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Inhibition of the Epstein–Barr virus lytic cycle by moronic acid

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ABSTRACT

Epstein–Barr virus (EBV) expresses two transcription factors, Rta and Zta, during the immediate-early stage of the lytic cycle to activate the transcription of viral lytic genes. Our immunoblotting and flow cytometry analyses find that moronic acid, found in galls of *Rhus chinensis* and *Brazilian propolis*, at 10 μM inhibits the expression of Rta, Zta, and an EBV early protein, EA-D, after lytic induction with sodium butyrate. This study also finds that moronic acids inhibits the capacity of Rta to activate a promoter that contains an Rta-response element, indicating that moronic acid interferes with the function of Rta. On the other hand, moronic acid does not appear to influence with the transactivation function of Zta. Therefore, the lack of expression of Zta and EA-D after moronic acid treatment is attributable to the inhibition of the transactivation functions of Rta. Because the expression of Zta, EA-D and many EBV lytic genes depends on Rta, the treatment of P3HR1 cells with moronic acid substantially reduces the numbers of EBV particles produced by the cells after lytic induction. This study suggests that moronic acid is a new structural lead for anti-EBV drug development.

1. Introduction

Epstein–Barr virus (EBV) is a human herpesvirus, which causes infectious mononucleosis and is associated with many malignant diseases, including nasopharyngeal carcinoma, B-cell and T-cell lymphomas (Henle and Henle, 1970; Jones et al., 1988; Wolf et al., 1973), and posttransplant lymphoproliferative diseases (Hopwood et al., 2002; Schwarzmann et al., 1998). When EBV enters the lytic cycle, the virus first expresses two transcription factors, Rta and Zta, which are encoded by BRLF1 and BZLF1, respectively (Biggin et al., 1987) to activate the transcription of EBV lytic genes (Holley-Guthrie et al., 1990; Kenney et al., 1989a,b). Earlier studies found that in the absence of either protein, the virus cannot complete its lytic cycle (Feederle et al., 2000; Chiu et al., 2007). As it is generally known, acyclovir and ganciclovir, two nucleotide analogs that inhibit the function of herpesvirus DNA polymerases, are used clinically to treat herpesvirus infections (Datta et al., 1980; Ernberg and Andersson, 1986). Our previous studies showed that (−)-epigallocatechin gallate (EGCG) from green tea and andrographolide from *A. paniculata* also inhibit the EBV lytic cycle (Chang et al., 2003; Lin et al., 2008). Recently, Wang et al. (2009) demonstrated that maribavir (MBV) is a potent and nontoxic drug that inhibits EBV lytic DNA replication. The mechanism of action of MBV may involve the inhibition of the transcription of EBV lytic genes.

Moronic acid, a triterpenoid keto acid (Fig. 1), is present abundantly in *Brazilian propolis* (Ito et al., 2001) and plant origins (Cao et al., 2004; Gu et al., 2007; Kurokawa et al., 1999; Rios et al., 2001). Hostettmann-Kaldas and Nakanishi (1979) demonstrated that moronic acid isolated from *Ozoroa mucronata* has antimicrobial activity. Meanwhile, moronic acid had anti-herpes simplex virus type 1 (HSV-1) activity in mice (Kurokawa et al., 1999). Our earlier investigation showed that moronic acid has anti-human immunodeficiency virus (HIV) activity with EC_{50} < 0.1 μg/ml and TI > 186 in H9 lymphocytes (Ito et al., 2001). Furthermore, one of the moronic derivatives, compound 20, has potent anti-HIV activity with EC_{50} values of 0.0156 and 0.0085 μM and TI > 2500 (Yu et al., 2006). So far, exactly how moronic acid inhibits HIV infection remains unknown. This study finds that moronic acid inhibits the function of Rta, thus blocking the progression of the EBV lytic cycle.

2. Materials and methods

2.1. Moronic acid

Moronic acid was isolated from galls of *Rhus chinensis*, which were purchased from the I-Chen Chinese Medicine Drugstore,

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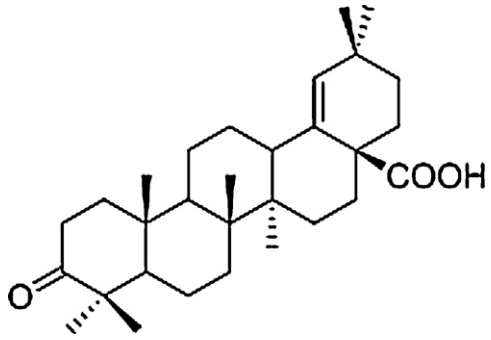

Fig. 1. Structure of moronic acid.

Kaohsiung. The voucher specimens were deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Taiwan. The galls (1 kg) were extracted three times with 2 L of methanol. The extract (150 g, wet weight) was then dried with a rotary evaporator. Moronic acid (20 mg) was isolated after repeated column chromatography procedures according to the method described by Ito et al. (2001). The structure of purified compound (Fig. 1) was verified by spectroscopic methods and mass spectrometry analysis.

