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Antiviral activity of topoisomerase II catalytic inhibitors against Epstein–Barr virus

Ting Wu [a,b], Yan Wang [a,c,*], Yan Yuan [a,b,d,*]

[a] Institute of Human Virology, Ministry of Education, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, China
[b] Key Laboratory of Tropical Disease Control, Ministry of Education, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, China
[c] Guanghua School of Stomatology, Sun Yat-Sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangzhou, China
[d] Department of Microbiology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA, USA

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ABSTRACT

Herpesviruses require several cellular proteins for their lytic DNA replication including topoisomerase II (Topo II). Thus, Topo II could be an effective drug target against herpesviral infection. In this study, we examined several Topo II catalytic inhibitors for their potentials in blocking EBV replication and becoming efficacious antiviral agents. Topo II catalytic inhibitors in general exhibited marked inhibition of EBV lytic replication and minimal cytotoxicity. In particular, (+)-rutamarin, with the best selectivity index (SI > 63) among the inhibitors tested in this study, is effective in inhibiting EBV DNA replication and virion production but shows little adverse effect on cell proliferation, suggesting its potential to become an efficacious and safe drug for the treatment of human diseases associated with EBV infection.

1. Introduction

Epstein–Barr virus (EBV) is a human gamma-herpesvirus that infects more than 90% of the human population (Cohen, 2000). EBV is an important pathogen in humans responsible for a number of diseases including infectious mononucleosis, oral hairy leukoplakia, AIDS immunoblastic lymphomas, posttransplant lymphoproliferative disease, 50% of Hodgkin's lymphomas, and the endemic forms of nasopharyngeal carcinoma and Burkitt's lymphoma (Young and Rickinson, 2004).

Like other herpesviruses, EBV exhibits two distinct life cycles: latency and lytic viral replication. In latency, only a few latent viral genes are expressed (dependent on types of EBV latency), which does not result in release of viral particles. In contrast, lytic viral replication leads to expression of all viral proteins and production of progeny virions (reviewed in Kieff and Rickinson, 2007). Although much of the EBV-induced pathology has been attributed to viral latency, the importance of EBV lytic replication in viral pathogenicity has been increasingly recognized. New studies are emerging which implicate reactivation of lytic virus, and specifically the lytic activator protein Zta, in tumorigenesis and autoimmune diseases (Gross et al., 2005; Hong et al., 2005; Ma et al., 2011). EBV lytic replication is directly linked to infectious mononucleosis, chronic active EBV infection (CAEBV), oral hairy leukoplakia in immunosuppressed individuals and to an increased risk of EBV-associated nasopharyngeal carcinoma (Al Tabaa et al., 2011; Dardari et al., 2000; Kimura et al., 2001; Lau et al., 1993). Therefore, inhibition of EBV lytic replication may be a strategy for treatment of some EBV-associated diseases.

A few drugs have been developed against alpha- and beta-herpesvirus (Andrei and Snoeck, 2011; Kimberlin and Whitley, 2007; Komatsu et al., 2014). However, there are no effective drugs available to treat gamma-herpesvirus (KSHV and EBV) infection. Given that EBV is associated with many malignant and non-malignant human diseases, efficacious drugs against EBV are very much needed.

EBV lytic DNA replication initiates at an origin (ori-Lyt) and requires trans-acting elements, both viral and cellular (Hammerschmidt and Sugden, 1988). Host cellular proteins topoisomerases I and II (Topo I and II) have been reported to be essential for gamma-herpesvirus lytic DNA replication (Kawanishi, 1993; Wang et al., 2009, 2008), raising a possibility that certain inhibitors to Topo I and II may have potentials to become antivirals against

* Corresponding authors. Address: Institute of Human Virology, Ministry of Education, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, China (Y. Wang). Address: Department of Microbiology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA, USA. Tel.: +1 2155737556; fax: +1 2158988385 (Y. Yuan).

E-mail addresses: wang93@mail.sysu.edu.cn (Y. Wang), yuan2@pobox.upenn.edu (Y. Yuan).

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EBV infection. We intended to explore the values of inhibitors of Topo II as potential antiviral substances against gamma-herpesviruses including EBV. Topoisomerase II inhibitors are split into two categories: Topo II poisons, which target the topoisomerase-DNA intermediate and Topo II catalytic inhibitors, which disrupt turnover of the enzyme (Gonzalez-Molleda et al., 2012). Etoposide and doxorubicin are Topo II poisons which have been used as antitumor drugs (Blum and Carter, 1974; Meresse et al., 2004). In our previous study, Topo II poisons were shown to effectively inhibit KSHV replication and virion production, but exhibiting considerable cytotoxicities (Gonzalez-Molleda et al., 2012). In contrast, Topo II catalytic inhibitors, novobiocin and merbarone, exhibited marked inhibition on KSHV replication and minimal cytotoxicity, suggesting that this category of Topo II inhibitors have better potentials to become antivirals against the infection of KSHV or other gamma-herpesviruses (Gonzalez-Molleda et al., 2012). In this report, we have been exploring the possibility of Topo II to serve as an anti-EBV drug target and the potential of their catalytic inhibitors to become therapeutic agents in treatment of EBV-associated diseases.

