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The multi-targeted kinase inhibitor sorafenib inhibits enterovirus 71 replication by regulating IRES-dependent translation of viral proteins

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ABSTRACT

The activation of ERK and p38 signal cascade in host cells has been demonstrated to be essential for picornavirus enterovirus 71 (EV71) replication and up-regulation of virus-induced cyclooxygenase-2 (COX-2)/prostaglandins E_2 (PGE_2) expression. The aim of this study was to examine the effects of sorafenib, a clinically approved anti-cancer multi-targeted kinase inhibitor, on the propagation and pathogenesis of EV71, with a view to its possible mechanism and potential use in the design of therapy regimes for Hand foot and mouth disease (HFMD) patients with life threatening neurological complications. In this study, non-toxic concentrations of sorafenib were shown to inhibit the yield of infectious progeny EV71 (clinical BC08 strain) by about 90% in three different cell types. A similar inhibitory effect of sorafenib was observed on the synthesis of both viral genomic RNA and the VP1 protein. Interestingly, sorafenib exerted obvious inhibition of the EV71 internal ribosomal entry site (IRES)-mediated translation, the first step in picornavirus replication, by linking it to a firefly luciferase reporter gene. Sorafenib was also able to prevent both EV71-induced CPE and the activation of ERK and p38, which contributes to up-regulation COX-2/PGE_2 expression induced by the virus. Overall, this study shows that sorafenib strongly inhibits EV71 replication at least in part by regulating viral IRES-dependent translation of viral proteins, indicating a novel potential strategy for the treatment of HFMD patients with severe neurological complications. To our knowledge, this is the first report that investigates the mechanism by which sorafenib inhibits EV71 replication.

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1. Introduction

Enterovirus 71 (EV71) is the major etiological agent of the large outbreaks of hand, foot and mouth disease (HFMD) that have occurred in recent years in the Asia–Pacific region, including mainland China (Solomon et al., 2010; Yang et al., 2009; Yi et al., 2011). Infection with the virus can give rise to the development of severe life-threatening neurological complications that are partially mediated through cyclooxygenase-2 (COX-2)/prostaglandins (PG) E_2 expression (Tung et al., 2010, 2011). There are currently no licensed preventative vaccines for HFMD and no effective anti-EV71 drugs commercialized (Shang et al., 2013).

EV71 belongs to the Enterovirus genus of the Picornaviridae (Nasri et al., 2007); it has a single stranded positive RNA genome with an internal ribosome entry site (IRES) located in the 5′-untranslated region (UTR) which can drive the initiation of translation of the viral polyprotein in a cap-independent manner (Belsham and Sonenberg, 1996; Etchison et al., 1982; Jackson et al., 1994). As obligate intracellular parasites, all viruses are reliant on host cell components and metabolism for their propagation and the host cell's extracellular regulated kinase (ERK) (Perkins et al., 2002) signal cascade, necessary for cellular proliferation, migration, division, has been shown in previous work from this lab and others to be essential for EV71 replication (Hunter et al., 1995; King et al., 1986; Moser and Schultz-Cherry, 2008; Perkins et al., 2002; Pleschka, 2008; Smith et al., 2000; Wang et al., 2012).

Sorafenib (previously known as BAY 43-9006 and marketed commercially as Nexavar) is a multi-target tyrosine and serine-threonine kinase inhibitor currently used in cancer therapy. Its primary targets are the Raf serine-threonine kinase and two growth factor receptors with tyrosine kinase activity named vascular

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endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) (Stein and Flaherty, 2007). This suggests that this drug may well interfere with other factors further down the mitogen-activated protein kinases (MAPKs) signaling cascade, for example, ERK, p38 MAPK (p38), c-Jun-N terminal kinase (JNK) and the transcription factor nuclear factor κB (NF-κB). In addition, sorafenib has recently been shown to efficiently inhibit the replication of hepatitis C virus (HCV) (Himmelsbach et al., 2009) and human cytomegalovirus (HCMV) (Michaelis et al., 2011) by inhibiting Raf-ERK activation. It has also been reported that the activation of ERK and p38 contributes to virus-induced COX-2/PGE_2 expression (Tung et al., 2010). Against this background, it would be of interest to know whether sorafenib can influence the propagation and pathogenesis of the picornavirus EV71, with a view to its possible use in the design of therapy regimes for HFMD patients with life threatening neurological complications.

