain lipid alcohols) are present in foods commonly consumed on a daily basis and that it has demonstrated no toxicity at doses of up to 5000 mg/kg[17,18]. We have documented that Metadichol inhibits parasitic infections (Malaria) and also affects bacteria such as Methicillin-Resistant Staphylococcus aureus (MRSA)[2]. The potential of Metadichol is largely based on its safety and broad range of activity against parasites, bacteria and viruses. Metadichol may serve as a preventive agent for many tropical diseases given that, it strengthens innate immunity through VDR binding, and represent a first key step in preventing diseases. Metadichol is ready for large scale testing in regions that are ravaged by viruses, bacteria or parasites. Once proven on large populations, Metadichol could be used as a preventive nutritional supplement in countries where viral fevers are widely prevalent to reduce diseases burden safely and effectively.

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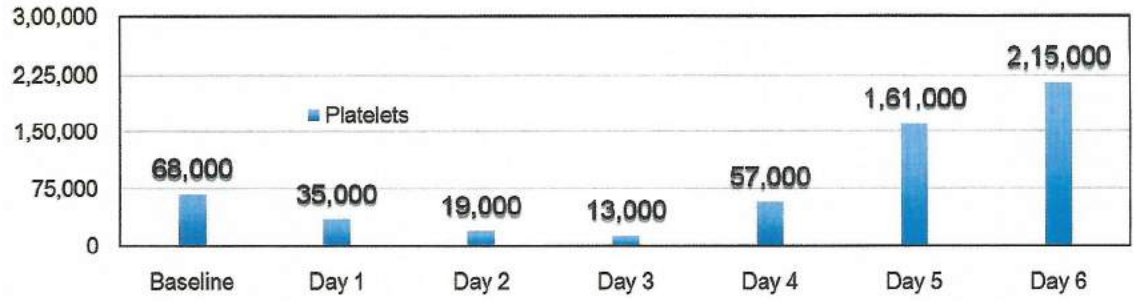


Figure 1a: Plot of Daily Platelet Count for Dengue Patient 1 (Female 34) treated with Metadichol

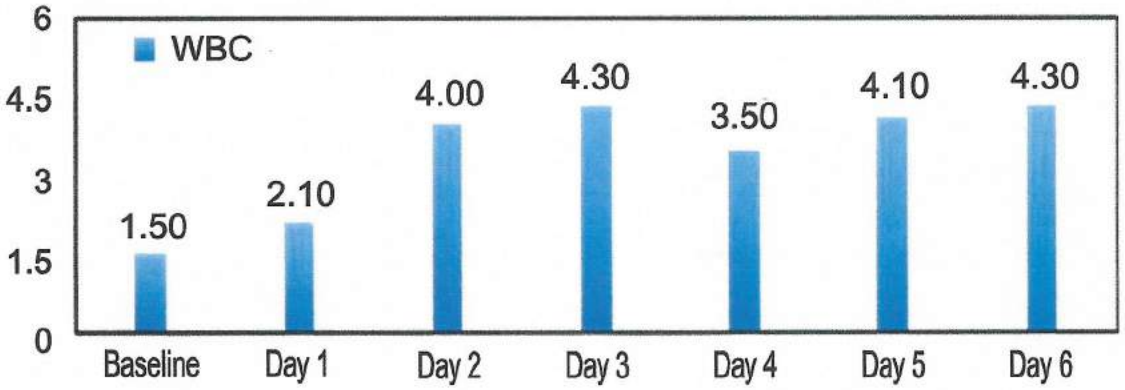


Figure 1b: Plot of Daily WBC for Dengue Patient 1 (Female 34) treated with Metadichol