2. Materials and methods

2.1. Cells and plasmids

The P3HR-1 is a EBV-positive Burkitt lymphoma cell line, a clonally derived subline of Jiyoye (Hinuma et al., 1967). Akata-Bx1 cell line carries a recombinant EBV where the thymidine kinase gene was replaced by a CMV immediate early promoter driven GFP (Guerreiro-Cacais et al., 2007) and is kindly provided by Dr. Musheng Zeng at Sun Yat-sen University Cancer Center. Cells were cultured in RPMI 1640 medium (Gibico-BRL, Gaithersburg, MD), supplemented with 10% FBS (Gibico-BRL), penicillin (100 U/ml), streptomycin (100 μg/ml).

The EBV ori-Lyt plasmid pEBV-oriL was constructed by cloning a 1434 bp fragment carrying EBV ori-Lyt (nucleotides 52385–53819 of EBV genome) into pUC18 vector. The plasmid McZ is a ZTA expression vector in the backbone of pBXG1 and a gift from Dr. George Miller at Yale University.

2.2. Chemicals and cell treatment

Novobiocin was purchased from Sigma–Aldrich (St. Louis, MO) and merbarone was purchased from Merck Millipore. (+)-Rutamarin (Fig. 1) is extracted from natural plant Ruta graveolens L, supplied by the Guangdong Small Molecule Tangible Library (GSMTL) (Gu et al., 2010). Novobiocin was prepared as aqueous stock, while the others were dissolved in dimethyl sulfoxide (DMSO).

For induction of EBV lytic replication, P3HR-1 cells (4×10^5 cells/ml of culture) were treated with 20 ng/ml 12-O-tetradecanoylphorbol 13-acetate (TPA; Sigma–Aldrich) and 3 mM sodium butyrate (Sigma–Aldrich). Novobiocin, merbarone, and (+)-rutamarin in different concentrations was added to P3HR-1 cells three hours after induction. Akata-Bx1 cells were resuspended at 2×10^6/ml in fresh medium and treated with 0.8%(V/V) goat anti-human IgG (Jackson ImmunoResearch). Three hours post-induction, Akata-Bx1 cells were adjusted to the density of 4×10^5 cells/ml and treated with various concentrations of novobiocin, merbarone, and (+)-rutamarin. The effects of these inhibitors on viral DNA content and virion production were assayed at different time points.

2.3. Analysis of intracellular EBV genomic DNA content and chemical effects

Two days post-treatment, cells were collected from induced and uninduced cultures and total DNA was purified from the cells using the TaKaRa MiniBEST Universal Genomic DNA Extraction Kit (TaKaRa). EBV genomic DNA was quantified by real-time PCR on a Roche Light Cycler II instrument using the Lightcycler 480 SYBR green I Master kit with primers directed to EBNA1 (forward, 5′-CATTGAGTCGTCTCCCCTTTGGAAT-3′; reverse, 5′-TCATAACAA GGTCCTTAATCGCATC-3′). The intracellular viral genomic DNA in each sample was normalized with the amount of GAPDH determined also by real-time DNA PCR by using primers directed to GAPDH (forward, 5′-AGCCACATCGCTCAGACAC-3′; reverse, 5′-GCCCAATACGACCAAATCC-3′). EBV DNA content value from induced cells was subtracted by that from uninduced cells. These corrected values were divided by those from the control, non-drug treatment and then represented on the y axes of dose–response curves: y axis value = $(\text{INDUCE}_X - \text{NONINDUCE}_X)/(\text{INDUCE}_0 - \text{NONINDUCE}_0)$, where INDUCE means TPA + Butyrate treatment or anti-IgG induction, X is any concentration of the drug and 0 represents non-treatment. The 50% DNA replication inhibitory concentration (IC_{50}) for each compound was calculated from the dose–response curve with the aid of Graphpad Prism software.

