Theo10® Oral Gel

Patented Anti-Viral Natural Product To Inhibit The Infectivity of Hand Foot Mouth Disease

(With reference from Journal of Virology written by Tao Meng[a,b], Qiang Jia[a], Sek-Man Wong[a,b,c], Kaw-Bing Chua[a]).
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Abstract:

Hand, foot, and mouth disease (HFMD), a highly contagious disease in children, is caused by human enterovirus, coxsackievirus A16 (CVA16) and coxsackievirus A6 (CVA6) and especially enterovirus 71 (EV71). Numerous sulfonated azo dyes, widely used as food addictive, were identified as having potent antiviral activities against human enteroviruses. Among them, brilliant black BN (E151) was able to inhibit all EV71, CVA16 and CVA6 strains tested.

In rhabdomyosarcoma cells, the 50% inhibitory concentrations of the dye E151 for various strains of EV71 ranged from 2.39μM to 28.12 μM, whereas its 50% cytotoxic concentration was 1,870 μM.

E151 interacted with the vertex of the 5-fold axis of EV71 and prevented viral entry. Their efficacy in viral inhibition was regulated by amino acids at VP1-98, VP1-145 and/or VP1-246. E151 also eluted attached viruses in a concentration-dependent manner. E151 also inhibited the interaction between EV71 and its cellular uncoating factor cyclophilin A.

In vivo studies demonstrated that E151 at a dose of 200mg/kg of body weight/day given on the initial 4 days of challenge protected AG129 mice challenged with 10x the 50% lethal dose of wild-type EV71 isolates.

(Introduction) Human enterovirus 71 (EV71) is one of the causative agents of hand, foot, and mouth disease in children and is responsible for thousands of deaths in the past 20 years. Food azo dyes have been widely used since the 19th century; however, their biological effects on human and microbes residing in humans are poorly understood. We discovered that brilliant black BN (E151), was particularly effective in inhibiting the infectivity of EV71 in both cell culture and mouse model studies. Mechanistic studies demonstrated that these sulfonated dyes mainly competed with EV71 attachment factors for viral binding to block viral attachment/entry to host cells.

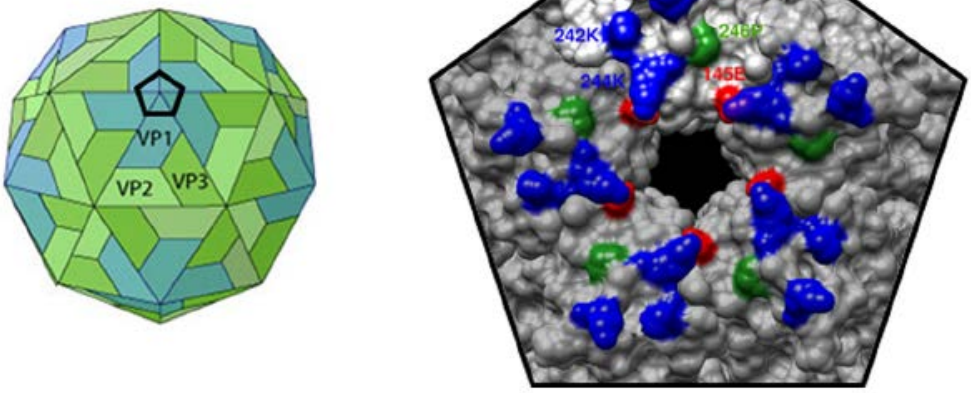
(Identifying the trackers) Human enteroviruses, belonging to the genus *Enterovirus* in the family *Picornaviridae*, have a positive-sense, single-stranded-RNA genome encapsidated in a nonenveloped, icosahedral particle with 60 copies of each of the 4 viral structural proteins, VP1, VP2, VP3 and VP4. VP1 is immunodominant and based on its nucleotide sequence. Every VP1 pentamer on the surface of an EV71 virion forms a prominent star-shaped plateau at the 5-fold axis of symmetry surrounded by a deep depression called the canyon region. (1,2) (*Fig 1*). Sulfated P-selectin glycoprotein ligand-1 (PSGL-1) and glycosaminoglycans (GAGs) on the cell surface interact with the positively charged vertex of the 5-fold axis of viral capsid to facilitate EV71 attachment and infection (3-8).

(How it works) Sulphated/sulfonated heparin, suramin, and suramin derivatives have been shown to bind to the vertex of the 5-fold axis to block EV71 attachment to host cells (9-11). The cellular protein cyclophilin A has been determined to be an EV71-uncoating regulating factor through interactive with and modifying the conformation of the H-I look of VP1 at the vertex of the 5-fold axis (12). Antibodies binding to the vertex od the 5-fold axis also prevent EV71 infection (13).