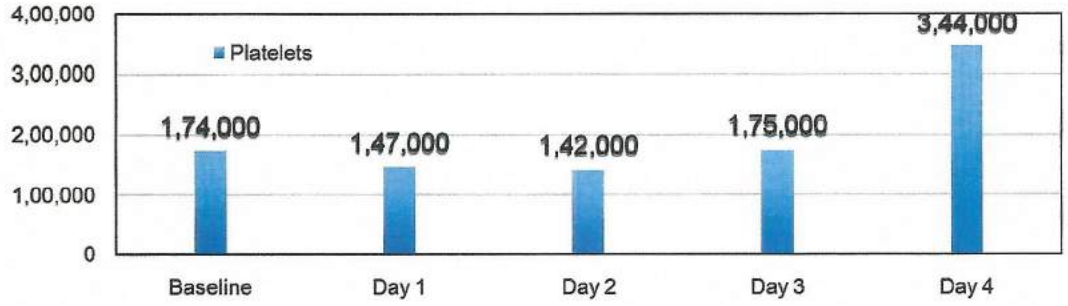


Figure 2a: Plot of Daily Platelet Count for Dengue Patient 2 (Male 35) treated with Metadichol

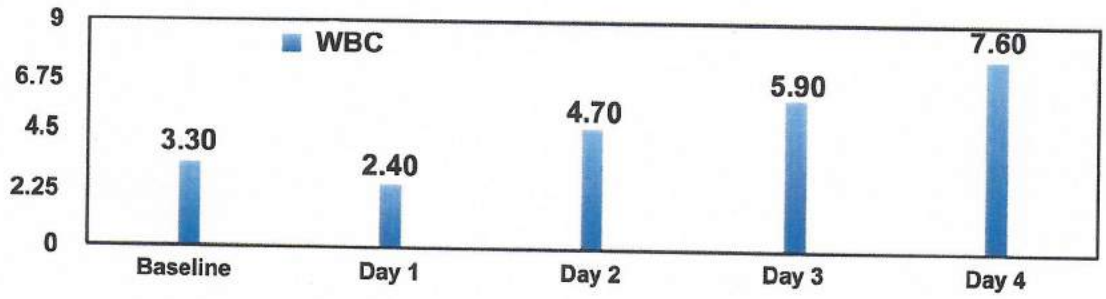


Figure 2b: Plot of Daily WBC for Dengue Patient 2 (Male 35) treated with Metadichol

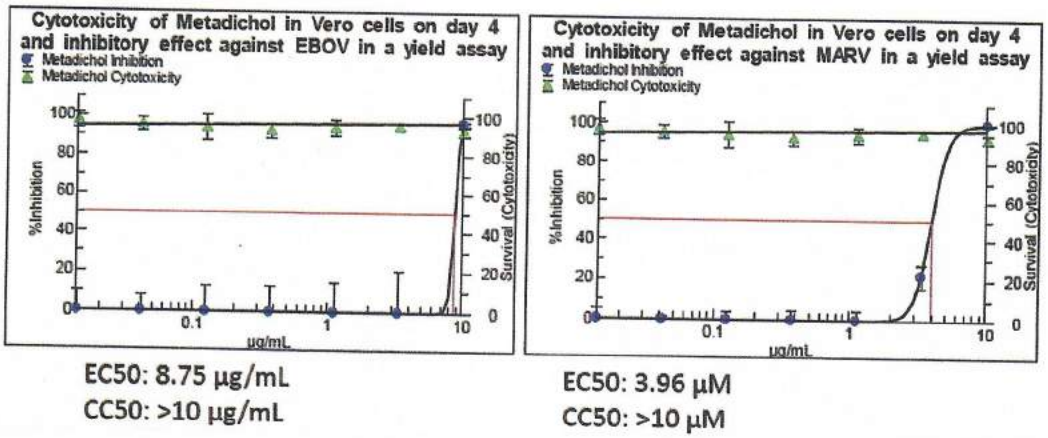


Figure 3. Cytotoxicity and antiviral activity of Metadichol (NanoRx, USA) in Vero E6 cells exposed to Ebola virus (EBOV) (Maringa) or Marburg virus (MARV) (Musoke)