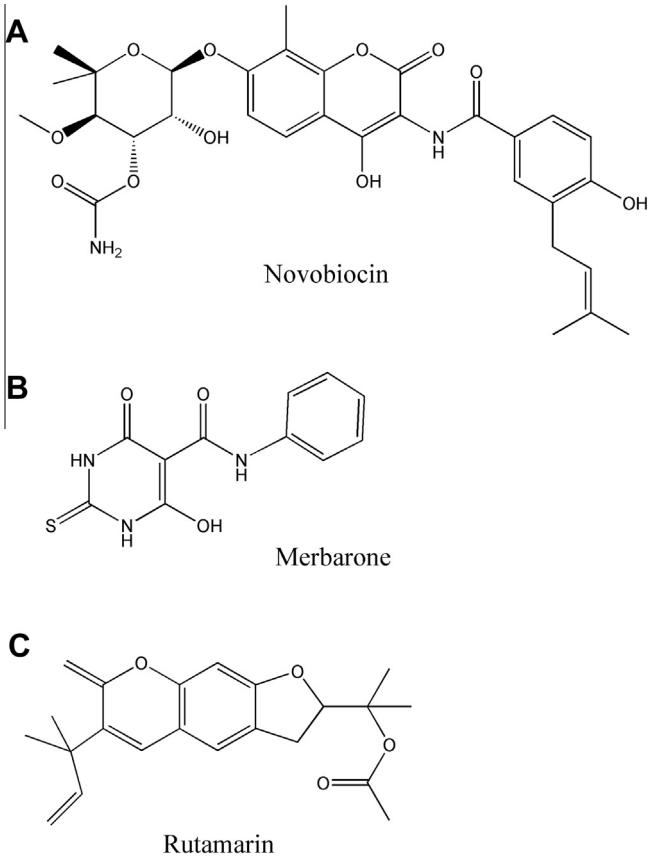

Fig. 1. Chemical structure of novobiocin, merbarone and (+)-rutamarin.

2.4. Analysis of extracellular EBV virion production and chemical effects

Five days post-induction, cell culture media were collected and virion particles were cleared by passing through 0.45 μm filters and extracellular virions were then pelleted from the medium supernatant of the cultures as detailed earlier (Gonzalez-Molleda et al., 2012) and were resuspended in 1× phosphate-buffered saline (PBS) in 1/100 of the original volume. To remove any contaminating DNA outside viral particles, the concentrated viruses were treated with DNase I (TaKaRa) at 37 °C for 1 h followed by

proteinase K digestion. The amounts of virion particles from the media were determined by quantifying encapsidated viral DNA by real-time PCR, and values were corrected as described above. The 50% antiviral effective concentration (EC_{50}) for each compound was calculated from the dose–response curve with the aid of GraphPad Prism software.

2.5. Cytotoxicity assay

The cell viabilities of P3HR-1 and Akata-Bx1 cells after treated or untreated with chemicals were assessed by counting Trypan blue stained cells 2 or 5 days post treatment using a light microscope. Cell viabilities were defined relative to control cells (non-drug treated) and represented on the *y* axes of dose–response curves: *y* axis value = $NONIDUCE_X/NONINDUCE_0$, where *X* is any drug concentration and 0 represents non-treatment. The 50% cytotoxic concentration (CC_{50}) for each compound was calculated from these dose–response curves with the aid of Graphpad Prism software.

2.6. Cell proliferation assay

P3HR-1 cells (starting with 2×10^5 cells/ml) were treated with topoisomerase inhibitors for 5 days at two different concentrations: IC_{50} and an excess concentration ($5 \times IC_{50}$). Cell samples were daily collected, stained with Trypan blue, and counted. To provide a constant cellular growth, fresh medium (supplemented with or without the drug) was added to these cultures every 2 days.

2.7. Transient-transfection DNA replication assay

To assay the effect of each compound on ori-Lyt-dependent DNA replication, P3HR-1 cells (1×10^7) were transfected with plasmids pEBV-oriL (2.5 μg) and McZ (2.5 μg) by nucleoporation (Amaxa) and cultured in the media with each drug in a wide range of concentration. Seventy-two hours post-transfection, extrachromosomal DNAs were prepared from cells using the Hirt DNA extraction method (Hirt, 1967). Cells were lysed in 700 μl lysis buffer (10 mM Tris–HCl [pH 7.4], 10 mM EDTA, and 0.6% SDS). Chromosomal DNA was precipitated at 4 °C overnight by adding 5 M NaCl to a final concentration of 0.85 M. Cell lysates were centrifuged at 4 °C at 14,000 rpm for 30 min. The supernatant containing extrachromosomal DNA was subjected to phenol–chloroform extraction, followed by ethanol precipitation. The DNA was treated with RNase A at 25 °C for 30 min and then with proteinase K at 50 °C for 30 min. Twelve micrograms of DNA was digested with EcoR I or EcoR I/DpnI (TaKaRa). The DNAs were separated by electrophoresis on 0.9% agarose gels and transferred onto GeneScreen PLUS membranes (Perkin Elmer, Boston, MA).

Southern blot was performed according to an optimized protocol using the DIG high prime DNA labeling and detection starter kit I (Roche). Probes were prepared by excising insert DNA from pEBV-oriL plasmid by restriction digestion and labeled with digoxigenin by random primed DNA synthesis with digoxigenin-dUTP. The membranes were prehybridization in 10 ml of the DIG Easy Hybridization solution, with 50 mg/ml denatured salmon sperm DNA for 4 h at 68 °C. Then, DIG labeled probe was added and hybridization was carried for overnight. The membranes were washed three times for 15 min at 65 °C with washing buffer (2× SSC, 0.1% SDS). Subsequently the membranes were blocked for 1 h and incubated in anti-DIG-AP solution for 1 h and then incubated in 10 ml color substrate solution in the dark.