(Why it is safe) Sulfonation of azo dyes increases their solubility in water and decreases their cellular absorption and enhances their urinary excretion in humans and animals. It can interact with positively charged amino acids, such as lysine and arginine, and possess antimicrobial activities (14-18). Azo dyes have been authorized to be used as additives in the F&B industry since the 19th century. Common food azo dyes labelled with an ‘E’ number can be found in the list of food additives from the European Food Safety Authority (EFSA)

(Complications of HFMD) Infection with EV71 occasionally leads to fatal pulmonary edema or neurological disorders like encephalitis, aseptic meningitis, and acute flaccid paralysis. Millions of cases and several thousands deaths have been reported during EV71 epidemics in the Asia-Pacific region since the 1990s. (19-23)

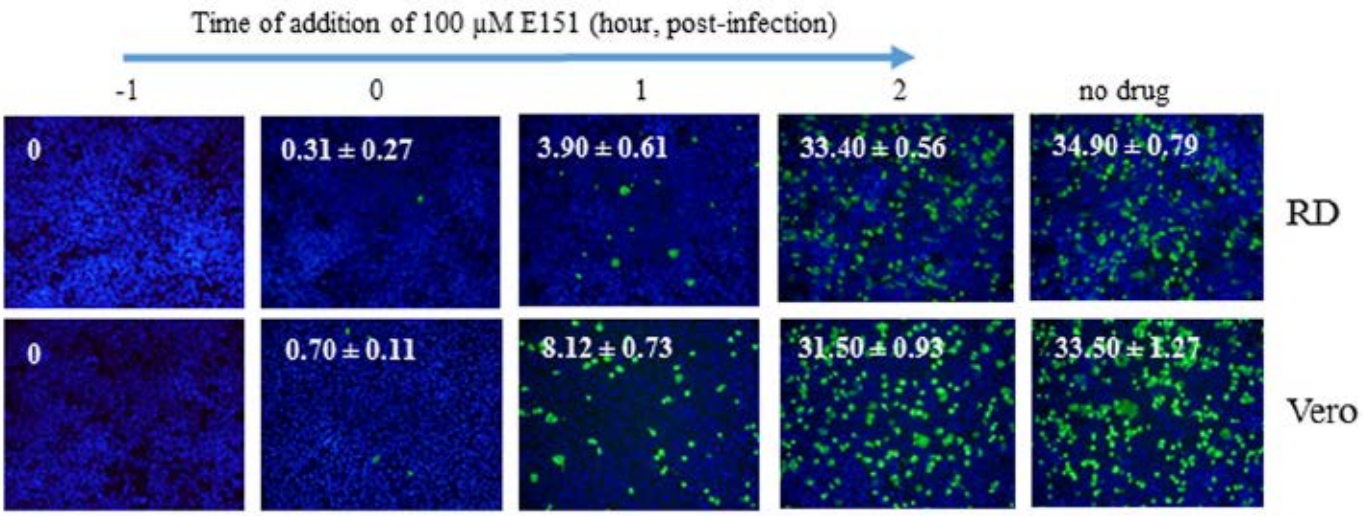
FIG 1



[Left] Cartoon image of the icosahedral EV71 virion with VP1 (blue), VP2 (light green), and VP3 (green) on viral surface. The regular black pentagon indicates the vertex of the 5-fold axis formed by a VP1 pentamer.

[Right] Three-dimensional surface of the vertex of an EV71 KE strain. Amino acids at VP1-145 (red) and VP1-246 (green) are critical for E151 binding to prevent EV71 infection. VP1-246P is conserved, while Vp1-145E is dominant. EV71 strains with VP1-145G/Q are highly sensitive to E151, whereas strains with VP1-145E have low sensitivity to E151. Positively charged VP1-98K, VP1-242K, and VP1-244K (blue) might also interact with E151 through electrostatic attraction.