Therefore in this study, the effect(s) of sorafenib on the replication of EV71 in rhabdomyosarcoma (RD) cells, Vero cells and SK-N-SH cells has been examined.

2. Materials and methods

2.1. Virus and cell culture

EV71 (a clinical strain denoted as EV71-BC08, JQ514785.1) (Liu et al., 2012) was propagated in three cell lines, RD cells (tumor cells), Vero cells (normal cells) and SK-N-SH cells (neuroblastoma cells), which are readily infected by the virus. The cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific Hyclone, USA) supplemented with 2% or 10% fetal bovine serum (FBS, Life Technologies Gibco, USA) at 37 °C in a humidified 5% CO_2 incubator.

2.2. Reagents and antibodies

Sorafenib was purchased from Sigma–Aldrich (USA). Phospho-ERK1/2 antibody was obtained from Cell Signaling Technology (USA). Polyclonal EV71 VP1 antibody was obtained from Abcam (UK) and β-actin antibody was obtained from Santa Cruz (USA) Biotechnology. In vitro transcription kit, the T7 MEGAscript High Yield Transcription Kit and mMESSAGE mMACHINE Kit were both obtained from Life Technologies Ambion (USA).

2.3. Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay (CHEMICON International, USA) was used to determine cell viability. Cells were grown in 96-well plates and the culture medium was changed to that containing sorafenib at different concentrations after overnight incubation. After further incubation at 37 °C for 24 h, MTT was added and cells were incubated for 2–4 h. DMSO was then added and absorbance at 570 nm was measured in an enzyme-linked immunosorbent assay plate reader. MTT assays were performed in triplicate. And the median cytotoxic concentration (CC_{50}) was calculated.

2.4. Quantitative PCR (q-PCR)

Cellular or viral RNAs were extracted using Trizol reagent (Life Technologies Invitrogen, USA). Then, total RNA was reverse transcribed into cDNA with the ReverAid First strand cDNA synthesis kit (Thermo Scientific, USA). q-PCR was performed in SYBR Green Supermix (F. Hoffmann-La Roche, Switzerland) with the Roche Light Cycler 480 system. The reactions were performed under the following thermal cycling conditions: 1 cycle of initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 30 s, extension at 72 °C for 30 s. PCR primers applied in this study are listed in Table 1. Quantified results were extrapolated from the CT value normalized to that of GAPDH whose expression levels were measured as an internal control. All assays were performed in triplicate.

2.5. Western blot analysis

Cells were lysed in a lysis buffer containing 100 mM NaCl, 30 mM NaF, 20 mM Hepes (pH 7.4), 5 mM EDTA (pH 7.4), 1 mM Na_3VO_4, 5% glycerol, 1% Triton X-100, 0.1% SDS, 10 mM p-nitrophenylphosphate, 1 mM glycerophosphate, supplemented with complete protease inhibitors (F. Hoffmann-La Roche, Switzerland). Protein concentration was determined with the Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific Pierce, USA). Proteins were resolved using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), and proteins transferred to PVDF membranes (EMD Millipore, USA). The membrane was blotted with a specific primary antibody, as indicated in the figure legends. This was followed by incubation with a secondary antibody conjugated with horseradish peroxidase, and the assay developed using an enhanced chemiluminescent substrate (ECL).

2.6. Construction of Luc mRNA reporters and mRNA transcription in vitro

A firefly luciferase (Fluc) open reading frame was linked to the 5′-UTR of EV71 genome (EV71-BC08, JQ514785.1) with primers containing BglII (sense) or SalI (antisense) sequences at each 5′ end. Then the 2.5 kb fusion gene (BglII-EV71 5′ UTR-Fluc-SalI) was cloned into the pGEM-Teasy vector. Then the linearized recombinant plasmid by SalI was in vitro transcribed for the synthesis of IRES-dependent Fluc mRNA reporter (IRES-Fluc mRNA) using the T7 MEGAscript High Yield Transcription Kit. While pRL-CMV plasmid (Promega Corporation, USA) which contains T7 promoter and a following Renella luciferase (Rluc) sequence was linearized by BamHI and then applied for in vitro transcription to synthesize capped mRNA reporter (cap-Rluc mRNA) by using mMESSAGE mMACHINE Kit. 1.5 μg of each mRNA transcript were co-transfected into cells using Lipofectamine 2000 (Life Technologies Invitrogen, USA).