3. Results

3.1. Evaluation of Topo II catalytic inhibitors for their effects on EBV DNA replication and virion production

In our previous work, we demonstrated that Topo II catalytic inhibitors, including novobiocin and merbarone, have potent antiviral activity halting KSHV DNA replication and virion production (Gonzalez-Molleda et al., 2012). In addition, a ligand-based viral screen using novobiocin as the template and an in-house 3D molecular superimposing algorithm led to identify a novel Topo II catalytic inhibitor, namely (+)-rutamarin. Our study demonstrated that (+)-rutamarin is more potent than novobiocin in blocking KSHV DNA replication and virion production (Xu et al., 2014). Given that EBV and KSHV share a similar lytic DNA replication mechanism and both require host Topo II for their DNA replication (Kawanishi, 1993; Wang et al., 2009, 2008), we decided to explore if these Topo II catalytic inhibitors can effectively block EBV lytic replication and have any potential to become antiviral drugs against EBV.

P3HR-1 cells that are latently infected by EBV were induced with TPA and butyrate for lytic viral replication. Three hours after induction, the cells were treated with various concentrations of novobiocin, merbarone, and (+)-rutamarin. The effects of these inhibitors on the viral DNA synthesis and on virion production were examined 2 days and 5 days post-induction, respectively. All three inhibitors were found to effective block EBV DNA synthesis as well as virion production (Fig. 2A–C and Table 1A). Among them, (+)-rutamarin revealed the highest potency in halting EBV DNA replication with a half-maximal inhibitory concentration (IC_{50}) of 3.78 μM and in blocking virion production with a half-maximal antiviral effective concentration (EC_{50}) of 5.40 μM (Fig. 2C and Table 1A).

To make sure that novobiocin, merbarone and (+)-rutamarin inhibit EBV lytic replication regardless of host cells and means of lytic cycle induction, we tested the effect of these inhibitors on EBV replication in Akata-Bx1 cells induced by anti-human IgG for lytic replication (Daibata et al., 1990). The inhibition of EBV lytic DNA replication and virion production by these three Topo II inhibitors in Akata-Bx1 cells are very similar to the results obtained with P3HR-1 cells. In particular, (+)-rutamarin again exhibited the highest antiviral potency in Akata-Bx1 cells with the IC50 and EC50 of (+)-Rutamarin of 2.38 μM and 2.94 μM, respectively (Fig. 3 and Table 1B).

3.2. Evaluation of Topo II inhibitors for their cytotoxicities and effects on host cell proliferation

Cytotoxicities of these three inhibitors were examined in parallel with their inhibition of EBV DNA replication and virion production in P3HR-1 and Akata-Bx1 cells. Cells treated with these inhibitors at different concentrations were subjected to the trypan blue exclusion method to assess the numbers of viable cells and nonviable cells in culture. The half-maximal cytotoxic concentrations (CC_{50}) were determined based on the results. Novobiocin and merbarone exhibited relatively low cytotoxicity to P3HR-1 cells with CC_{50} values of 518.5 μM and 137.6 μM, respectively (Fig. 2A and B). For (+)-rutamarin, the highest concentration used in this study (150 μM) showed little cytotoxicity in 2 and 5 days culture, suggesting that the CC_{50} value of (+)-rutamarin to P3HR-1 cell is greater than 150 μM (Fig. 2D and E). Similar results were obtained with Akata-Bx1 cells (Fig. 3D and E). The low cytotoxicities and high inhibition rates for both viral DNA replication and virion production, represented by the selectivity indices (CC_{50}/IC_{50}) higher than 39.6 in P3HR-1 and 63 in Akata-Bx1 cells