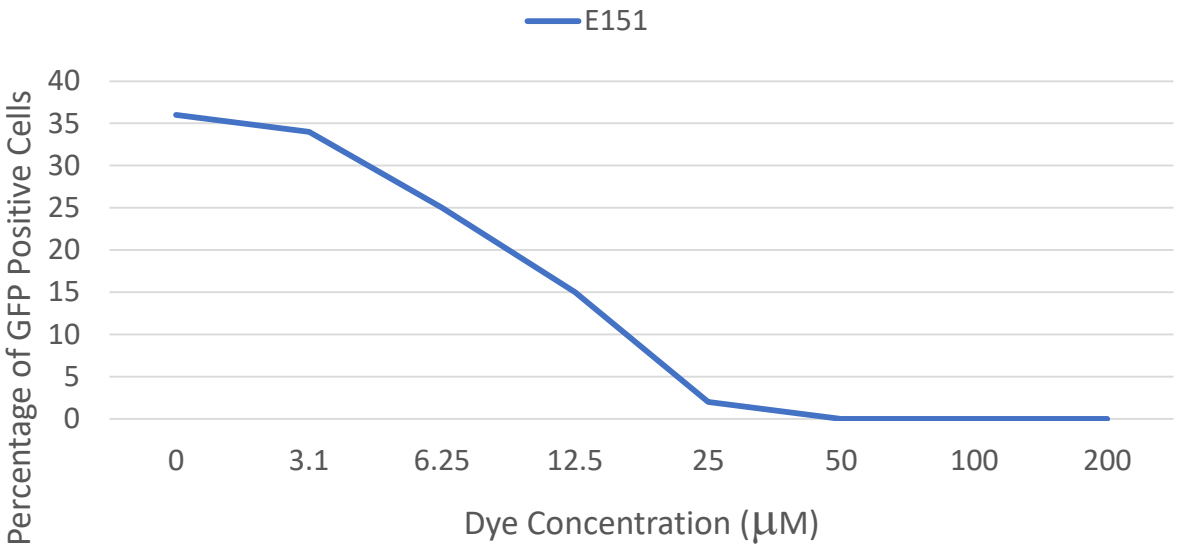
FIG 2



To investigate which steps of the EV71 life cycle were affected by E151, E151 was added to RD or Vero cells at an MOI of 1 at different time points during EV71-GFP infection, and infected cells were quantified at 12hrs postinfection. The percentage of infected cells with positive GFP signals was significantly reduced when E151 was added at -1, - or 1hrs but not at 2hrs postinfection. Therefore, the inhibitory effects of E151 occurred at the early stages of EV71 infection, which include viral attachment, internalization and uncoating but not viral replication.

To further dissect the inhibitory mechanisms of the dye E151, assays of the attachment of EV71 to cells and attachments factors were performed in the absence or presence of E151. in a cell-virus binding assay, E151 prevented EV71 attachment to RD cells in a concentration-dependent manner. The attachment of EV71-B4 (highly sensitive to E151) and EV71-C1 (slightly sensitive to E151) to RD cells was significantly blocked by E51 at concentration of 30μM and 100μM, respectively, based on the results of the Western blot analysis using a specific anti-VP1 monoclonal antibody, 1D9(24).

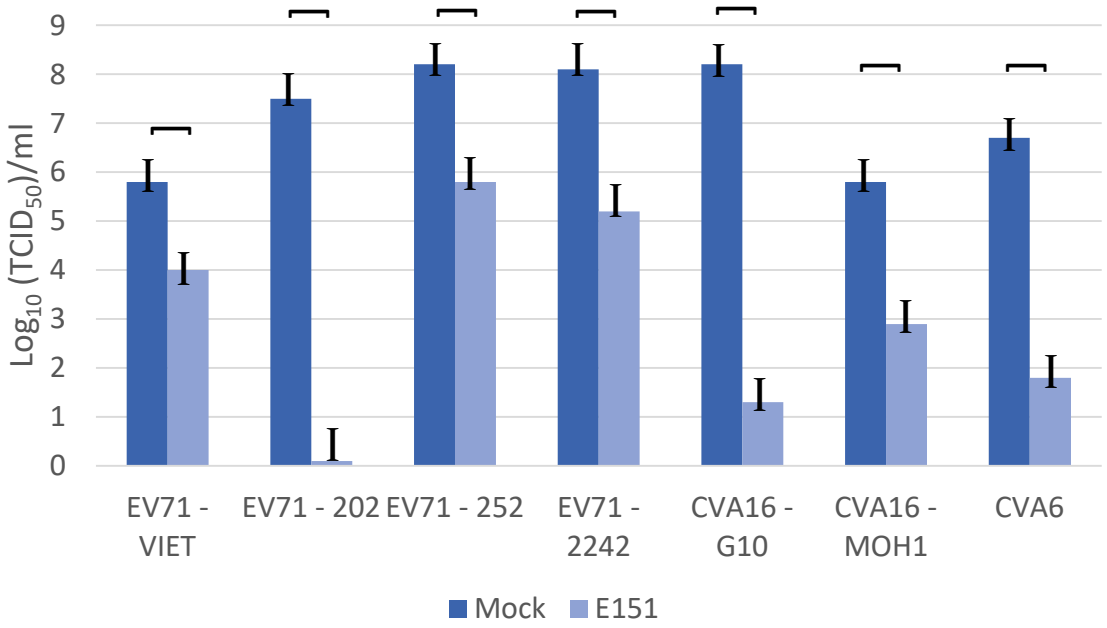
Fig 3



Percentages of infected cells (GFP positive) after treatment with E151 at the indicated concentrations from 0 to 200 μM. The average value ± standard error of 3 random spots for each concentration is presented.

