Virus Research 270 (2019) 197653

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

# Conflicting effects of poly(ADP-ribose) polymerase inhibitor on cell-mediated and virion-mediated HTLV-1 infection

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ARTICLE INFO

*Keywords:*
HTLV-1
Viral infectivity
Poly ADP ribosylation
PARP inhibitor

ABSTRACT

Adult T-cell leukemia and human T-cell leukemia virus type 1 (HTLV-1) – associated myelopathy/tropical spastic paraparesis, which develop after HTLV-1 infection, are difficult to cure. In particular, the mode of HTLV-1 propagation is not well understood. Poly (ADP-ribose) polymerase-1 is reported to be a co-activator of HTLV-1 Tax protein; however, the effects of polyADP-ribosylation on infectivity of HTLV-1 have not been fully clarified. We studied the effects of a PARP inhibitor on two modes of HTLV-1 transmission: through cell adhesion between MT-2 cells (an HTLV-1-infected cell line) and uninfected cells and through virus particles produced by HTLV-1-producing c77 cells. Although the PARP inhibitor decreased HTLV-1 infection through cell adhesion, it increased HTLV-1 infection through virion production and caused apoptosis of HTLV-1-infected cells. Thus, careful consideration is required for clinical application of PARP inhibitors in HTLV-1 patients.

## 1. Introduction

Human T-cell leukemia virus type 1 (HTLV-1) was the first human oncogenic retrovirus to be characterized as an etiologic agent of adult T-cell leukemia/lymphoma (ATL), a malignancy of T lymphocytes (Uchiyama et al., 1977). HTLV-1 infection between individuals occurs through the transmission of HTLV-1-infected lymphocytes contained in mother's milk, transfused blood, and sexually transmitted seminal fluids (Yamaguchi et al., 2002). However, it is not clear how HTLV-1 is transmitted to other cells and disseminates within the body. As compared with other retroviruses, HTLV-1 virions are not efficiently released from infected cells and are poorly infectious (Clapham et al., 1983; Derse et al., 1995). HTLV-1 virions seem to be quite unstable in vitro, and they inefficiently bind and penetrate host cells (Fan et al., 1992). On the other hand, cell-free HTLV-1 infects dendritic cells, leading to transmission and transformation of CD4 + T cells (Jones et al., 2008). Thus, understanding the spread of HTLV-1 infection within the body through cell-free HTLV-1 virions cannot be neglected. Furthermore, retroviral integration into host DNA, which is essential for virus replication and propagation, involves DNA strand breaks and the cellular DNA damage repair mechanism (Skalka et al., 2005). However, the importance of cellular recovery from DNA damage during retroviral infection is not yet understood.

Poly (ADP-ribose) polymerase-1 (PARP-1) is one of the key factors in DNA damage repair, and it synthesizes a negatively charged polymer, poly (ADP-ribose), using $NAD^+$ as its substrate. Poly (ADP-ribose) influences not only regulation of DNA repair but also transcription, cell proliferation, and cell fate (Miwa and Masutani, 2007). PARP-1 can bind to various DNA structures including strand breaks and modify histones in nucleosomes, which are involved in the regulation of retroviral integration (Gäken et al., 1996; Ha et al., 2001). Host factors are required for the integration of the retroviral genome into chromosomal DNA (Brown et al., 1997). These events can either support or inhibit viral replication; however, ultimately there is selection for survival of both the virus and infected cells. Previously, it was reported that a PARP inhibitor induced transcription of mouse mammary tumor virus

*Abbreviations:* 3-AB, 3-aminobenzamide; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; FBS, fetal bovine serum; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; PARG, poly (ADP-ribose) glycohydrolase; PARPs, poly (ADP-ribose) polymerases; PBS, phosphate-buffer saline

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https://doi.org/10.1016/j.virusres.2019.197653
Received 22 November 2018; Received in revised form 16 April 2019; Accepted 8 July 2019
Available online 09 July 2019