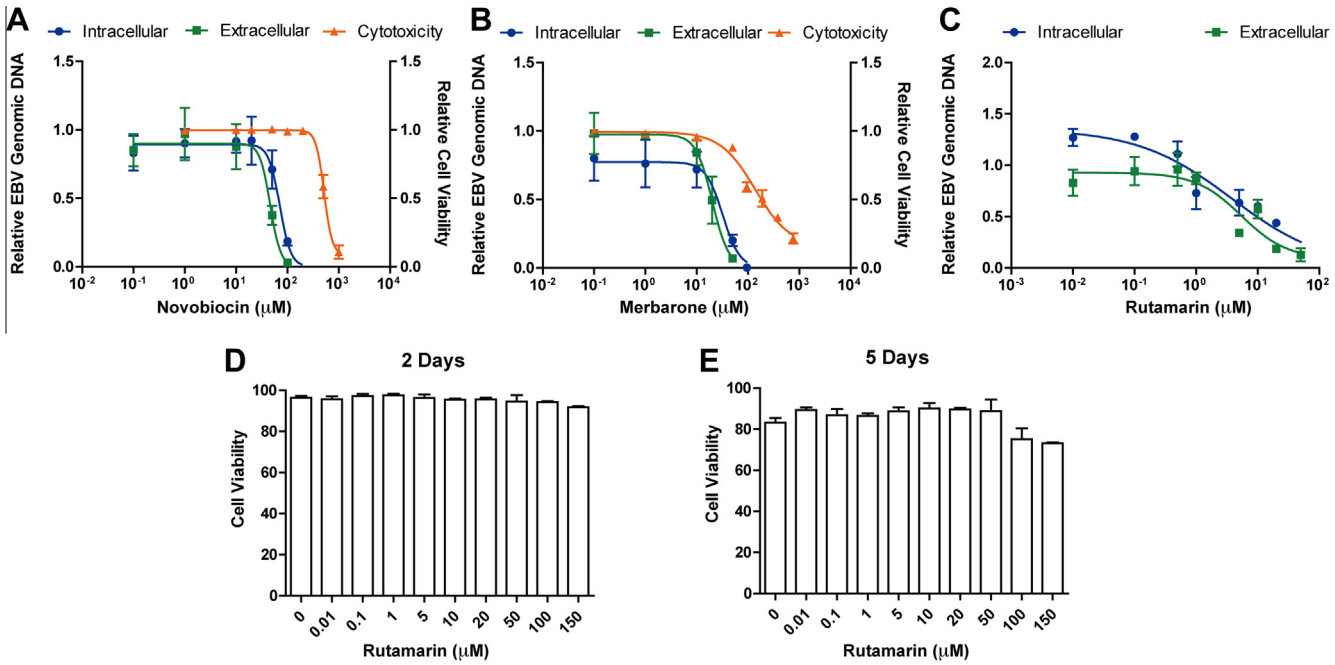

Fig. 2. Effects of Topo II inhibitors on EBV replication and their associated cytotoxicity in P3HR-1 cells. EBV lytic replication in P3HR-1 was induced with TPA and sodium butyrate. Topo II catalytic inhibitors, novobiocin (A), merbarone (B) and (+)-rutamarin (C) were added to the cell culture 3 h after the induction. The concentration ranges tested for different inhibitors are: 0.1–1000 μM (novobiocin), 0.1 −760 μM (merbarone), and 0.01−150 μM ((+)-rutamarin). Intracellular EBV DNA (blue), extracellular virion DNA (green), and cell viability (orange) were determined for each concentration point as described in Section 2. These values were compared to those from the control cells (non-drug treatment). Mean values of results from at least three independent experiments and standard deviations are presented on the *Y* axes of dose–response curves. Topo II inhibitor doses are indicated on the *x* axes as logarithmic scales. The effect of (+)-rutamarin on cell viability of P3HR-1 cells on 2 and 5 days exposure were shown in panels D and E.

Table 1
Antiviral activities of Topo II inhibitors and their associated cytotoxicities[a] in P3HR-1 cells induced with TPA/butyrate and Akata-Bx1 cells induced with Anti-IgG.

Inhibitor	IC_{50}[a]	R^2	EC_{50}	R^2	CC_{50}[b]	R^2	SI[c]	CC_{50}[d]	R^2
A. P3HR-1 cells									
Novobiocin	70.70	0.992	46.21	0.988	518.5	0.999	7.33	137.6	0.996
Merbarone	32.15	0.994	20.12	0.999	137.6	0.979	4.27	27.5	0.984
(+)-Rutamarin	3.78	0.931	5.43	0.879	>150	N/A[e]	>39.6	>150	N/A[e]
B. Akata-Bx1 cells									
Novobiocin	41.05	0.975	68.60	0.982	616.7	0.992	14.8	339.4	0.978
Merbarone	42.76	0.959	22.19	0.998	103.2	0.957	2.4	69.98	0.997
(+)-Rutamarin	2.38	0.912	2.94	0.980	>150	N/A[e]	>63.0	>150	N/A[e]

[a] IC_{50} represents the half maximal inhibitory concentration for EBV DNA replication. EC_{50} denotes the half maximal effective concentration for blocking EBV virion production. CC_{50} refers to the concentration of the compound that causes 50% cell death after specific exposure time. They were determined by nonlinear regression analysis of dose response curves and are expressed as mean values of results from at least three independent experiments. All these parameters are presented in μM units. R^2: correlation coefficient.
[b] Cytotoxicity was measured after 2 days of drug treatment.
[c] SI (selectivity index) was calculated as the ratio of CC_{50}/IC_{50}.
[d] Cytotoxicity was measured after 5 days of drug treatment.
[e] NA means not available.

for (+)-rutamarin (Table 1A and B), suggest the potential of (+)-rutamarin to be an effective anti-EBV drug candidate for treatment of EBV-associated diseases.

To further investigate the potentials of Topo II catalytic inhibitors to become safe anti-EBV drug candidates, we assessed the effects of these inhibitors on cell proliferation. P3HR-1 cells were cultured in the absence and the presence of novobiocin, merbarone and (+)-rutamarin at two different concentrations, the IC_{50} and an excess concentration ($5 \times IC_{50}$) for 5 days. Cell samples were daily collected and counted. Merbarone was found to be able to inhibit cell proliferation in both concentrations; novobiocin did not exhibit much inhibitory effect on cell growth at the concentration of IC_{50} but the cell proliferation rate was dramatically decreased at the excess concentration ($5 \times IC_{50}$). In contrast, little effect was observed with (+)-rutamarin at both IC_{50} and $5 \times IC_{50}$ concentrations on cell proliferation (Fig. 4).