Green fluorescent protein (GFP) as a reporter, the percentage of GFP-positive cells, the 50% inhibitory concentration (IC_{50}) of E151 was determined to be 10.1μM.

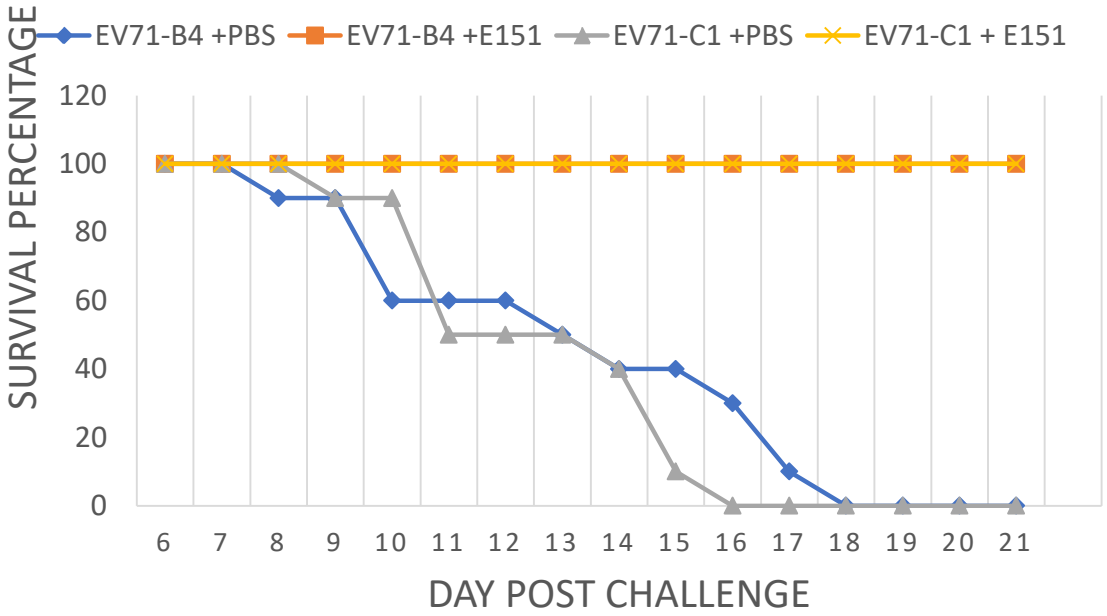
FIG 4



By using a virus titer reduction assay, the inhibitory effects of the 4 azo dyes on the infectivity of wild-type human enteroviruses were investigated in RD cells. E151 at 100μM significantly prevented cytopathic effect (CPE) induced by viral infection and reduced the titers of all 8 RD cell-adapted EV71 strains, belonging to different sub-genogroups and 4 clinical EV71 isolates. E151 inhibited the infectivity for all 3 CVA16 and 1 CVA6 virus.

In the presence of E151. Brackets indicate P values of >0.01 (one-way ANOVA with Dunnett's posttest in panel A and two-tailed Student's *t* test in panel B)

FIG 5



The efficacy of E151 in preventing EV71 infection *in vivo* was assessed in 14-day-old AG129 mice, which were intraperitoneally challenged with 10 50% lethal doses (LD_{50}) of highly E151-sensitive EV71-B4 (3 X 10^{10} 50% tissue culture infective dose [$TDIC_{50}$]) or slightly E151-sensitive EV71-C1 (1.5X 10^{8} $TCID_{50}$).

The mice (8 pups per group) were then treated with 200mg E151/kg of body weight/day or 200µl PBS/day through intraperitoneal inoculation from the 14th to the 17th day (0 to 30 days postchallenge). The PBS-treated mice in control groups began to exhibit clinical symptoms, such as hunched back and limb paralysis, from as early as day 6 postchallenge and succumbed to infection from day 8 postchallenge. Eventually they all died, with survival percentage declining to 0. In the E151 treatment groups, some challenged mice only exhibited mild illness with clinical scores of less than 3 and subsequently recovered. All E151-treated mice were completely protected throughout the experiment.

To further evaluate the protective efficacy of E151, the EV71-C1-challenged mice treated with PBS or E151 were euthanized at 4, 8, or 12 days postchallenge (5 mice per group per time point), and titers of progeny virus in the brain and hind limb muscle tissues were determined. For the PBS-treated mice, viruses were detected in the muscle and brain from day 4 postchallenge. The viral titers in brains continually increased with time, while those in muscle tissues reached the highest levels at day 4 postchallenge and then declined, as previously reported (25).

In contract, significantly lower virus titers in both brain and muscle tissues were observed in the mice treated with E151. although the viral titers in muscle of E151-treated mice increased after the cessation of E151 treatment from day 4 postchallenge, those in brains did not increase to the detectable level ($10^{3.5}$ $TCID_{50}$/g).

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