2.2. Cell lines and induction of the EBV lytic cycle

An EBV-containing Burkitt's lymphoma cell line, P3HR1, was cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. An epithelial cell line from human kidney, 293T, was cultured in DMEM medium that was supplemented with 10% fetal calf serum. The EBV lytic cycle was induced by treating the cells with 3 mM sodium butyrate (SB) (Luka et al., 1979).

2.3. Cytotoxicity test

Toxicity of moronic acid to P3HR1 cells was determined using an LDH cytotoxicity detection kit (Clontech). P3HR1 cells (5×10^4) were seeded in wells of a 96-well plate and treated with different concentrations of moronic acid for 24 h. The cells were centrifuged and then 100 μl of cell-free supernatant was mixed with 100 μl of assay solution for 20 min at room temperature. LDH activity was determined spectrophotometrically at 490 nm. The reactions with added RPMI 1640 medium was used as a background control. The amount of LDH released from cells cultured in RPMI 1640 medium was used as a low control. The amount of LDH released from cells what were treated with 1% Triton X-100 was used as a high control. The percent cytotoxicity was calculated according to the instruction provided by the manufacturer, using an equation, (triplicate absorbance − low control/high control − low control) × 100. All assays were repeated at least three times.

2.4. Detecting EBV lytic proteins

Cell lysate was prepared from 3×10^6 P3HR1 cells with 100 μl lysis buffer that contained 50 mM Tris–HCl, pH 7.8, 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100 and 0.5% NP40 according to a method described elsewhere (Chang et al., 2004). Immunoblot analysis was performed according to a method reported previously (Chang et al., 2003) with monoclonal anti-Rta (Argene, Varilhes, France), anti-Zta (Argene) and anti-EA-D (Advanced Biotechnologies) antibodies. Polyclonal anti-EBNA-1 antibody was provided by M. Chao, Chang-Gung University. Monoclonal anti-α-tubulin antibody was purchased from Sigma (St. Louis, MO).

2.5. Flow cytometry analysis

P3HR1 cells (5×10^6) were treated with sodium butyrate and moronic acid for 24 h and washed with phosphate-buffered saline (PBS), followed by fixing with 4% paraformaldehyde for 30 min. Cells were then treated with PBS containing 0.1% Triton X-100 for 5 min, washed with PBS, treated with 1% BSA in PBS for 1 h, and incubated with 1:200-diluted monoclonal antibody anti-Rta antibody, monoclonal anti-EA-D antibody, or polyclonal rabbit anti-EBNA-1 antibody for 1 h at room temperature. Next, cells were washed with 0.5% Tween 20 in PBS, and incubated with 1:200-diluted goat anti-mouse IgG (Alexa Fluor 594, Invitrogen) to detect Rta and EA-D. Alternatively, cells were incubated with 1:200-diluted FITC-conjugated goat anti-rabbit immunoglobin G (Alexa Fluor 488, Invitrogen) to detect EBNA-1. Finally, cells were suspended in 1% paraformaldehyde and analyzed using a FACScan cytofluorometer (Bacton-Dickinson, USA).

2.6. RNA analysis

RNA was isolated from cells with RNeasy mini kit (Qiagen) according to the method recommended by the manufacturer. Reverse transcription was performed with random hexamers and M-MLV reverse transcriptase (Promega). PCR was performed by using primers 5′-CCATACAGGACACAACACCTCA and 5′-ACTCCCG-GCTGTAAATTCCT for the BRLF1–BZLF1 bicistronic mRNA; primers 5′-GAAGGTGAAGGTCGGAGT and 5′-GAAGATGGTGATGGGATTTC for GAPDH. The PCR was performed for 28–32 cycles under the conditions of 30 s at 94, 52 and 72 °C.

2.7. Plasmids

Plasmid pRRE, pCMV-3, pCMV-R and pCMV-Z were described earlier (Chang et al., 2005, 1998). Plasmid pZRE was constructed by inserting a PCR-amplified DNA fragment from the BHLF1 promoter (EBV genome 52,769–52,931; GenBank accession no. V01555) into the HindIII and XhoI sites in pGL2-Basic.

2.8. Transient transfection and luciferase assay

293T cells (1×10^4) were transfected with 0.2 μg of pRRE and pCMV-R or pZRE and pCMV-Z with Lipofectamin 2000 (Invitrogen). Cells were harvested by centrifugation and were washed with phosphate-buffered saline at 24 h after transfection. Cells were then lysed in 30 μl of lysis buffer containing 25 mM Tris-phosphate (pH 7.8), 2 mM dithiothreitol, 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid, 10% glycerol, and 1% Triton X-100. The lysate was centrifuged with a microcentrifuge at 11,750 × *g* for 5 min at room temperature. The activity of luciferase in the supernatant was determined using a luminometer (Orion II, Berthold) according to a method described elsewhere (Chang et al., 1998). Each transfection experiment was performed at least three times, and each sample in the experiment was prepared in duplicate.