2.7. Transfection and dual-luciferase assay

RD cells were cultured to 80% confluency in 96-well plates and transfected with both EV71 IRES-Fluc mRNAs and cap-Rluc mRNAs using Lipofectamine 2000 according to the manufacturer's protocol. The amount of Fluc or Rluc mRNAs detected respectively by q-RT-PCR 6 and 12 h post transfection was used as an internal control to gauge transfection efficiency. Transfected cells were lysed by incubation for 5 min in a lysis buffer containing 25 mM

Table 1
The PCR primers applied in this study.

Primer name		Sequences(5′–3′)
EV71	Sense	GCA GCC CAA AAG AAC TTC AC
	Antisense	ATT TCA GCA GCT TGG AGT GC
RLuc	Sense	TCCTGGATCACTACAAGTACCTCACCG
	Antisense	ATCTTGCTTGGGAGCATGGTCTCG
FLuc	Sense	GAACAGCATGGGCATCAGC
	Antisense	CAAATGGGAAGTCACGAAGGT
GAPDH	Sense	TGT TCC AAT ATG ATT CCA CCC
	Antisense	CTT CTC CAT GGT GCG TGA AGA

Table 2
Effect of sorafenib on EV71-induced CPE and cell viability.

Cell types	Sorafenib (μM)		
	IC_{50}	CC_{50}	TI
RD cells	1.50 ± 0.13	7.05 ± 0.28	4.7
Vero cells	6.58 ± 0.33	26.37 ± 0.39	4.0
SK-N-SH cells	1.34 ± 0.11	7.90 ± 0.36	5.9

CC_{50} is the concentration of sorafenib that reduced cell viability by 50% measured by an MTT assay. IC_{50} represents the sorafenib concentration that inhibits virus induced CPE by 50%. All three cell types were infected with EV71 at an MOI of 2. TI = CC_{50}/IC_{50}. Data shown are the mean ± SD.

Tris–HCl, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid, 10% glycerol, 1% Triton X-100 (Promega Corporation, USA) 6 or 12 h post transfection, and luciferase reporter activity was assayed using a dual-luciferase assay kit (Promega Corporation, USA) according to the manufacturer's instructions in a Multimode Plate Reader (PerkinElmer, USA).

2.8. Morphological analysis

RD cells, Vero cells and SK-N-SH cells were examined for the cytopathic effect (CPE) induced by EV71 infection every 12 h after infection using phase-contrast microscopy.

2.9. Statistics

Statistical difference analysis was carried out using the Student's *t*-test or ANOVA as indicated in figure legends.

3. Results

3.1. Sorafenib inhibits EV71 propagation in different cells

In order to evaluate the efficacy of sorafenib on EV71 amplification in RD, Vero and SK-N-SH cells, the therapeutic index (TI) for drug treatment was measured (Table 2). The TI was defined as the ratio of the concentration of sorafenib that reduced cell viability by 50% (CC_{50}) and caused a 50% reduction in virus induced CPE (IC_{50}). Sorafenib treatment inhibited EV71 propagation in the three types of cells with different TI values. No effect on cell viability was found when RD and SK-N-SH cells were treated with 3 μM sorafenib, or Vero cells with 10 μM of the drug (Fig. 1a). However treatment with these drug concentrations did reduce the yields of both released infectious virus particles in the supernatant medium and virus in infected cell lysates by about 90% (Fig. 1b). These observations indicated that sorafenib efficiently inhibited the production of infectious EV71 progeny virions.