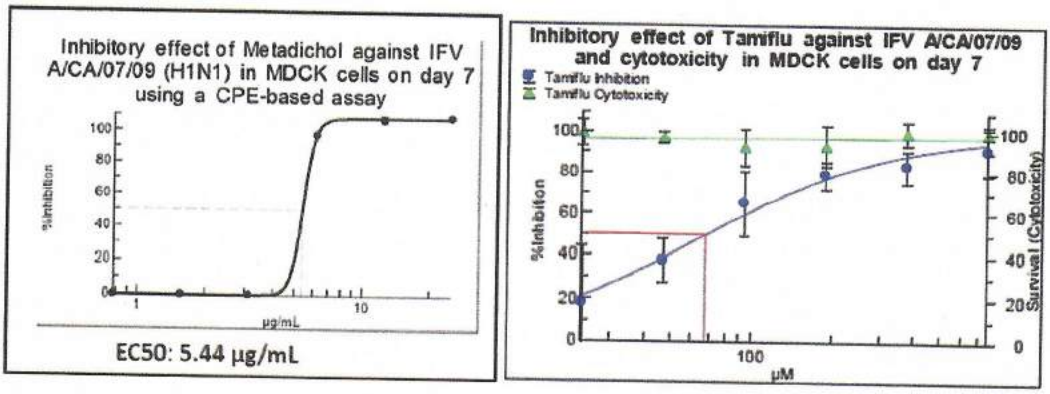


Figure 4. Cytotoxicity and antiviral activity of Metadichol and Oseltamivir in MDCK cells exposed to Influenza A CA/07/09

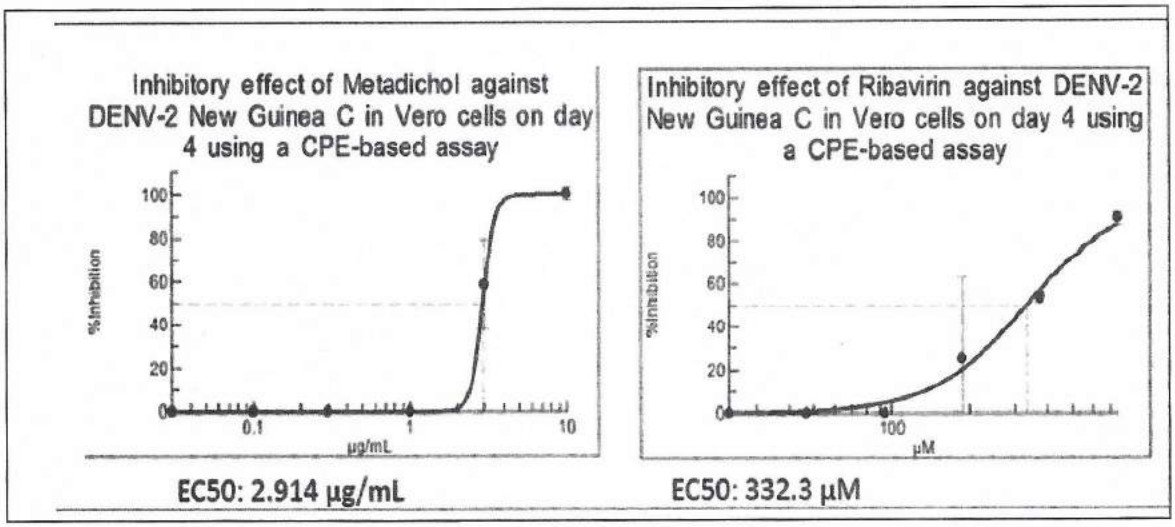


Figure 5. Cytotoxicity and antiviral activity of Metadichol and Ribavirin in MDCK cells exposed to DENV-2 New Guinea C