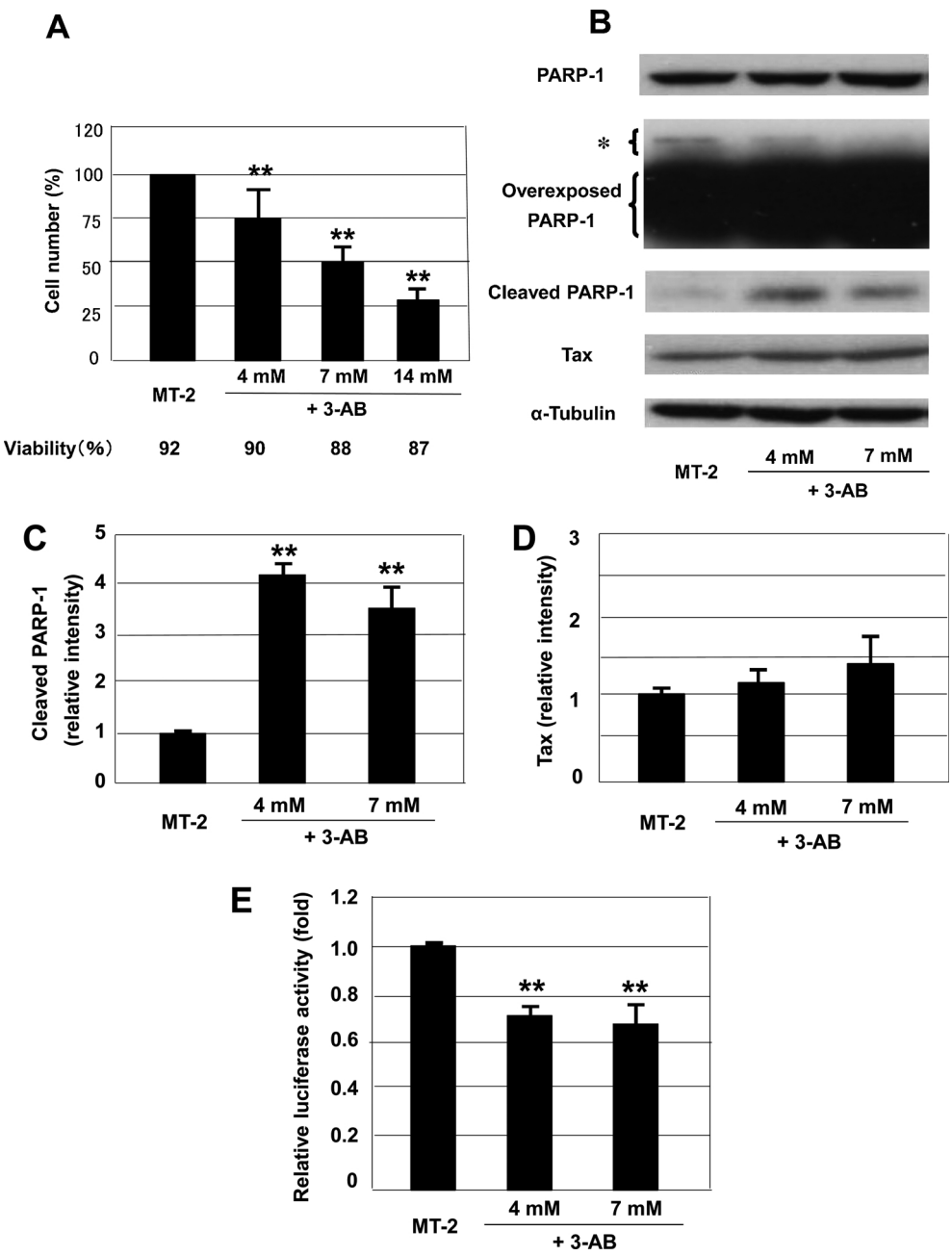


**Fig. 1.** The PARP inhibitor, 3-AB, decreases the proliferation and HTLV-1 infectivity in MT-2 cells. (A) The proliferation of MT-2 cells was determined with or without 3-AB for 48 h. Cell viability was calculated as the number of cells that did not stain with Trypan Blue divided by the total number of cells. (B) Cellular proteins (PARP-1, cleaved PARP-1, and HTLV-1 Tax) in MT-2 cells were detected by western blotting, and α-tubulin was used as a loading control. *: PolyADP-ribosylated PARP-1. (C, D) The levels of cleaved PARP-1 and Tax provided in Fig. 1B were taken by Image-J and digitized. **p < 0.01, compared with control. (E) MT-2 cells treated with or without 3-AB were co-cultured for 24 h with Jurkat cells. Following cell fusion, cells were lysed and luciferase activity was measured using a luminometer. The mean and standard deviation is shown for four independent experiments. **p < 0.01, compared with control.

(Johnson et al., 1986) and suppresses virus production at the level of transcription in HIV-1 (Kameoka et al., 1999). Therefore, poly (ADP-ribose) metabolism may participate in the life cycle of other retroviruses such as HTLV-1.

Recently, we established a sensitive indicator system to detect cell-free infection of HTLV-1 virions produced from the feline kidney cell line c77 (Tanaka et al., 2010). In the present work, we demonstrated that although PARP inhibition decreased infectivity through cell adhesion, it increased viral transmission through cell-free HTLV-1 virions.

## 2. Materials and methods

### 2.1. Cells and reagents

MT-2 cells are a T-cell strain derived from human umbilical cord white blood cells established by co-cultivation with ATL cells (Miyoshi et al., 1981; Kobayashi et al., 1984). MT-2 cells were maintained in RPMI1640 medium supplemented with 10% fetal calf serum (FCS). The feline kidney cell line c77 (Melanesian subtype of HTLV-1) was used as an HTLV-1 virion–producing line [Tanaka et al., 2010; Hoshino et al., 1985; Haraguchi et al., 1997) and maintained in Eagle's minimum essential medium supplemented with 10% FCS. A human T-cell line, Jurkat, was transfected with the luciferase gene (described below) and used as the target cells for HTLV-1 infection (Schneider et al., 1977); Jurkat cells were maintained in RPMI 1640 medium supplemented with 10% FCS. 3-Aminobenzamide (3-AB), a general inhibitor of polyADP-ribosylation, was purchased from Tokyo-Kasei [MacLaren et al., 1989; Morgan et al., 1983], and doxorubicin, a DNA damaging agent, was purchased from Sigma. Antibodies against the following proteins were used: PARP-1 (BD Transduction Laboratories), p53 (FL-393; Santa Cruz Biotechnology), Tax (LT-4; gifted from Prof. Y Tanaka at the University of the Ryukyus, Japan), and α-tubulin (DM-1A; Sigma).