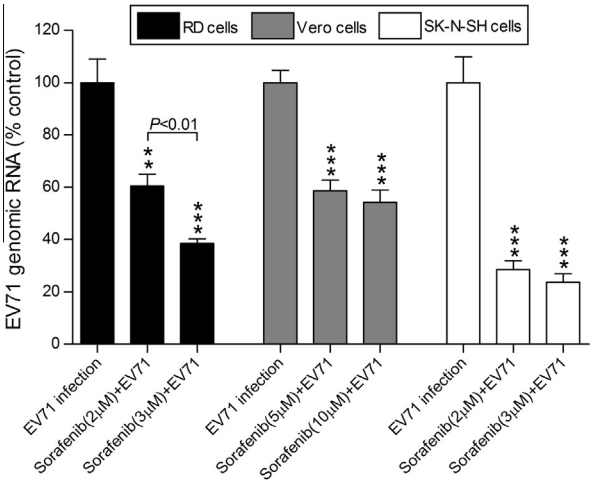

Fig. 2. The effect of sorafenib treatment on EV71 genome replication in RD, Vero and SK-N-SH cells. The level of EV71 RNA in the overlay medium and virus infected cells treated with sorafenib was monitored for the three types of cells by relative q-RT-PCR. Cells were treated with sorafenib at the indicated doses from 1 h, before being infected with EV71 (MOI of 2). EV71 infection cells without sorafenib treatment were used as control. The virus infected cells and supernatant medium were collected together at 14 h p.i. for q-RT-PCR detection of viral genomes as described in Section 2. $^{**}P < 0.01$, $^{***}P < 0.001$ when compared with respective EV71 infection by Student's *t*-test.

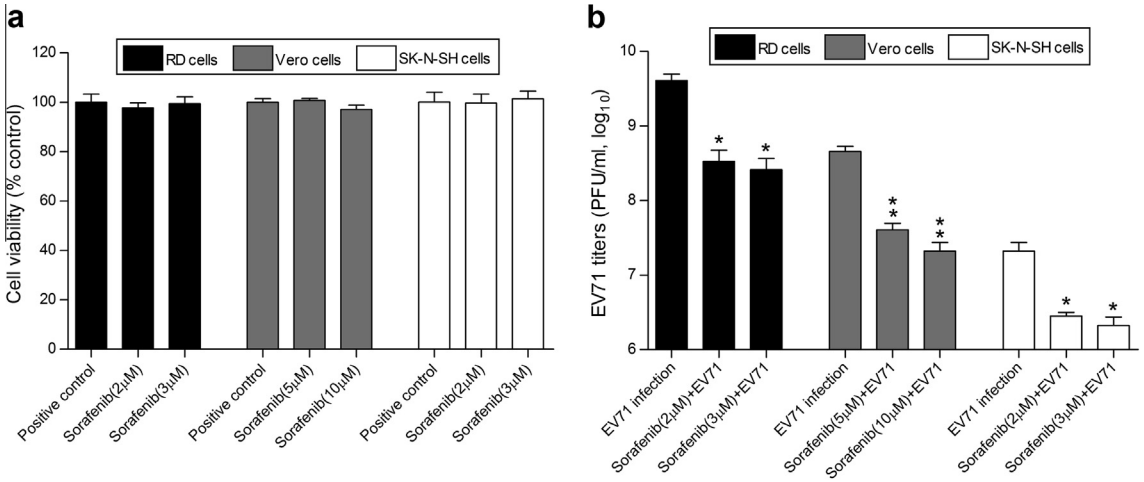

Fig. 1. The effect of sorafenib treatment on cell viability and EV71 propagation. (a) Effect of sorafenib treatment on cell survival. RD cells, Vero cells and SK-N-SH cells were treated with sorafenib at the indicated doses for 24 h, and cell viability was measured by MTT assay. Background absorbance given by a blank control was subtracted from all measurements before expressing viability as a percentage of that of the positive control (non-drug treated cells) set at 100%. Statistical difference analysis was carried out using ANOVA ($P > 0.05$). (b) Effect of sorafenib treatment on infectious virus yield of EV71. The three cells were pre-treated with sorafenib at the indicated dose for 1 h, followed by infection with EV71 (MOI of 2) and maintenance in sorafenib until 24 h post-infection (p.i.). EV71 infection cells without sorafenib treatment were used as a control. Culture supernatants and infected cells were collected together at 24 h p.i. The titer of infectious virus was measured by plaque assay and expressed as p.f.u./ml. The *y*-axis shows logarithmic values. The data were shown as the means ± standard deviations ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with respective EV71 infection by Student's *t*-test.