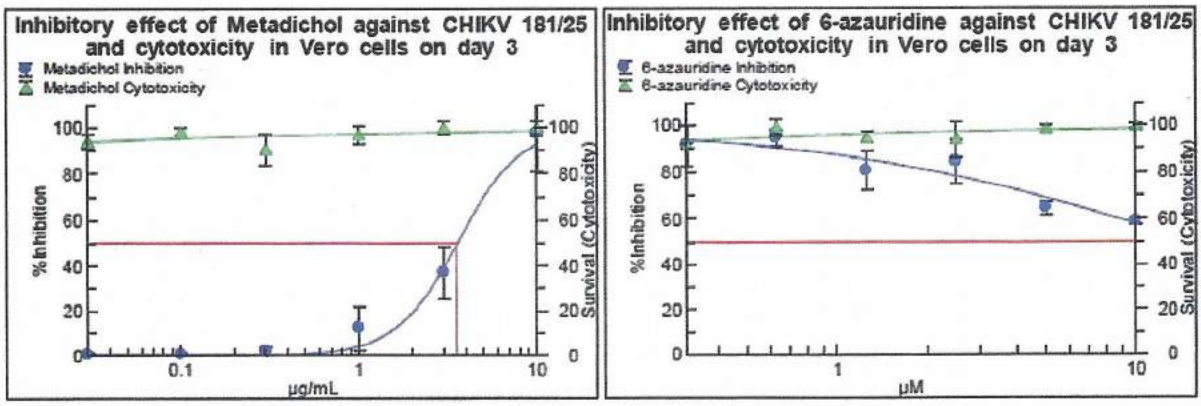
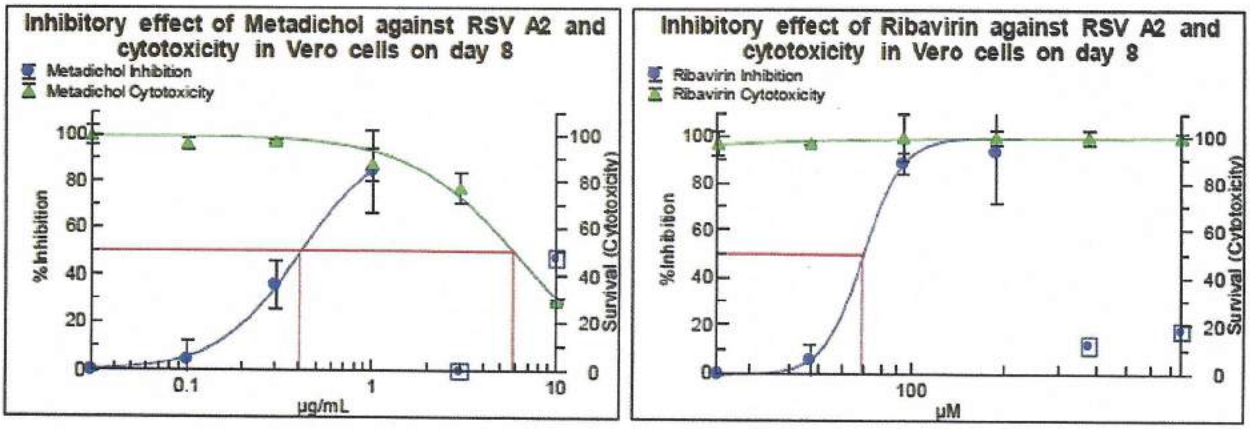


Figure 6. Cytotoxicity and antiviral activity of Metadichol and 6-azauridine in Vero cells exposed to CHIKV 181/25



EC50: 0.41 µg/mL
CC50: 5.89 µg/mL

EC50: 69.6 µM
CC50: >750 µM

Figure 7. Cytotoxicity and antiviral activity of Metadichol and Ribavirin in Vero cells exposed to HRSV A2

µg /mL Metadichol	Adenovirus cell type A-549	Tacaribe (TRVL-11573)	Rift Valley Fever (MP-12)	SARS (Co-V)	Japanese Encephalitis (SA-14)	West Nile	Yellow Fever (17D vaccine strain)	Powassan Virus
5	100%	31%	100%	0%	56%	84%	70%	53%
1.6	100%	69%	100%	52%	87%	100%	73%	100%
0.5	100%	97%	100%	100%	100%	100%	95%	100%
0.16	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**96%**	**100%**

Table 1. Percent cytopathic effect (CPE) reduction by Metadichol as measured by neutral red assay

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