### 2.2. Effect of drugs on cell proliferation and cell viability

MT-2 cells were seeded at $3 \times 10^5$ cells/well together with 3-AB.

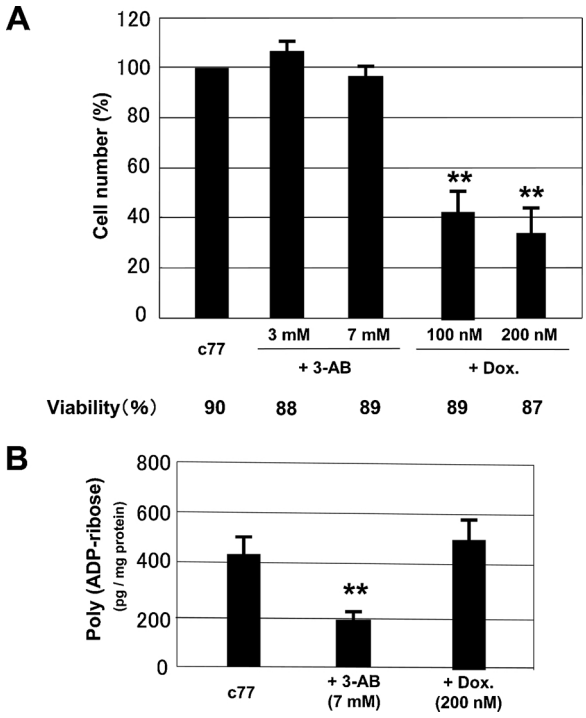


**Fig. 2.** The PARP inhibitor 3-AB does not inhibit proliferation of HTLV-1-producing c77 cells. (A) The proliferation of HTLV-1-producing c77 cells after treatment with 3-AB. The experiments were carried out in triplicate, and the standard deviation is represented by bars. HTLV-1-producing cells were treated with 3-AB or doxorubicin (Dox.) for 48 h. Cell viability was calculated as the number of cells that did not stain with Trypan Blue divided by the total number of cells. (B) Poly (ADP-ribose) levels of c77 cells treated with 3-AB or doxorubicin for 48 h. The experiments were carried out in triplicate by ELISA. $^{**}p < 0.01$, compared with control.

c77 cells were seeded at $2 \times 10^4$ cells/well, and 3-AB was added 24 h later. The cells were collected after 48 h and stained with 0.4% Trypan Blue to measure viable cells. Viable cells were those that excluded Trypan Blue.

### 2.3. Quantification of poly (ADP-ribose) levels

Because of the low level of poly (ADP-ribose) and the rapid turnover of poly (ADP-ribose) with PARPs and poly (ADP-ribose) glycohydrolase (PARG), the level of poly (ADP-ribose) under physiological conditions has been difficult to determine (Mullen et al., 2000; Okajima et al., 2013). When disrupting cultured cells, PARP-1 is easily activated during the collection and lysis of cells using ordinary buffers such as RIPA buffer, presumably owing to DNA strand breakage. Thus, different amounts of poly (ADP-ribose) are artificially produced during these steps, implying that the true cellular poly (ADP-ribose) level cannot be assessed with western blotting. We found that trichloroacetic acid is useful to immediately fix PARPs and PARG in cultured cells and developed a sensitive ELISA to measure the physiological level of poly (ADP-ribose) (Ida et al., 2016).

Quantification of poly (ADP-ribose) levels in treated cells was analyzed by sandwich ELISA system (Ida et al., 2016). Microtiter 96 well plates (Thermo Fisher Scientific, 442,404) were coated with a mouse monoclonal antibody against PAR (10 H) as the PAR-capturing antibody. The rabbit anti-PAR polyclonal antibody was used as the detection antibody. All the experiments were performed in triplicate.

### 2.4. Western blotting

To assay for apoptosis by PARP-1 cleavage, cells were lysed in isotonic lysis buffer containing 140 mM NaCl, 50 mM Tris − HCl pH 8.0, 5 mM EDTA, 0.5% (w/v) Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 1 × protease inhibitor mixture (Sigma-Aldrich). Samples were processed for western blotting.

Virion-containing culture medium was loaded onto a 20–65% discontinuous sucrose gradient and centrifuged at 30,000 × *g* for 2 h. The interphase fraction of the sucrose gradient–containing virions was collected and centrifuged at 30,000 rpm for 2 h at 4 °C. The virion pellet was resuspended in RIPA buffer (50 mM Tris − HCl pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1% (w/v) NP-40, 0.5% sodium deoxycholate, 2 mM sodium orthovanadate, 10 mM NaF, 1 × protease inhibitor cocktail [Sigma-Aldrich]).