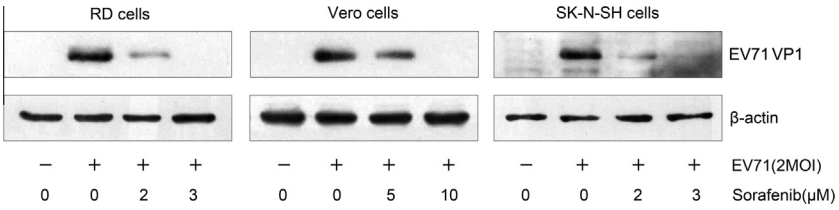

Fig. 3. The effect of sorafenib treatment on synthesis of EV71 VP1 protein in virus infected cells. RD cells, Vero cells and SK-N-SH cells were treated with sorafenib at the indicated doses from 1 h before infection with EV71 (MOI of 2). Mock and EV71 infection cells without sorafenib treatment were used as controls. Infected cells were collected at 24 h p.i. Proteins were fractionated by SDS–PAGE and the VP1 protein detected on Western blots of cell lysates all as described in Section 2. β-Actin was used as a loading control for the SDS–PAGE.

Table 3
Effect of sorafenib at 3 μM on infectious EV71 titers and genomic RNA in RD cells.

Sorafenib	Titers of EV71 (PFU/ml × 10^8)	EV71 genomic RNA (2-dt/GAPDH)
None treatment	40.62 ± 0.91	1.15 ± 0.10
2 h pretreatment	39.12 ± 0.52	1.10 ± 0.04
Addition during virus adsorption	38.56 ± 1.05	1.05 ± 0.10
1 h treatment post-infection	3.80 ± 0.26	0.57 ± 0.05
3 h treatment post-infection	4.32 ± 0.97	0.62 ± 0.31

Cells were infected with EV71 for 1 h. Then virus-containing medium was replaced by fresh virus-free cell culture medium. "2 h pretreatment" means that cells were incubated with sorafenib for 2 h prior to virus infection; "addition during virus adsorption" means that sorafenib was only present during the 1-h infection period; "1-h and 3-h treatment post-infection" means that sorafenib was added 1-h or 3-h after the adsorption period of RD cells infected with EV71 at MOI 2. Samples collected at 24 h p.i. and 8 h p.i. were applied for PFU and q-RT-PCR assays, respectively. Data shown are the mean ± SD.

3.2. Sorafenib reduces the genome replication of EV71

To determine the impact of sorafenib on RNA genome replication of EV71, a quantitative reverse transcription polymerase chain reaction (q-RT-PCR) with viral capsid protein 1 (VP1) primers (Table 1) was used to monitor RNA levels for the VP1 as an indicator of the level of EV71 genomic RNA in infected cells. Fig. 2 shows that the level of VP1 RNA was significantly reduced in sorafenib-treated cells, when compared to that seen in the control (untreated) virus infected cells. These data indicated that sorafenib treatment reduced the replication of viral RNA.

3.3. Sorafenib inhibits EV71 protein synthesis

The effect of sorafenib treatment on viral VP1 protein as an indicator of the level of EV71 protein in infected cells was monitored by Western blot analysis. Fig. 3 shows that treatment with sorafenib resulted in a reduction in the synthesis of VP1 compared to that seen in control (untreated) infected cells. This reduction in viral protein synthesis was more marked as the concentration of sorfenib used was increased (Fig. 3). These data showed that sorafenib inhibited viral protein synthesis in EV71 infected cells.

3.4. Sorafenib blocks post-entry event(s) in EV71 replication cycle

In order to determine which point(s) of the EV71 replication cycle sorafenib might act, sorafenib treated with a time-of-addition manner in infected RD cells were performed. As shown in Table 3, a significant reduction of infectious EV71 titers and genomic RNA is observed in infected cells with sorafenib "1 h and 3 h treatment post-infection", as compared to that seen in sorafenib none treatment cells ($P < 0.01$), but no difference when compared sorafenib "2 h pre-treatment" and "addition during virus adsorption" with sorafenib none treatment in infected cells ($P > 0.05$). These results suggest that sorafenib efficiently blocks one or more post-entry events in the EV71 replication cycle.