2.9. Determining the DNA replication of the EBV genome

Cells (1×10^5) were treated with sodium butyrate and moronic acid at 5–15 μM for 48 h. EBV replication assay was performed as described (Wiedmer et al., 2008). Real-time PCR by using the StepOne real-time PCR system (Applied Biosystems) was conducted with primers 5′-TCGCCTTCTTTTATCCTCTTTTTG and 5′-CCCAACGGGCTAAAATGACA that amplified the *oriLyt* region in EBV. Actin DNA was amplified with primers 5′-ATTGCC-GACAGGATGCAGAA and 5′-GCTGATCCACATCTGCTGGAA.

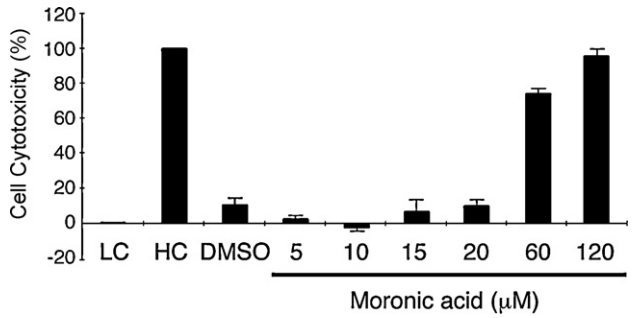

Fig. 2. Toxicity of moronic acid to P3HR1 cells. Cell cytotoxicity was determined using the LDH method. P3HR1 cells were treated with different concentrations of moronic acid for 24 h and the viability of the cell was determined by LDH assay. DMSO was used as a negative control. Low control (LC) is the LDH release from untreated cells; high control (HC) is the maximum LDH activity that is exhibited by the cells that are treated with 1% of Triton X-100. The data are presented as mean ± S.D. from at least three independent experiments.

2.10. Determining the copy number of the EBV genome

After lytic induction, P3HR1 cells were cultured for 5 days. EBV particles released into the culture medium were harvested by ultracentrifugating at 25,000 × *g* for 2 h. The pellet was suspended in TNE buffer (10 mM Tri–HCl pH 7.4, 100 mM NaCl and 1 mM EDTA), treated with DNase I and proteinase K before the lysate was extracted with phenol. EBV DNA in the aqueous fraction was precipitated with ethanol and the amount of EBV DNA was determined by real-time PCR according to a method described previously (Chiu et al., 2007). The copy number of the EBV genome was calculated using maxi-EBV DNA extracted from *Escherichia coli* as a reference. The molecular weight of maxi-EBV is about 1.2×10^7 and 1 ng of maxi-EBV equals to 5.05×10^6 copies of the maxi-EBV genome.

2.11. Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) using the SAS JMP 6.0 software package. Data are presented as means ± S.D. and a p value of <0.05 was regarded significant.

3. Results

3.1. Toxicity of moronic acid to P3HR1 cells

The toxicity of moronic acid to P3HR1 cells was determined by using the LDH cytotoxicity assay. Moronic acid at 15 and 20 μM killed 6.5% and 11.8% of P3HR1 cells, respectively (Fig. 2). The CC_{50} against P3HR1 cells is 46.67 μM (Fig. 2).

3.2. Inhibition of the expression of EBV lytic proteins by moronic acid

To investigate how moronic acid affected the EBV lytic cycle, P3HR1 cells (1×10^6) were treated with sodium butyrate for 24 h. As expected, three EBV lytic proteins, Rta, Zta, and EA-D, were expressed after induction and detectable by immunoblotting (Fig. 3A and B, lane 2). Meanwhile, adding 1 and 5 μM of moronic acid partially inhibited the expression of these three proteins (Fig. 3A, lanes 3 and 4); the expression of Rta, Zta and EA-D was substantially reduced by the treatment with 10 and 20 μM moronic acid (Fig. 3A, lanes 5 and 6). On the other hand, the expression of an EBV latent protein, EBNA-1, was unaffected by the treatment (Fig. 3A). This study also showed that treating P3HR1 cells with moronic acid alone did not result in the expression of Rta, Zta and EA-D (Fig. 3B, lanes 3–6), indicating that moronic acid does not lytically activate EBV. In addition, moronic acid inhibited Rta expression in lytically induced-P3HR1 cells with an EC_{50} value of 3.153 μM and a TI value of 14.8.

3.3. Flow cytometry analysis of EBV proteins

The inhibitory effects of moronic acid on the expression of EBV proteins were examined by quantitative flow cytometry analysis. The population of P3HR1 cells that expressed Rta and EA-D was 17.6% and 13.7%, respectively, following sodium butyrate treatment (Fig. 4). Adding 5 μM of moronic acid before lytic induction decreased the population that expressed Rta and EA-D to 13.8% and 12.7%; adding 15 μM, 6.1% and 5.6%, respectively (Fig. 4). However, the expression of EBNA-1 was unaffected if the cells were treated with sodium butyrate or moronic acid (Fig. 4).