Proteins of cell extracts and virion suspensions were separated via SDS-polyacrylamide gel electrophoresis (PAGE, 10% polyacrylamide). The separated proteins were transferred to Immobilon-P membrane (Millipore, Bedford, MA, USA, treated with respective antibodies and visualized with chemiluminescence detection reagents (Roche Diagnostics, Mannhaim, Germany). The ATL patient serum used in this study was obtained and anonymized before enforcement of Ethical Guidelines for Clinical Research (Public Notice of the Ministry of Health, Labour and Welfare No. 255 of 2003). Additionally, since it is used as an antibody to investigate viral antigen proteins of cultured cells and is not used for genomic/genetic analyses, it is excluded from these guidelines.

### 2.5. Apoptosis assays

Annexin V–positive cells were quantified using the Annexin-V-FLUOS Staining kit (Roche). Cells were seeded in 24-well tissue culture plates at $5 \times 10^4$ cells/well, 24 h prior to drug treatment, and were incubated with the drug for an additional 48 h. Cells were collected by centrifugation at 300 × *g* for 10 min. Flow cytometry was performed with a FACSCalibur (BD Bio-sciences).

### 2.6. Preparation of cell-free HTLV-1 virions

Cell-free HTLV-1 virions were prepared as described (Tanaka et al., 2010) with slight modification. In brief, $4 \times 10^6$ c77 cells in 8 ml of medium were seeded in a 10-cm diameter plastic dish and cultured for 48 h with or without 3-AB. The medium was replaced with fresh medium and cultured for 24 h. The culture supernatant was harvested and was centrifuged at low speed to remove cells and debris. The non-concentrated and concentrated virus fractions were passed through 0.45-μm pore-size cellulose acetate membrane filters to prepare cell-free HTLV-1 virions. The supernatant was centrifuged on a cushion of 20% sucrose with a SW28 rotor at 100,000 × *g* for 2 h at 4 °C (Beckman Coulter Co., Ltd., Japan). The pellet was suspended with 1 ml of RPMI medium as a concentrated virus fraction.

### 2.7. Viral infectivity assay

It has been reported that HTLV-1 Tax regulates virus infectivity in the early viral replication phase (Matsuoka et al., 2007). We performed virus recovery time only 48 h later. Jurkat cells were seeded at $1.5 \times 10^6$ cells/well in 2 ml of complete media on the day of transfection in 6-well plates. For each well, 2.5 μg DNA (2.25 μg HTLV-1 long terminal repeat (LTR) GL4 vector and 0.25 μg RL-RSV vector) and 2.5 μl Plus reagent (Invitrogen, Japan) were taken up in 500 μl serum-free media for 5 min, then 5 μl Lipofectamine LTX (Invitrogen, Japan) was added, mixed, and incubated for an additional 25 min before being added dropwise to the cells. The cells were incubated with occasional agitation at 37 °C in an atmosphere of 5% $CO_2$ for up to 24 h and then transferred to a 5-ml flask. These cells were used as indicator cells for viral infectivity.

To determine cell-to-cell infectivity, we treated MT-2 cells with or without 3-AB. After incubation for 48 h, we ensured equal numbers of