3.3. Validation of Topo II inhibitors for their effects on EBV ori-Lyt-dependent DNA replication

The topoisomerase II inhibitors tested in this study have been demonstrated to inhibit EBV DNA synthesis and virion production. We asked whether these inhibitors indeed block viral ori-Lyt-dependent DNA replication. To address this question, P3HR-1 cells were cotransfected with an EBV ori-Lyt-containing plasmid (pEBV-oriL) and a ZTA expression vector (McZ). Expression of ZTA sufficiently drives latent EBV into lytic replication cycle (Countryman and Miller, 1985; Rooney et al., 1988). The transfected cells were

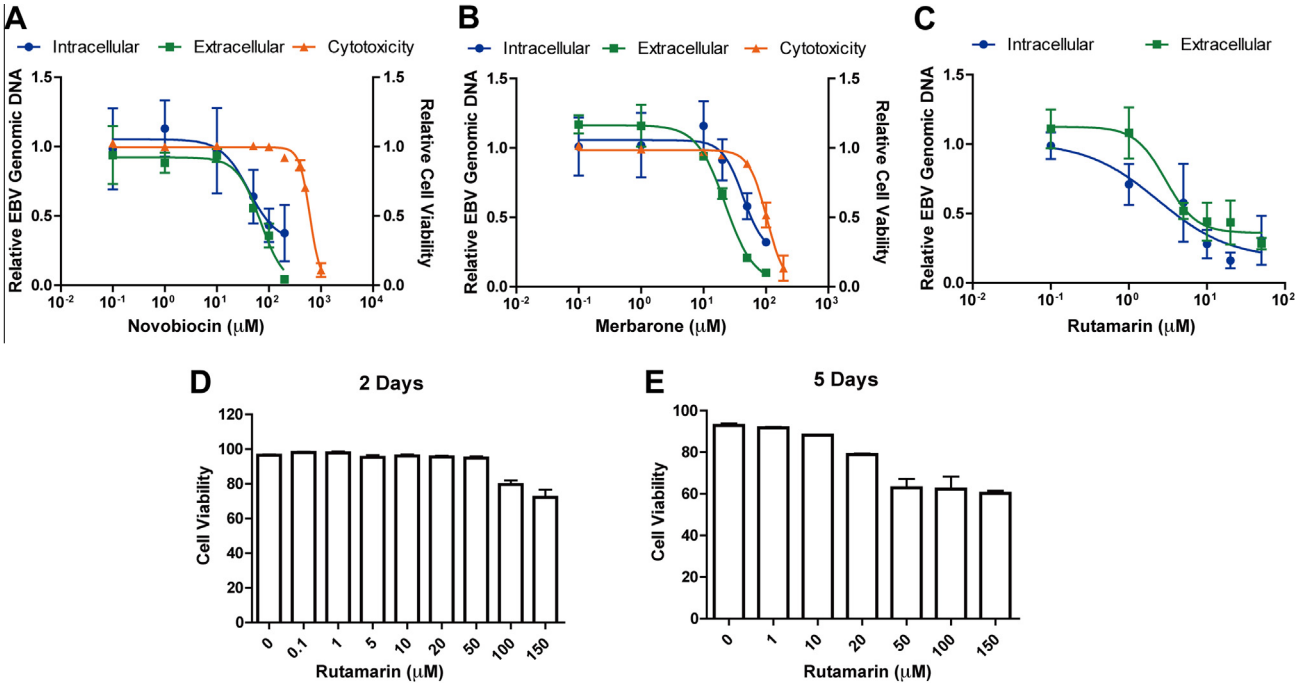

Fig. 3. Effects of Topo II inhibitors on EBV replication and their associated cytotoxicity in Akata-Bx1 cells. EBV lytic replication in Akata-Bx1 was induced by treatment of cells with anti-IgG. Topo II catalytic inhibitors, novobiocin (A), merbarone (B) and (+)-rutamarin (C) in wide ranges were added to the cell culture 3 h after the induction. Intracellular EBV DNA (blue), extracellular virion DNA (green), and cell viability (orange) were determined for each concentration point as described in Section 2. These values were compared to those from the control cells (non-drug treatment). Mean values of results from at least three independent experiments and standard deviations are presented on the *Y* axes of dose–response curves. Topo II inhibitor doses are indicated on the *x* axes as logarithmic scales. The effect of (+)-rutamarin on cell viability of Akata-Bx1 cells on 2 and 5 days exposure were shown in panels D and E.