3.4. Inhibition of BRLF1 transcription by moronic acid

This study performed RT-PCR to examine if moronic acid affects the transcription of bicistronic BRLF1–BZLF1 mRNA. P3HR1 cells were treated with sodium butyrate for 24 h to induce the lytic cycle. As expected, the BRLF1–BZLF1 mRNA was transcribed after the treatment (Fig. 5, lane 2). However, treating the cells with 5–15 μM of moronic acid resulted in a decrease of the amount of BRLF1–BZLF1 mRNA in the cell in a dose dependent manner (Fig. 5, lanes 3–5). Expression of GAPDH was unaffected by the treatment of moronic acid (Fig. 5).

3.5. Influence of the transactivation activity of Rta by moronic acid

To elucidate the mechanism by which moronic acid inhibits the EBV lytic cycle, pCMV-R and a reporter plasmid pRRE that contains an Rta-response element (RRE) were cotransfected into 293T cells. Assaying the luciferase activity exhibited by the cells revealed that

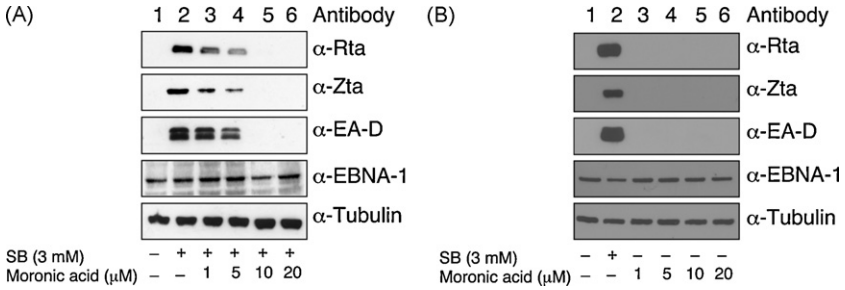

Fig. 3. Effect of moronic acid treatment on the expression of Rta, Zta, EA-D, and EBNA-1 by EBV. P3HR1 cells were treated with moronic acid and sodium butyrate (SB) (A). Cells were also untreated with SB but treated with moronic acid (B). Expression of Rta, Zta, EA-D, and EBNA-1 was examined by immunoblotting at 24 h after the treatment. Amount of α-tubulin was used as a control.