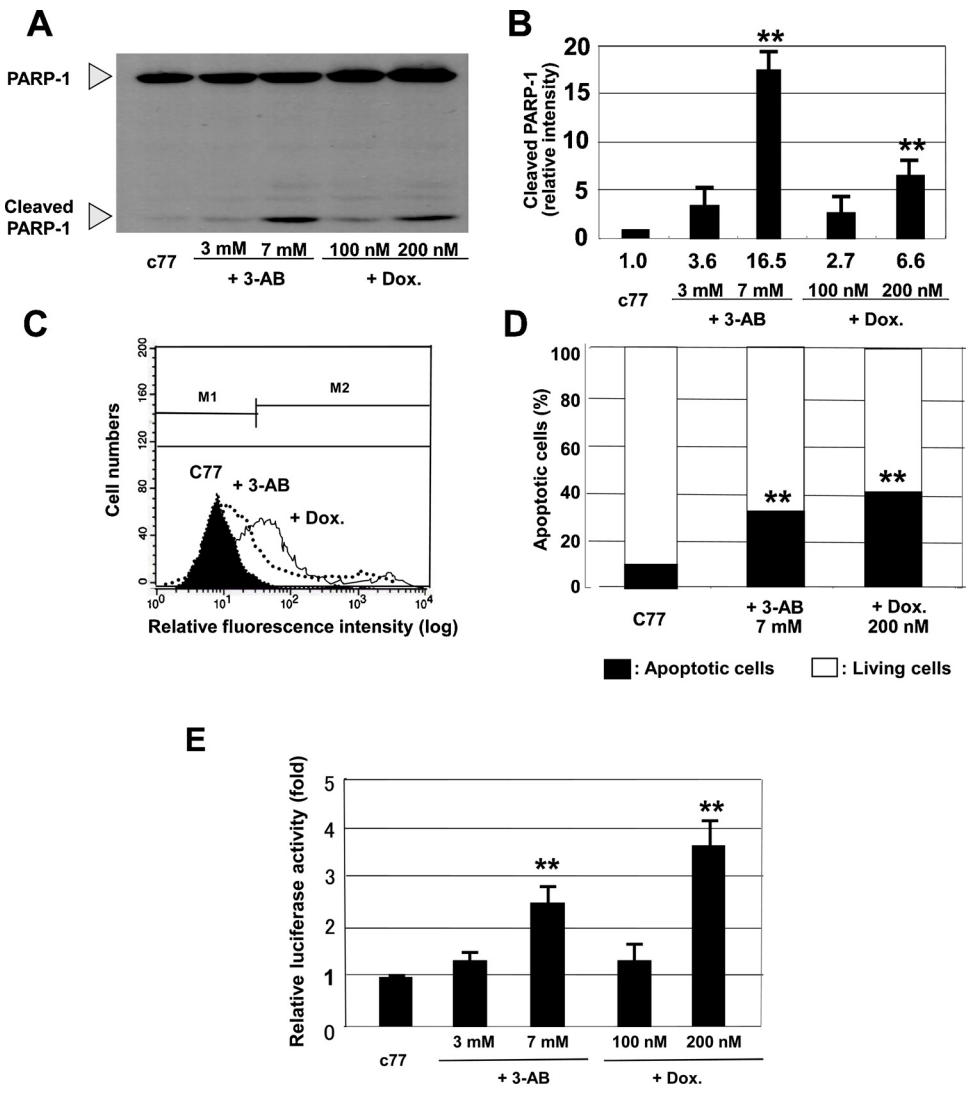


**Fig. 3.** The PARP inhibitor 3-AB induces apoptosis and increases viral infectivity in c77 cells. (A) 3-AB and doxorubicin induced PARP-1 cleavage in c77 cells with doxorubicin treatment after incubation for 48 h. Western blotting of cell lysates revealed increased cleavage of PARP-1 into the apoptosis-specific PARP-1 fragment. (B) The levels of cleaved PARP-1 provided in Fig. 3A were taken by Image-J and digitized. **p < 0.01, compared with controls, c77 cells without treatment. (C) HTLV-1-producing cells were treated with 7 mM 3-AB or 200 nM doxorubicin for 48 h. c77 cells were left untreated (control; filled histogram) or were treated with 3-AB (bold dotted-line histogram) or doxorubicin (Dox.; thin dotted-line histogram) and were stained for annexin V. (D) The percentage of cells in each phase of apoptosis is shown. This graph was derived from the results shown in panel C. **p < 0.01, compared with control. (E) Virions recovered from c77 cells underwent multiple rounds of replication in Jurkat T cells harboring the HTLV-1 long terminal repeat connected to the luciferase gene. The early phase (from attachment to integration) of virus replication was monitored for 48 h. The results from at least three independent experiments are shown. Error bars indicate standard deviation. **p < 0.01, compared with control.

MT-2 cells and indicator cells. At 24 h post-infection, the cells were harvested and analyzed for luciferase expression with the Dual-Luciferase Assay System (Toyo-ink, Tokyo).

To determine the infectivity of cell-free HTLV-1 virions, the harvested HTLV-1 virions (25 ng p19) were inoculated immediately onto Jurkat cells, serving as indicator cells as described above. After incubation for 5 h, fresh growth medium (3.5 ml) was added to the indicator cells. The amount of input cell-free HTLV-1 virions was adjusted based on the level of HTLV-1 core protein p19 via ELISAs (RETRO-TEK). After infection for 24 h, the cells were harvested and analyzed for luciferase expression using the Dual-Luciferase Assay System. Relative luciferase activity was calculated as the ratio of the luciferase activity of firefly to that of Renilla.

### 2.8. Statistical analysis

The Student's *t*-test was used to evaluate the statistical significance of the difference between the control group and each test group.

## 3. Results

### 3.1. Cell proliferation, apoptosis induction, and cell-to-cell HTLV-1 infectivity after treatment of MT-2 cells with PARP inhibitor

We used MT-2 cells, which are known to have high virus production and high viral infectivity among HTLV-1 infected cells, and investigated the cytostatic effect of the PARP inhibitor 3-AB. We found that proliferation was suppressed in a concentration-dependent manner (Fig. 1A). It was shown that 3-AB efficiently inhibited PARP activity by disappearance of polyADP-ribosylated PARP-1 (Fig. 1B, asterisk). In addition, we investigated the cleavage of PARP-1, which is an index of apoptosis, and the expression of HTLV-1 Tax protein following treatment with 3-AB. PARP-1 cleavage was significantly increased in the presence of 3-AB (Fig. 1B and C). However, there was no significant change in Tax levels after treatment with 3-AB (Fig. 1B and D). Furthermore, when the infectivity of HTLV-1 infected positive adherent cells was examined using the luciferase assay, the infectivity was significantly decreased upon treatment with 3-AB (Fig. 1E).