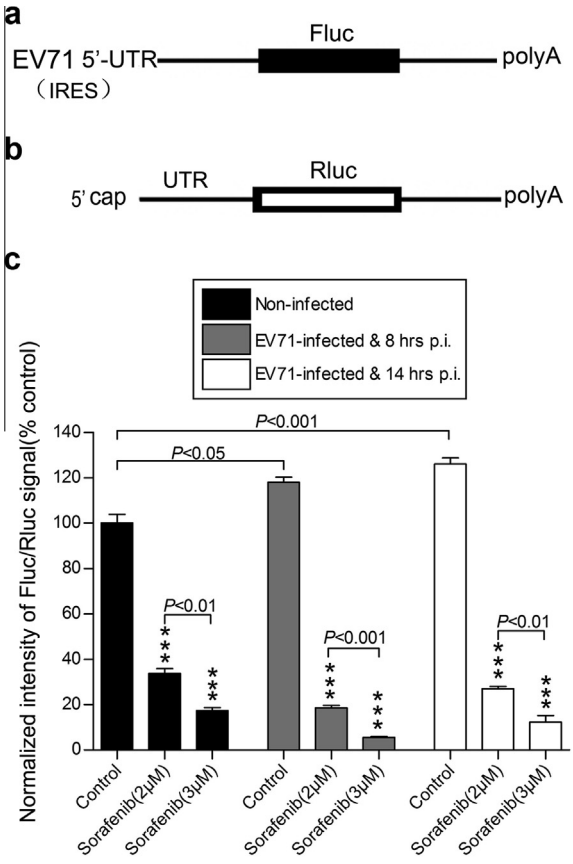

Fig. 4. The effect of sorafenib treatment on the expression of Luc reporter mRNAs. (a) The structures of the EV71 IRES-Fluc reporter mRNAs used in this study. (b)The structures of the cap-Rluc reporter mRNAs used in this study. (c) Effect of sorafenib treatment on the level of Luc reporter mRNAs expressed in EV71 infected or uninfected RD cells. Cells were infected with EV71 for 1 h, then virus-containing medium was replaced by fresh virus-free cell culture medium. Cells were incubated with sorafenib at the indicated doses for 1 h prior to co-transfection with IRES-fluc mRNA and cap-Rluc mRNA. In addition, transfected cells with non-sorafenib treatment or non-EV71 infection were used as controls. Samples collected at 8 and 14 h p.i. were applied for *quantitation of luminescent* signal from each of the luciferase reporter detected by a dual-luciferase assay. The normalized intensity of Fluc related to Rluc signal on the *y*-axis is expressed as a relative percentage to the intensity of luminescence seen in control untreated cells, latter was set at 100% in each experiment. The data are shown as the means ± standard deviations ($n = 3$). $^{***}P < 0.001$ when compared with respective control by Student's *t*-test.

3.5. Sorafenib impairs IRES-driven translation

The first step in the post-entry of picornavirus replication cycle is the IRES-dependent translation of the viral polyprotein,