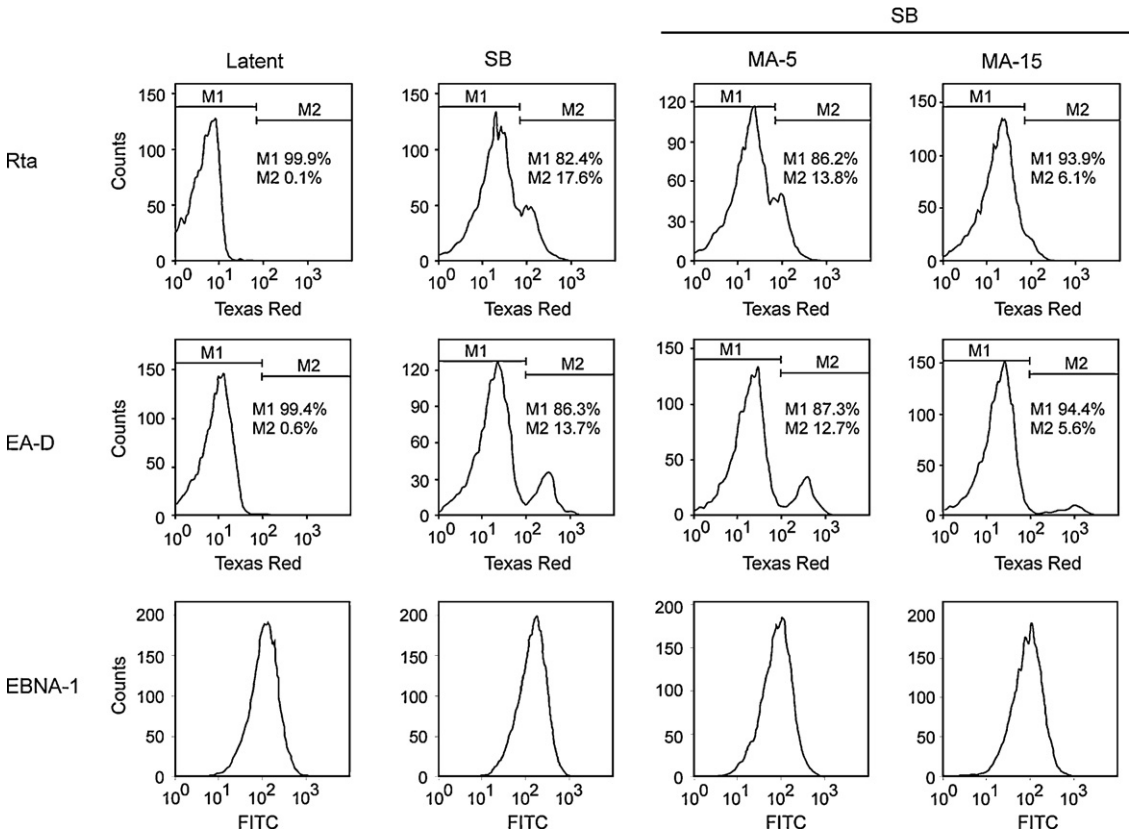

Fig. 4. Flow cytometry analysis of the expression of Rta, EA-D and EBNA-1. P3HR1 cells were treated with 5 μM (MA-5) or 15 μM (MA-15) moronic acid and 3 mM sodium butyrate (SB). After culturing for 24 h, cells were incubated with monoclonal anti-Rta and monoclonal anti-EA-D antibodies, and finally stained with secondary anti-mouse IgG-Texas Red-conjugated antibody. Cells were also incubated with polyclonal anti-EBNA-1 antibody and finally stained with secondary anti-rabbit IgG-FITC-conjugated antibody. M1 and M2 gate on uninduced- and induced-P3HR1 cells, respectively.

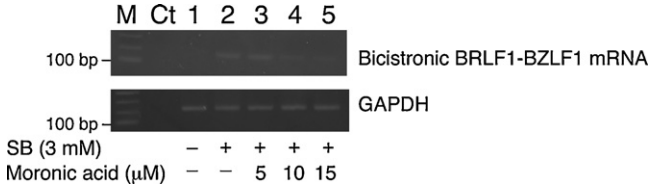

Fig. 5. Effect of moronic acid on the transcription of BRLF1–BZLF1 mRNA. P3HR1 cells (5×10^6) were treated with 5–15 μM of moronic acid before lytic induction of sodium butyrate (SB). Total RNA was extracted and reversed transcribed into cDNA. PCR was performed to examine the expression of bicistronic BRLF1–BZLF1 mRNA. The expression of GAPDH was used as an internal control. Ct: PCR reactions without reverse transcriptases; M: 100 bp marker.

adding 5, 10 and 15 μM of moronic acid decreased the capacity of Rta to activate the promoter 17%, 32% and 59%, respectively (Fig. 6A). On the other hand, moronic acid at these concentrations affected little of Zta's capacity to activate a reporter plasmid that contains a Zta-response element (ZRE) (Fig. 6B). These results showed that moronic acid inhibits the transactivation activity of Rta but not Zta.

3.6. Inhibition of the lytic replication by moronic acid

P3HR1 cells were treated with sodium butyrate and 5–15 μM of moronic acid to examine whether moronic acid influences viral lytic DNA replication. The result showed that replication of viral DNA decreased 16%, 47%, and 61% after the treatment with 5, 10, and 15 μM of moronic acid, respectively (Fig. 7A).

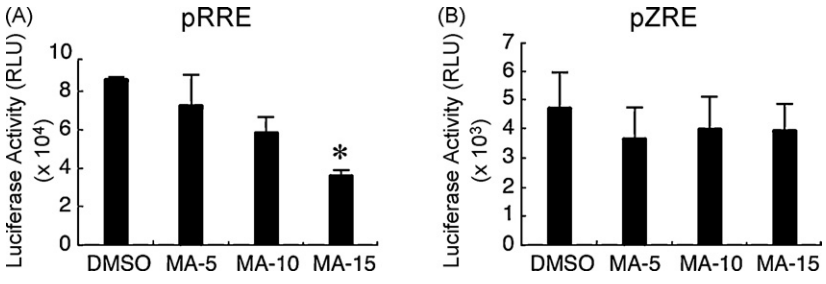

Fig. 6. Influence of moronic acid on the transactivation activities of Rta and Zta. Plasmids pRRE (A) and pZRE (B) were cotransfected with pCMV-R and pCMV-Z, respectively, into 293T cells in the presence of 5 μM (MA-5), 10 μM (MA-10), and 15 μM (MA-15) of moronic acid. Luciferase activity exhibited by the plasmids was monitored at 24 h after treatment. Each transfection experiment was repeated three times and each sample was prepared in duplicate in the experiment. $^*p < 0.05$, which represents a significant difference from the value of the control group; RLU: relative light units.

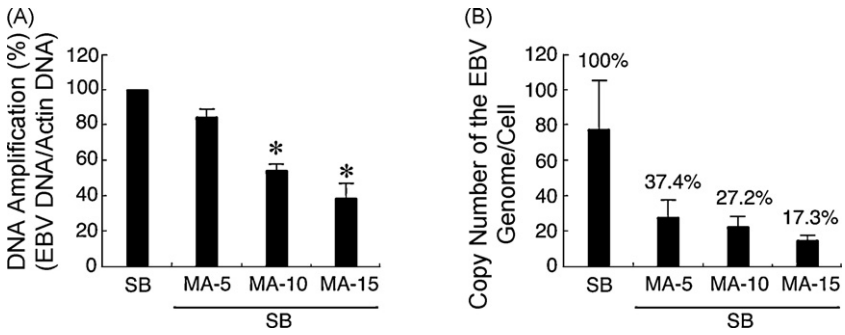

Fig. 7. Effect of moronic acid on EBV lytic cycle. (A) P3HR1 cells were treated with sodium butyrate (SB) and 5–15 μM of moronic acid for 48 h. Cells were lysed and EBV lytic DNA replication was assayed by real-time PCR analysis. The amount of EBV DNA was normalized with the amount of actin DNA that was determined in the same assay. *$p < 0.05$, which represents a significant difference from the value of the control group. (B) P3HR1 cells were treated with 5–15 μM of moronic acid in the presence of sodium butyrate for five days. EBV DNA from viral particles that were released into the culture medium was determined by real-time PCR after the DNA extraction. The copy number of EBV genome was calculated by using maxi-EBV that had been isolated from *E. coli* as a standard.