### 3.2. Cell proliferation and poly ADP-ribosylation analysis after treatment of c77 cells with PARP inhibitor or doxorubicin

HTLV-1 virions are believed to be weakly infectious under ordinary cell culture conditions. HTLV-1 Tax controls many critical cellular pathways including the host-cell DNA damage response (Chandhasin et al., 2008; Kinjo et al., 2010; Chlichlia and Khazaie, 2010). Because HTLV-1 virion particle infection and how the spread of HTLV-1 is in vivo, we used our established HTLV-1 cell-free infection system (Tanaka et al., 2010) to assess the effect of 3-AB and doxorubicin, a DNA damaging agent, on anti-HTLV-1 activity. We performed a cell

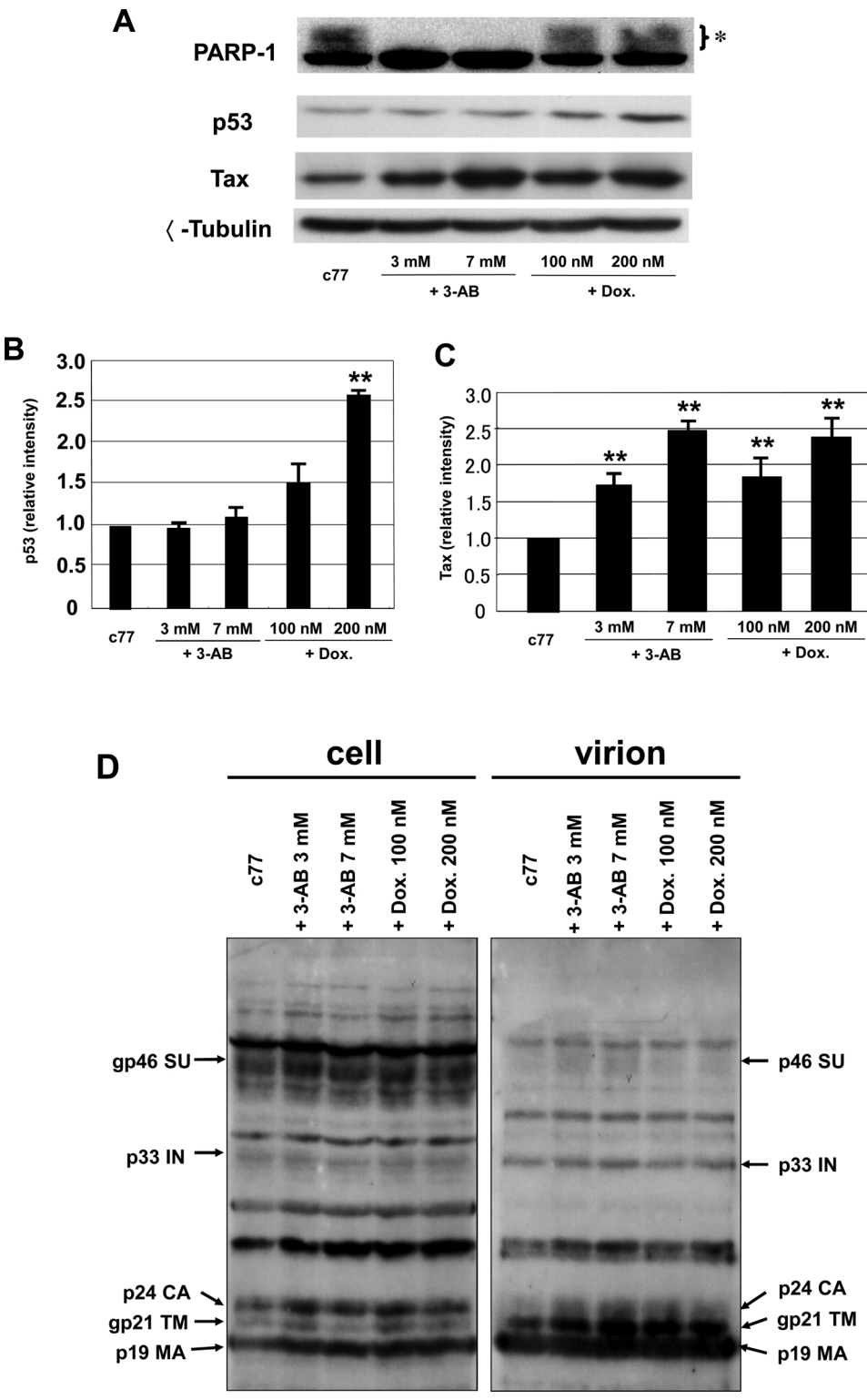


**Fig. 4.** The PARP inhibitor 3-AB increases the level of Tax and alters the composition of viral proteins in c77 cells. (A) PARP-1, P53, and HTLV-1 Tax proteins in infected cells treated with 3-AB or with doxorubicin were detected by western blotting after incubation for 48 h, and α-tubulin was used as a loading control. *: PolyADP-ribosylated PARP-1. (B) The amount of p53 in cells after doxorubicin in c77 cells. The level of each protein was calculated by Image-J and was digitized. **$p < 0.01$, compared with control. (C) Tax accumulation after PARP inhibition in c77 cells. The level of each protein was calculated by Image-J and was digitized. **$p < 0.01$, compared with control. (D) Viral protein expression in c77 cells and the virions produced were analyzed by SDS-PAGE followed by western blotting with anti-HTLV-1-positive serum from an ATL patient. Whole-cell extracts were subjected to SDS-PAGE after adjusting for total protein. Virion lysates were applied after adjusting for the volume of culture medium. Arrowheads indicate the positions of the major viral components.