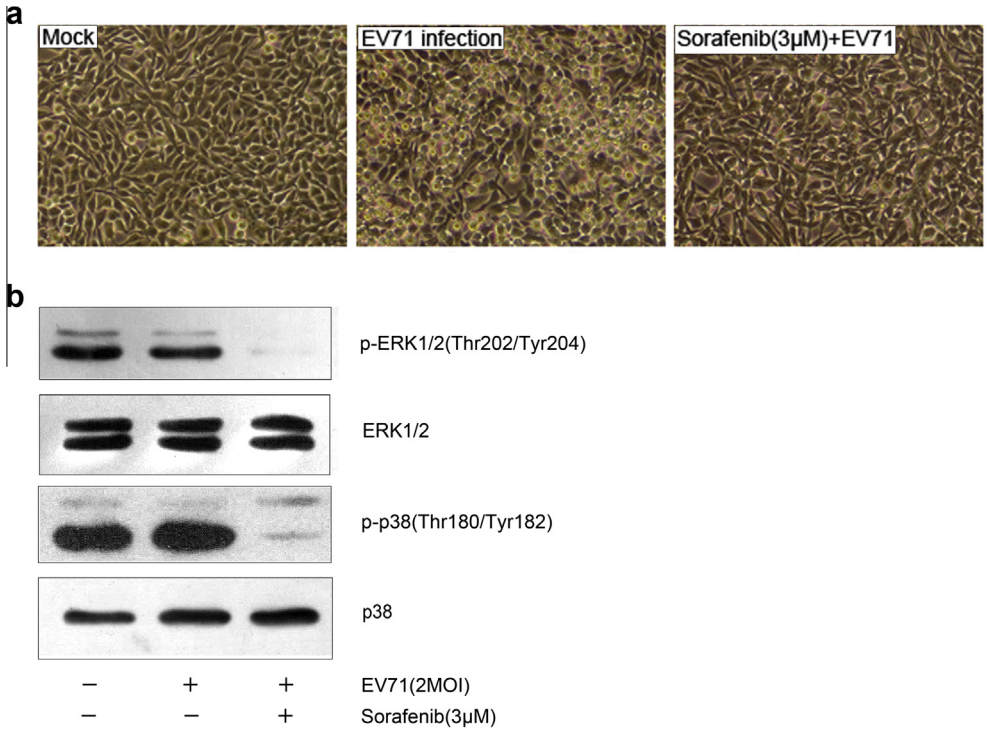

Fig. 5. The effects of sorafenib treatment on EV71 induced CPE and activation of the ERK1/2 and p38 proteins in RD cells. RD cells were pre-treated with sorafenib at the indicated dose for 1 h, followed by infection with EV71 (MOI of 2) and maintenance in sorafenib until 24 h p.i. Mock infected and EV71 infected cells not treated with sorafenib were used as controls. (a) Phase contrast microscopy of the effect of sorafenib treatment on EV71 induced CPE. The effects of sorafenib were monitored by phase-contrast microscopy (100-fold magnification) at 12 and 24 h p.i. Cell monolayers were photographed at 24 h p.i. to show the effects of sorafenib treatment. (b) Effect of sorafenib treatment on activation of the ERK1/2 and p38 proteins. Cells were collected at 24 h p.i. The cellular p-ERK1/2 and p-p38 proteins were detected on Western blots of cell lysates with specific antibodies.

consequently as a first step in unraveling the mechanism of action of sorafenib, it was important to investigate sorafenib's impact on this first step in the post-entry of EV71 replication cycle. To do this a reporter gene construct named EV71 IRES-Fluc mRNA was employed (Fig. 4a). In addition, cap-Rluc mRNA reporter was done in parallel as the control of translation initiation in a cap-dependent manner (Fig. 4b).

To determine if sorafenib treatment does interfere with IRES-mediated translation, both IRES-Fluc mRNA and cap-Rluc mRNA were simultaneously co-transfected into RD cells and reporter gene expressions in the presence and absence of the drug were compared. The normalized intensity of IRES-Fluc/cap-Rluc luminescence signal in uninfected RD cells was decreased by about 66% and 83% in the presence of 2 and 3 μM sorafenib, respectively (Fig. 4c, $P < 0.001$). A similar inhibition following sorafenib treatment in infected RD cells was also seen after transfection of IRES-Fluc mRNA into EV71 (Fig. 4c, $P < 0.001$). Notably, as transfection internal controls, the level of Fluc mRNA and Rluc mRNA in transfected cells detected by q-RT-PCR at 6 and 12 h post transfection, had no discernible difference in EV71 infected or uninfected RD cells (data not shown), indicating that viral infection did not alter the copies of mRNA reporter genes. Therefore these data showed that sorafenib treatment specifically inhibited IRES driven translation irrespective of whether or not the cells were infected with EV71.