3.7. Inhibition of the production of the EBV virion

EBV particles produced by P3HR1 cells were determined five days after lytic induction. As expected, treating P3HR1 cells with sodium butyrate caused the production of EBV particles (Fig. 7B). Treating the cells with 5, 10 and 15 μM of moronic acid reduced the numbers of viral particles 62.6%, 72.8% and 82.7%, respectively, indicating that moronic acid inhibits the production of EBV particles (Fig. 7B).

4. Discussion

Drugs that inhibit the function of herpesvirus-encoded DNA polymerase, including acyclic nucleoside analogues, acyclic nucleotide analogues and pyrophosphate analogues, are commonly used for inhibiting the productive cycle of EBV (Gershburg and Pagano, 2005). On the other hand, drugs that target proteins other than DNA polymerases have also been demonstrated to be effective in inhibiting the EBV lytic cycle. These drugs include maribavir, which inhibits the phosphorylation of EA-D, a DNA replication accessory protein that is needed for EBV lytic DNA replication (Gershburg and Pagano, 2002; Wang et al., 2009), indolocarbazole (K252a), which inhibits autophosphorylation of the EBV protein kinase (Gershburg et al., 2004). Additionally, EGCG and andrographolide inhibit the expression of EBV immediate-early proteins (Chang et al., 2003; Lin et al., 2008).

Earlier studies demonstrated that moronic acid has an $EC_{50} < 0.1$ μg/ml, TI > 186 and inhibits HIV activity in H9 lymphocytes (Ito et al., 2001; Yu et al., 2006). This work finds that moronic acid has cytotoxicity toward P3HR1 cells, with the values of EC50 = 3.153 μM, TI = 14.8 (Fig. 2). Although the EC50 value in P3HR1 cells is higher than that in H9 lymphocytes, the working concentrations of many anti-EBV drugs are higher than that of moronic acid at the values of 50 μM for ACV and 20 μM for MBV (Wang et al., 2009).

This study performed immunoblotting and flow cytometry analyses and verifies that the synthesis of three EBV lytic proteins, e.g. Rta, Zta and EA-D, is inhibited by moronic acid (Figs. 3 and 4). Additionally, this study examines the transcription of the BRLF1–BZLF1 bicistronic mRNA to assess the inhibitory effects of moronic acid on the expression of EBV lytic genes. The BRLF1–BZLF1 bicistronic mRNA is transcribed from the BRLF1 promoter and the transcription is strongly activated by Rta (autoregulation) and Zta (Biggin et al., 1987; Manet et al., 1989; Ragoczy and Miller, 2001; Sinclair et al., 1991). The transcription of this mRNA species leads to the expression of Rta and Zta, which is critical to the activation and expression of EBV lytic genes (Holley-Guthrie et al., 1990; Kenney et al., 1989a,b). The fact that moronic acid inhibits the transcription of BRLF1–BZLF1 mRNA (Fig. 5) suggests that the compound interferes with the immediate-early functions of EBV at the onset of the lytic cycle. Moreover, the fact that the transactivation activity of Rta is inhibited by moronic acid (Fig. 6) explains why the transcription of BRLF1–BZLF1, which is activated by Rta, is inefficient (Fig. 5), the level of EBV lytic DNA replication decreases (Fig. 7A), and the production of the viral particles reduces (Fig. 7B). An earlier study (Manet et al., 1993) showed that when binding to an RRE, Rta directly interacts with TBP and TFIIB to activate transcription. Moronic acid likely inhibits the function of Rta directly rather than that of TBP or TFIIB because moronic acid has little effects on the transcription promoted by Zta, which also interacts with basal transcription factors (Lieberman, 1994). This study shows that moronic acid has a mode-of-action differs from that of ACV and MBV to inhibit the EBV lytic cycle.

The intensive use of nucleoside analogs to treat herpesvirus infection will inevitably create virus resistant to these drugs (Field and Biron, 1994; van der Horst et al., 1991). Thus, moronic acid, which targets the function of an important EBV transcription factor, Rta, is an alternative to nucleotide analogs for treating herpesvirus infections.

Acknowledgments

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References

Biggin, M., Bodescot, M., Perricaudet, M., Farrell, P., 1987. Epstein–Barr virus gene expression in P3HR1-superinfected Raji cells. J. Virol. 61 (10), 3120–3132.

Cao, S., Guza, R.C., Miller, J.S., Andriantsiferana, R., Rasamison, V.E., Kingston, D.G., 2004. Cytotoxic triterpenoids from *Acridocarpus vivy* from the Madagascar rain forest. J. Nat. Prod. 67 (6), 986–989.