viability assay using the HTLV-1-producing c77 cells, which can produce high titers of HTLV-1 virions. The viability of c77 cells was not affected by 200 nM doxorubicin treatment and 3-AB did not influence proliferation at all, as well as viability (Fig. 2A). Poly ADP ribosylation is known to be rapidly and greatly increased by DNA strand breaks and is easily detected (de Murcia and Menissier de Murcia, 1994). The level of poly (ADP-ribose) was significantly decreased in cells treated with 7 mM 3-AB, showing continuous inhibition for 48 h (Fig. 2B). Doxorubicin-treated cells did not show an increased poly (ADP-ribose) level, indicating that doxorubicin could be inactivated and/or that DNA repair might have occurred by 48 h after treatment, consistent with the rapid turnover of poly (ADP-ribose).

### *3.3. PARP inhibition induces apoptosis and enhances infectivity through cell-free virions*

PARP cleavage has been used as a marker for apoptosis caused by caspases (Soldani and Scovassi, 2002). Western blotting with a PARP-1

**Table 1**
Quantification of viral protein components in virion released from c77 cells, shown in Fig. 4D, were calculated and digitized by Image-J. The amount of each viral protein in virion from control c77 cells was normalized as 1.

| | c77 | 3-AB 3mM | 3-AB 7mM | Dox. 100 nM | Dox. 200 nM |
|---|---|---|---|---|---|
| gp46 SU | 1.0 | 2.0 | 2.2 | 1.3 | 1.3 |
| p33 IN | 1.0 | 1.4 | 1.5 | 1.1 | 1.6 |
| p24 CA | 1.0 | 1.6 | 1.9 | 1.9 | 1.8 |
| gp21 TM | 1.0 | 1.4 | 1.6 | 1.6 | 1.5 |
| p19 MA | 1.0 | 1.0 | 1.2 | 1.1 | 1.2 (48 hr) |

antibody revealed that PARP-1 cleavage was significantly increased in c77 cells treated with 7 mM 3-AB or 200 nM doxorubicin for 48 h (Fig. 3A and B). Next, we analyzed another apoptotic marker, annexin V, using flow cytometry. Doxorubicin increased the number of annexin V–positive cells as expected. Consistent with the above PARP cleavage observation, 3-AB also increased annexin V–positive cells, demonstrating the significant increase of apoptosis in HTLV-1-producing c77 cells (Fig. 3C and D). However these increases of these apoptotic markers are not so large as compared with the cell death determined with Trypan Blue dye exclusion test, the decrease of uncleaved PARP-1 level is not so apparent.

Then, we examined the effect of PARP inhibition and DNA damage on viral infectivity through virions. Virions were prepared from c77 HTLV-1-producing cells, and their infectivity was determined using the luciferase assay. Transfection efficiency was monitored by the expression of green fluorescent protein, and the samples showing over 60% transfection efficiency were used for the assay. When the infectivity was expressed as the number of infected cells per 50 ng p19 antigens of virions, treatment with 7 mM 3-AB significantly increased the infectivity by 2.5-fold. Treatment with 200 nM doxorubicin significantly increased the infectivity by 3.8-fold, whereas 3 mM 3-AB or 100 nM doxorubicin did not significantly increase infectivity (Fig. 3E). Similarly, 10 Gy γ-irradiation increased the infectivity by 2.8-fold (data not shown).

### *3.4. Inhibition of poly ADP ribosylation increases Tax levels with consequent changes in the composition of virion proteins*

The increased infectivity observed with 3-AB or doxorubicin might be caused by increased production of Tax (Fig. 4A). We assessed change in p53 levels because p53 is induced by treatment with DNA damaging agents (Datta and Nicot, 2008). Although treatment with doxorubicin increased both p53 and Tax levels, treatment with 3-AB did not change total p53 levels, but the Tax levels significantly increased in c77 cells after incubation for 48 h (Fig. 4A–C).

Next, we examined whether the composition of viral proteins both in cells and the virion extract was affected by treatment with 3-AB or doxorubicin (Fig. 4D). In the cell extract, gp46 SU, p24 CA, and p19 MA appeared to accumulate following drug treatment. In virions, additionally, the gp21 TM and p24 TM level also increased (Table 1). These results indicated that the enhancement of infectivity found following the drug treatments was a consequence of increased Gag and Env production in c77 cells and the incorporation of these two proteins in virions.