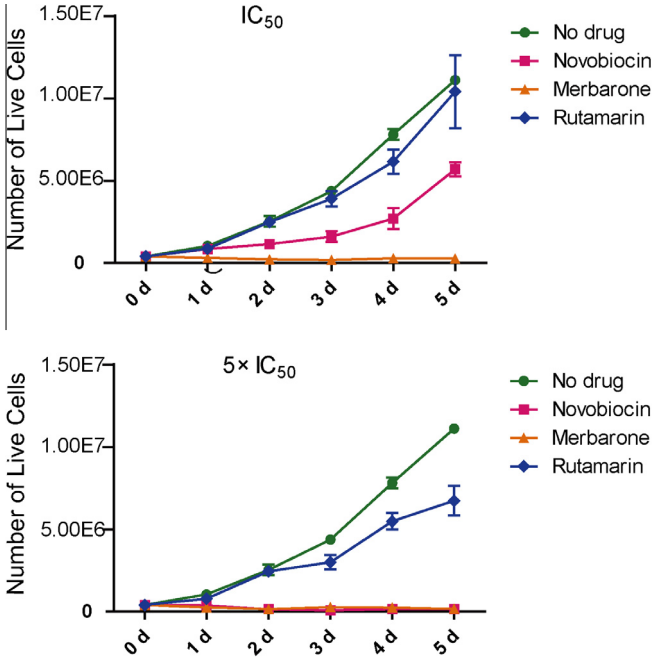

Fig. 4. Effects of Topo II inhibitors on P3HR-1 cell proliferation. P3HR-1 cells (starting with 2×10^5 cells/ml) were exposed to different inhibitors as indicated at two concentrations (IC_{50} and $5 \times IC_{50}$) and counted every day for five days. Data were obtained from three independent determinations and presented as means with standard deviations.

cultured in the absence or the presence of each inhibitor at various concentrations, usually with two concentration lower than IC_{50}, and two higher concentrations. The ori-Lyt-dependent DNA replication and the drug effects were measured by a Dpn I assay (Gonzalez-Molleda et al., 2012). In brief, DNA was isolated from the treated cells 72 h post-transfection and digested with EcoR I and EcoR I/Dpn I. Replicated plasmid DNA can be distinguished from input plasmid by DpnI restriction digest, which cleaves input DNA that has been dam + methylated in Escherichia coli but leaves intact the DNA that has been replicated at least one round in eukaryotic cells (Gonzalez-Molleda et al., 2012). Thus, only newly replicated plasmid DNA in P3HR-1 cells is resistant to Dpn I digestion and can be detected in Southern blot analysis. Replicated DNA was detected in the cells that were cotransfected with pEBV-oriL and ZTA expression vector (Fig. 5A–C). EcoR I digested Hirt DNAs served as controls for equal transfection of input plasmids in each chemical concentration. Novobiocin, merbarone and (+)-rutamarin potently blocked the newly replicated DNA in a dose-dependent manner (Fig. 5A–C). In conclusion, Topo II catalytic inhibitors that we tested in this study were found to be able to inhibit the ori-Lyt-dependent DNA replication, suggesting that these inhibitors act in blocking EBV ori-Lyt-dependent DNA replication.

4. Discussion

EBV is associated with a number of human diseases. The lytic replication of EBV is directly linked to infectious mononucleosis, chronic active EBV infection (CAEBV), oral hairy leukoplakia and to an increased risk of EBV-associated nasopharyngeal carcinoma (Al Tabaa et al., 2011; Dardari et al., 2000; Lau et al., 1993). For example, CAEBV is an often fatal disorder that is rare in USA but occurs more frequently in Asia and South America (Katano et al., 2004). Chronic active EBV infection is characterized by chronic or recurrent infectious mononucleosis-like symptoms that persist for a long time and by an unusual pattern of anti-EBV antibodies,