3.6. Sorafenib prevents EV71 induced pathogenesis

EV71 virus has a lytic life cycle and its pathogenesis embodies two aspects, direct virus-mediated cyto-pathology (CPE) and virus induced inflammatory responses. In addition, a recent study has shown that activation of ERK and p38 contributes to EV71-induced COX-2/PGE_2 expression, and this is thought to be an important factor in the more severe cases of EV71 disease where there is neurological damage (Solomon et al., 2010; Tung et al., 2011). Consequently, the influence of sorafenib inhibition of virus replication on EV71 induced-CPE and the activation of ERK and p38 was assayed. EV71 induced-CPE evident at 24 h p.i. was sharply inhibited by 3 μM sorafenib treatment of virus infected cells (Fig. 5a). Activation of ERK and p38 was also inhibited in virus infected cells treated with 3 μM sorafenib (Fig. 5b). These data indicate that sorafenib treatment is able to block the direct EV71 mediated CPE and through blocking virus induced activation of the ERK and p38 signaling pathways may alleviate EV71-induced inflammatory responses.

4. Discussion

The current absence of antiviral strategies to treat HFMD patients suffering life threatening neurological complication has stimulated work to develop novel approaches to combat this growing healthcare problem. In this context the present study focused on the effect on EV71 replication of treating cells with sorafenib, an anti-cancer drug already licensed for use in humans (Karaman et al., 2008). The treatment of EV71 infected cells with non-toxic concentrations of sorafenib inhibited the synthesis of both viral genomic RNA and viral proteins, and consequently the yield of infectious virions in each of the three different cell types tested in this study, indicating that its antiviral effects are not limited to a particular cell type.

As far as the mechanism underlying sorafenib's inhibition of EV71 is concerned, our results demonstrated that sorafenib efficiently blocks post-entry event(s) in the EV71 life cycle, both at viral protein translation and genomic RNA replication levels. This study focused on exploring the IRES within viral 5′-UTR, because IRES-dependent translation of the viral polyprotein is a key initial

step of post-entry events in the replication cycle of picornaviruses such as EV71 (Belsham and Sonenberg, 1996; Etchison et al., 1982; Jackson et al., 1994). The results obtained confirmed that an important component of sorafenib based inhibition of virus replication appears to operate at the level of viral mRNA translation through inhibiting IRES driven expression of EV71 proteins. This suppression effect on IRES activity is consistent with recent report showing that sorafenib down-regulates the anti-apoptotic protein c-IAP1 expression in HCC cells by targeting the cellular IRES within c-IAP1 mRNA (Li et al., 2012). Notably, it has been reported (Grainger et al., 2010; Honda et al., 1996) that an IRES-mediated translation initiation was involved in both HCMV and HCV amplification, the inhibition activity of sorafenib on the IRES element of two viruses' proliferation needs to be further investigation.

The effects of infection by a wide variety of viruses on COX-2/PGE_2 expression relate to inflammation have been demonstrated in many cell types (Steer and Corbett, 2003). A recent study has shown that EV71 infection induced COX-2/PGE_2 expression via MAPKs including ERK and p38, and further that inhibition of EV71-induced COX-2/PGE_2 expression may reduce CNS inflammation (Tung et al., 2010). In the present study, activation of both ERK and p38 was inhibited by sorafenib treatment in virus infected cells. These results are consistent with recent reports in other virus systems and cancer cells (Himmelsbach et al., 2009; Ma et al., 2012; Michaelis et al., 2011). It is believed that blocking the ERK and p38 signal pathways with sorafenib may alleviate the expression of the COX-2/PGE_2 inflammatory factors. In addition, sorafenib markedly suppressed the CPE induced by EV71. Our finding provides a cluing to find anti-EV71 drug in clinical treatment of HFMD patients with severe CNS diseases, which acts on inhibition both viral replication and inflammatory factor expression. Validating this approach in mouse models prior to clinical trials on sick people is the next step, which is required to determine whether the proposed reduction in neurological complications is due to reduced COX2/PGE_2 expression and the drug is effective in reducing viral titer without adverse toxic effects in vivo.

In conclusion, this study has provided evidence that the clinically approved anti-cancer kinase inhibitor sorafenib inhibits EV71 replication and that one aspect of this is the drug's activity in regulating the IRES-dependent translation of viral proteins. Therefore, the findings offer a novel potential strategy for inhibiting EV71 replication which should be further evaluated with respect to its clinical potential for the treatment of HFMD patients with severe neurological complications.

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