## 4. Discussion

PARP-1 has long been recognized as a key component of immunity and inflammatory responses (Rosado et al., 2013). Treatment of mice with a PARP inhibitor suppresses lymphocyte proliferation and lymphokine induction (Weltin et al., 1995). Durkacz et al. reported that the synergistic potentiation of cell killing by PARP inhibitors and alkylating agents might be used to treat human leukemia (Durkacz et al., 1980). “In our experiments, 3-AB at 7 mM decreased the proliferation rate of MT-2 cells and doxorubicin at 200 nM decreased the proliferation rate of c77 cells. These effects by 3-AB and doxorubicin are not toxic on the basis of Trypan Blue dye exclusion, since 10 mM 3-AB was not toxic to CHO-K1 cells (Tsuda et al. 2012) and the concentration of doxorubicin is one order lower than the effective concentration used in various human cells (Oliveira et al., 2014). Our experiments and the previous report (Tsuda et al. 2012) clearly showed that the decrease of proliferation rate with 3-AB in MT-2 cells and CHO-K1 cells is due to the decrease of cell cycle speed and not due to the increase of cell death. c77 cells showed less sensitivity to 3-AB with respect to cell proliferation, although the *in vivo* level of poly(ADP-ribose) was inhibited more than 50%.

There is some discrepancy between the viability assayed by Trypan Blue dye exclusion and that by apoptosis markers namely PARP-1 cleavage and showing annexin V on the cell surface. Since some of the cells treated with 3-AB or doxorubicin at these concentrations and showing apoptotic markers still excluded Trypan Blue, suggesting that they might not be really dying. On the contrary, even if they are really dying cells, the majority of the cell population is still proliferating in the long term culture. It is worth mentioning that about 8–10% of the control cells were stained with Trypan Blue dye. In any way our results revealed that although the PARP inhibitor induces apoptosis markers at certain level in HTLV-1-producing cells and hence might alleviate HTLV-1-associated diseases, we demonstrated in this work that the PARP inhibitor at nontoxic dose enhanced HTLV-1 virion production from HTLV-1-producing c77 cells.”

Genomic instability is the currently favored mechanism of transformation by HTLV-1. It is reported that HTLV-1 encodes a regulatory protein, Tax, which is associated with a generalized inability to repair DNA mutations, adducts, and breaks (Chaib-Mezrag et al., 2014). Tax-expressing cells may incur damage under a variety of environmental or physiological conditions. We clarified that a general PARP inhibitor, 3-AB, and a DNA damaging agent, doxorubicin, induced expression of Tax and highly increased HTLV-1 virus particles infectivity (Figs. 3E and 4 A and B). Retrovirus infectivity is controlled by the efficiency of integration into host DNA. Host DNA repair machinery that involves DNA strand breaks and rejoining are required for efficient retrovirus integration (Marriott et al., 2005; Matsuoka et al., 2011). Therefore, any DNA damaging agents such as doxorubicin that cause DNA strand breaks could be expected to increase the frequency of retrovirus integration. 3-AB, an inhibitor of PARP1 that is important for DNA base excision repair, could increase the abundance of broken DNA before the rejoining step of DNA repair, might be also expected to increase retrovirus integration frequency. These results might partially explain the acquisition of drug tolerance and various complications associated with HTLV-1 infection, given that CHOP therapy, which is representative chemotherapy for malignant lymphoma, is temporarily effective for ATL as ATL relapses.

We reported previously that 3-AB induces the DNA damaging signal pathway without exogenous DNA damage (Tsuda et al., 2012). However, here, we showed that treatment with 3-AB slightly increased the apoptotic cells in HTLV-1-producing cells (Fig. 1B and C). These results implied that DNA repair capacity is compromised in HTLV-1-infected cells (Tada et al., 2015). Furthermore, it is well established that many DNA repair proteins are recruited to sites of DNA damage, rich in poly ADP-ribosylated proteins (Tallis et al., 2014). Therefore, DNA repair proteins could not be recruited by treatment with 3-AB, which might cause induction of apoptosis.

Although UV irradiation is known to directly damage DNA, Shimizu and colleagues reported that HTLV-1 is more susceptible to UV irradiation than other retroviruses (Shimizu et al., 2004). We used doxorubicin because this drug alters DNA conformation by stabilizing the DNA topoisomerase II complex and induces DNA damage. We confirmed that doxorubicin increased the amount of p53 in cells and increased viral infectivity. On the other hand, although the PARP inhibitor did not alter p53 levels, it increased infectious viral discharge.

The mechanism of increase of viral production by PARP inhibitor awaits further study. Various kinds of PARP inhibitors are being considered as therapeutics for conditions such as inflammation, neurodegeneration, and cancers (Jagtap et al., 2005). Now PARP inhibitor is approved of treatment for ovarian and breast cancer with BRCA1/2 deficient genetic background. However, careful evaluation including proviral load measurement before and during the administration of PARP inhibitors or doxorubicin to HTLV-1- infected patients, especially in the endemic areas of HTLV-1 infection.

## Funding information

This work was supported partly by KAKENHI Grant Number 20590291 from JSPS, and the Emerging/Re-emerging Infectious Diseases Project of Japan Grant Number JP17fk0108111j0101 from the Japan Agency for Medical Research and Development (AMED). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

## Acknowledgements

We thank Dr. Jun-ichi Fujisawa, Kansai Medical University, for useful comments. The authors would like to thank Enago (www.enago.jp) for the English language review.

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