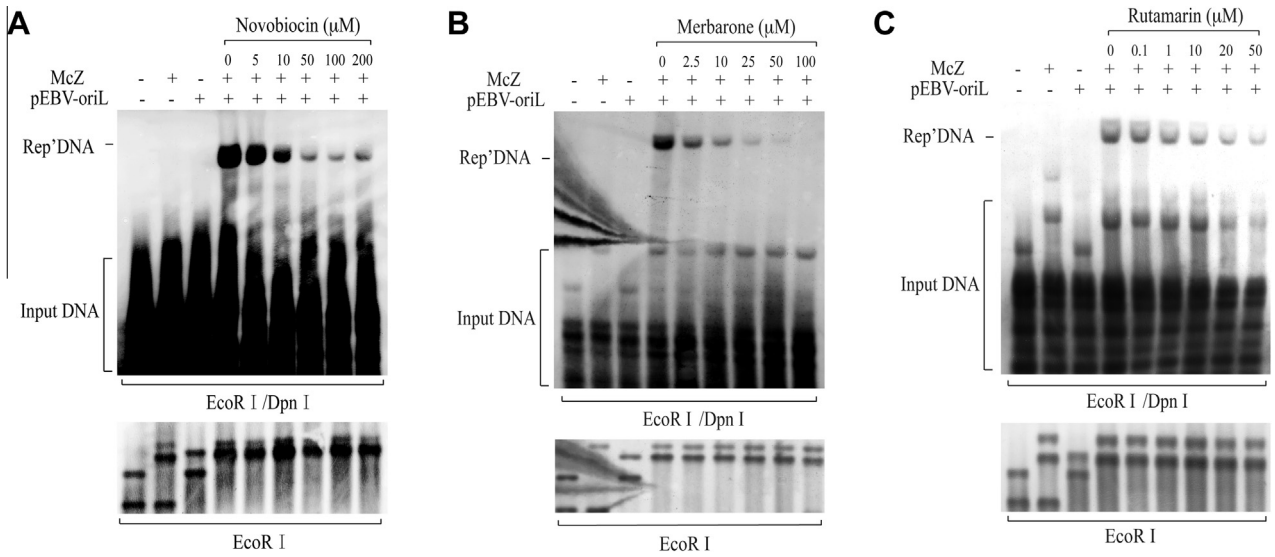

Fig. 5. Inhibition of EBV ori-Lyt-associated DNA replication with Topo II inhibitors. P3HR-1 cells were transfected with an ori-Lyt-containing plasmid (pEBV-oriL) and ZTA expression vector (McZ). Transfected cells were cultured in the absence or presence of increasing concentrations of novobiocin (A), merbarone (B), and (+)-rutamarin (C). After 72 h of incubation, hirt DNAs were extracted from the cells and digested with EcoRI or EcoRI/Dpn I as described in Section 2. DpnI-resistant products of DNA replication (Rep'd DNA) were detected by Southern blotting with digoxigenin – labeled EcoR I – Hind III fragment from the pEBV-oriL plasmid.

with life-threatening complications, such as virus-associated hemophagocytic syndrome and lymphoma (Kimura et al., 2001). The patients usually have a markedly elevated EBV DNA level in the blood (10^3–10^7 copies/ml), indicating active lytic viral replication. So far no satisfactory therapy is available for CAEBV. Antiviral or immunomodulatory agents, such as acyclovir, ganciclovir, vidarabine, interferon-α, and interleukin-2, have been trialed for CAEBV with limited success (Kimura et al., 2003). Acyclovir (ACV), which inhibits herpesviral DNA polymerase, is commonly used for treatment of CAEBV (Gershburg and Pagano, 2005) but found generally inefficient for this disease. Moreover, drug resistance frequently occurs as a major problem among immunosuppressed hosts, mainly those who have received prolonged ACV therapy (Andrei et al., 2012; Field and Vere Hodge, 2013). Some studies suggested that ganciclovir, which is a guanosine analog, might be a more effective drug than ACV for treating lytic EBV infection in patients (Andrei et al., 2012; Keever-Taylor et al., 2003). Unfortunately, the use of ganciclovir is limited by nephrotoxicity as well as hematological toxicity (Gilbert et al., 2002; Williams et al., 2003). Thus, there is an urgent need for efficient antivirals against EBV for treatment of EBV-associated human diseases.

EBV as well as other herpesviruses do not encode topoisomerases and rely on host cell topoisomerases activity for their DNA replication (Kawanishi, 1993). Thus topoisomerases could be therapeutic targets for blocking replication of herpesviruses including EBV and treatment of the infection-associated human diseases. It has been reported previously that Topo I inhibitor camptothecin is able to inhibit replication of herpes simplex virus type 2 (Yamada et al., 1990). Both Topo II catalytic inhibitor ICRF193 and Topo II poison teniposide (VM26) were found to be able to block herpes simplex virus type 1 from replication (Ebert et al., 1990; Hammarsten et al., 1996). Recently we reported that KSHV replication can be effectively blocked by Topo I inhibitor camptothecin, Topo II poisons etoposide and ellipticine as well as Topo II catalytic inhibitors novobiocin, merbarone and (+)-rutamarin (Gonzalez-Molleda et al., 2012; Xu et al., 2014). However, Topo I inhibitors and Topo II poisons in general possess considerable toxicities to host cells (Gonzalez-Molleda et al., 2012). In the current study we demonstrated that human topoisomerase II catalytic inhibitors are potent in inhibiting EBV replication and some of them exhibit little cytotoxicities, suggesting some Topo II catalytic inhibitors are promising antiviral candidates for EBV-associated human diseases. Furthermore, viruses have tendencies to mutate their genome and therefore develop drug resistance. An antiviral that targets a cellular protein such as Topo II offers the advantage of minimizing drug resistance and hence constitutes an important, novel therapeutic strategy.

Novobiocin is an old antibiotic drug against staphylococcal infection (Kirby et al., 1956). Novobiocin has also been used in combination with chemotherapeutical agents for the treatment of several cancers (Eder et al., 1991; Kennedy et al., 1995). Our study revealed its antiviral potency against EBV, suggesting a new usage for an old drug.

(+)-Rutamarin is a natural product and newly identified Topo II catalytic inhibitor (Xu et al., 2014). Among three inhibitors tested in this study, (+)-Rutamarin exhibited the highest potency in anti-EBV activity. (+)-Rutamarin effectively inhibits EBV replication with an IC_{50} of 2.38 μM and an EC_{50} of 2.94 μM (in Akata-Bx1 cells). It possesses relative low cytotoxicity to P3HR-1 cells with a CC50 greater than 150 μM. Currently, we are attempting to optimize the (+)-Rutamarin structure in order to further elevate its antiviral activity and improve its solubility, hopefully making it promising anti-EBV drug lead.

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