Chang, L.K., Chung, J.Y., Hong, Y.R., Ichimura, T., Nakao, M., Liu, S.T., 2005. Activation of Sp1-mediated transcription by Rta of Epstein–Barr virus via an interaction with MCAF1. Nucleic Acids Res. 33 (20), 6528–6539.

Chang, L.K., Lee, Y.H., Cheng, T.S., Hong, Y.R., Lu, P.J., Wang, J.J., Wang, W.H., Kuo, C.W., Li, S.S., Liu, S.T., 2004. Post-translational modification of Rta of Epstein–Barr virus by SUMO-1. J. Biol. Chem. 279 (37), 38803–38812.

Chang, L.K., Wei, T.T., Chiu, Y.F., Tung, C.P., Chuang, J.Y., Hung, S.K., Li, C., Liu, S.T., 2003. Inhibition of Epstein–Barr virus lytic cycle by (−)-epigallocatechin gallate. Biochem. Biophys. Res. Commun. 301 (4), 1062–1068.

Chang, P.J., Chang, Y.S., Liu, S.T., 1998. Role of Rta in the translation of bicistronic BZLF1 of Epstein–Barr virus. J. Virol. 72 (6), 5128–5136.

Chiu, Y.F., Tung, C.P., Lee, Y.H., Wang, W.H., Li, C., Hung, J.Y., Wang, C.Y., Kawaguchi, Y., Liu, S.T., 2007. A comprehensive library of mutations of Epstein–Barr virus. J. Gen. Virol. 88, 2463–2472.

Datta, A.K., Colby, B.M., Shaw, J.E., Pagano, J.S., 1980. Acyclovir inhibition of Epstein–Barr virus replication. Proc. Natl. Acad. Sci. U.S.A. 77 (9), 5163–5166.
Ernberg, I., Andersson, J., 1986. Acyclovir efficiently inhibits oropharyngeal excretion of Epstein–Barr virus in patients with acute infectious mononucleosis. J. Gen. Virol. 67, 2267–2272.
Feederle, R., Kost, M., Baumann, M., Janz, A., Drouet, E., Hammerschmidt, W., Delecluse, H.J., 2000. The Epstein–Barr virus lytic program is controlled by the co-operative functions of two transactivators. EMBO J. 19 (12), 3080–3089.
Field, A.K., Biron, K.K., 1994. "The end of innocence" revisited: resistance of herpesviruses to antiviral drugs. Clin. Microbiol. Rev. 7 (1), 1–13.
Gershburg, E., Hong, K., Pagano, J.S., 2004. Effects of maribavir and selected indolocarbazoles on Epstein–Barr virus protein kinase BGLF4 and on viral lytic replication. Antimicrob. Agents Chemother. 48 (5), 1900–1903.
Gershburg, E., Pagano, J.S., 2002. Phosphorylation of the Epstein–Barr virus (EBV) DNA polymerase processivity factor EA-D by the EBV-encoded protein kinase and effects of the L-riboside benzimidazole 1263W94. J. Virol. 76 (3), 998–1003.
Gershburg, E., Pagano, J.S., 2005. Epstein–Barr virus infections: prospects for treatment. J. Antimicrob. Chemother. 56 (2), 277–281.
Gu, Q., Wang, R.R., Zhang, X.M., Wang, Y.H., Zheng, Y.T., Zhou, J., Chen, J.J., 2007. A new benzofuranone and anti-HIV constituents from the stems of *Rhus chinensis*. Planta Med. 73 (3), 279–282.
Henle, G., Henle, W., 1970. Observations on childhood infections with the Epstein–Barr virus. J. Infect. Dis. 121 (3), 303–310.
Holley-Guthrie, E.A., Quinlivan, E.B., Mar, E.C., Kenney, S., 1990. The Epstein–Barr virus (EBV) BMRF1 promoter for early antigen (EA-D) is regulated by the EBV transactivators, BRLF1 and BZLF1, in a cell-specific manner. J. Virol. 64 (8), 3753–3759.
Hopwood, P.A., Brooks, L., Parratt, R., Hunt, B.J., Bokhari, M., Thomas, J.A., Yacoub, M., Crawford, D.H., 2002. Persistent Epstein–Barr virus infection: unrestricted latent and lytic viral gene expression in healthy immunosuppressed transplant recipients. Transplantation 74 (2), 194–202.
Hostettmann-Kaldas, M., Nakanishi, K., 1979. Moronic acid, a simple triterpenoid keto acid with antimicrobial activity isolated from *Ozoroa mucronata*. Planta Med. 37 (4), 358–360.
Ito, J., Chang, F.R., Wang, H.K., Park, Y.K., Ikegaki, M., Kilgore, N., Lee, K.H., 2001. Anti-AIDS agents. 48. (1) Anti-HIV activity of moronic acid derivatives and the new melliferone-related triterpenoid isolated from *Brazilian propolis*. J. Nat. Prod. 64 (10), 1278–1281.
Jones, J.F., Shurin, S., Abramowsky, C., Tubbs, R.R., Sciotto, C.G., Wahl, R., Sands, J., Gottman, D., Katz, B.Z., Sklar, J., 1988. T-cell lymphomas containing Epstein–Barr viral DNA in patients with chronic Epstein–Barr virus infections. N. Engl. J. Med. 318 (12), 733–741.
Kenney, S., Holley-Guthrie, E., Mar, E.C., Smith, M., 1989a. The Epstein–Barr virus BMLF1 promoter contains an enhancer element that is responsive to the BZLF1 and BRLF1 transactivators. J. Virol. 63 (9), 3878–3883.
Kenney, S., Kamine, J., Holley-Guthrie, E., Mar, E.C., Lin, J.C., Markovitz, D., Pagano, J., 1989b. The Epstein–Barr virus immediate-early gene product, BMLF1, acts in trans by a posttranscriptional mechanism which is reporter gene dependent. J. Virol. 63 (9), 3870–3877.
Kurokawa, M., Basnet, P., Ohsugi, M., Hozumi, T., Kadota, S., Namba, T., Kawana, T., Shiraki, K., 1999. Anti-herpes simplex virus activity of moronic acid purified from *Rhus javanica* in vitro and in vivo. J. Pharmacol. Exp. Ther. 289 (1), 72–78.
Lieberman, P., 1994. Identification of functional targets of the Zta transcriptional activator by formation of stable preinitiation complex intermediates. Mol. Cell. Biol. 14, 8365–8375.
Lin, T.P., Chen, S.Y., Duh, P.D., Chang, L.K., Liu, Y.N., 2008. Inhibition of the Epstein–Barr virus lytic cycle by andrographolide. Biol. Pharm. Bull. 31 (11), 2018–2023.
Luka, J., Kallin, B., Klein, G., 1979. Induction of the Epstein–Barr virus (EBV) cycle in latently infected cells by n-butyrate. Virology 94 (1), 228–231.
Manet, E., Gruffat, H., Trescol-Biemont, M.C., Moreno, N., Chambard, P., Giot, J.F., Sergeant, A., 1989. Epstein–Barr virus bicistronic mRNAs generated by facultative splicing code for two transcriptional trans-activators. EMBO J. 8, 1819–1826.
Manet, E., Allera, C., Gruffat, H., Mikaelian, I., Rigolet, A., Sergeant, A., 1993. The acidic activation domain of the Epstein–Barr virus transcription factor R interacts in vitro with both TBP and TFIIB and is cell-specifically potentiated by a proline-rich region. Gene Exp. 3, 49–59.
Ragoczy, T., Miller, G., 2001. Autostimulation of the Epstein–Barr virus BRLF1 promoter is mediated through consensus Sp1 and Sp3 binding sites. J. Virol. 75, 5240–5251.
Rios, M.Y., Salina, D., Villarreal, M.L., 2001. Cytotoxic activity of moronic acid and identification of the new triterpene 3,4-seco-olean-18-ene-3,28-dioic acid from *Phoradendron reichenbachianum*. Planta Med. 67 (5), 443–446.
Sinclair, A.J., Brimmell, M., Shanahan, F., Farrell, P.J., 1991. Pathways of activation of the Epstein–Barr virus productive cycle. J. Virol. 65, 2237–2244.
Schwarzmann, F., Jager, M., Hornef, M., Prang, N., Wolf, H., 1998. Epstein–Barr viral gene expression in B-lymphocytes. Leuk. Lymphoma 30 (1–2), 123–129.
van der Horst, C., Joncas, J., Ahronheim, G., Gustafson, N., Stein, G., Gurwith, M., Fleisher, G., Sullivan, J., Sixbey, J., Roland, S., et al., 1991. Lack of effect of peroral acyclovir for the treatment of acute infectious mononucleosis. J. Infect. Dis. 164 (4), 788–792.
Wang, F.Z., Roy, D., Gershburg, E., Whitehurst, C.B., Dittmer, D.P., Pagano, J.S., 2009. Maribavir inhibits epstein–Barr virus transcription in addition to viral DNA replication. J. Virol. 83 (23), 12108–12117.
Wiedmer, A., Wang, P., Zhou, J., Rennekamp, A.J., Tiranti, V., Zeviani, M., Lieberman, P.M., 2008. Epstein–Barr virus immediate-early protein Zta co-opts mitochondrial single-stranded DNA binding protein to promote viral and inhibit mitochondrial DNA replication. J. Virol. 82 (9), 4647–4655.
Wolf, H., zur Hausen, H., Becker, V., 1973. EB viral genomes in epithelial nasopharyngeal carcinoma cells. Nat. New Biol. 244 (138), 245–247.
Yu, D., Sakurai, Y., Chen, C.H., Chang, F.R., Huang, L., Kashiwada, Y., Lee, K.H., 2006. Anti-AIDS agents 69. Moronic acid and other triterpene derivatives as novel potent anti-HIV agents. J. Med. Chem. 49 (18), 5462–5469.