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Aflatoxin B_1 Inhibits the Replication of Chandipura Virus in Human Hepatoma Cells

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Abstract

We previously showed that aflatoxin B_1 inhibited the antiviral type 1 interferon signalling response pathway as a mechanism of hepatocellular carcinoma. The type I interferon signalling is part of the innate immune system that defends the body against invading microorganisms. The consequence of compromising or suppressing the immune system include increased susceptibility to infectious diseases, reactivation of chronic diseases as well as increased susceptibility to cancers. Therefore, the chandipura virus was selected as a model virus to test the hypothesis that aflatoxin B_1 would enhance viral replication in patients presenting with viral infections and long exposure to dietary aflatoxin. The chandipura viral titre was quantified in the presence or absence of aflatoxin B_1 by plaque assay using L929 cells and stained with crystal violet. The data presented in this research notes is a side product of another research of which the results have been published. The current study showed aflatoxin B_1 (10 μM) reduced the replication of chandipura virus and the rate of reduction was exacerbated in the presence of interferon. The results open avenues for further studies to understand the interplay between viral infections and aflatoxins in accelerating cancers such as the hepatocellular carcinoma.

Keywords: Aflatoxin B_1; Chandipura virus; HepG2; Type I interferon pathway

Introduction

Aflatoxin B_1 (AFB_1) a lethal mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* has been reported to inhibit the type I IFN response signalling pathway [1]. The type I IFN signalling response pathway is part of the innate immune system that is responsible for combating disease causing microorganisms and also initiate the process for the development of the adaptive immune response. The consequence of compromising the immune system include increased susceptibility to infectious diseases, reactivation of chronic diseases as well as increased susceptibility to cancers. What this suggests is that any agent that is capable of inhibiting the type I IFN response signalling pathway could significantly suppress the immune system and make the individual more prone to infections caused by microorganisms.

The current study aims to test the hypothesis that AFB_1 would enhance virus growth to higher titres in individuals with long term dietary exposure to aflatoxins. The data presented in this research notes is a side product of another research project of which the results have been published [1].

Materials and Methods

The human hepatoma cell line HepG2 (ECACC 85011430) and mouse fibroblast cells (L929) L929 (NCTC) (ECACC 85103115) were generously donated by Professor David J. Blackbourn of the University of Surrey, UK. The cells were cultured in growth medium containing DMEM high glucose, L-glutamine, sodium pyruvate and HEPES (Sigma Aldrich, Germany) supplemented with 10% v/v heat inactivated Foetal Bovine Serum (FBS) (Sigma Aldrich, Germany), 1% v/v MEM non-essential amino acids (Sigma Aldrich, Germany), 100 IU/mL of penicillin and 100 μg/mL of streptomycin (Gibco life technologies, UK). The cultures were maintained at 37°C in 5% carbon dioxide (CO_2). In order to prevent mycoplasma infection and also to ensure that the cells were free of mycoplasma, occasionally actively dividing culture of cells were treated with 25 μg/mL of Plasmocin (cat no ant-mpt, invivogen, USA) an anti-mycoplasma agent. The lyophilized AFB_1 used in the study was commercially purchased from Sigma-Aldrich, USA (cat no A6636) and reconstituted as previously described [1].The human interferon-alpha (IFN-α) used in the study was purchased from PBL interferon source (PBL, USA) and prepared as previously described [1]. The chandipura virus used in the study was generously donated by Professor David J. Blackbourn.

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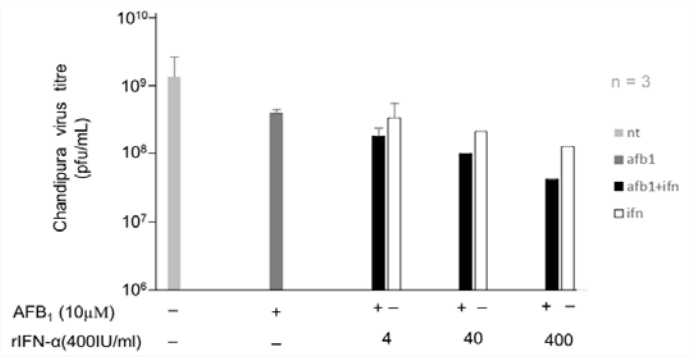

Figure 1: AFB_1 alone is lethal to chandipura virus and the lethality is exacerbated by IFN-α. Monolayer's of HepG2 cells were pre-treated with AFB_1 (10µM) and simultaneously treated with increasing concentrations of rIFN-α (4, 40 & 400 IU/ml) for 24 hours. The cells were infected with chandipura virus at MOI of 0.1. The infected cells were incubated for one hour to allow the viruses to adsorb onto the cells. The virus containing media were removed and the cells treated with fresh media (without AFB_1 and or rIFN-α) for 24 hours. The tissue culture supernatants containing the virus were collected and the virus titre was quantified by plaque assay using L929 cells. After incubation for 3 days, plaques were identified by crystal violet staining and counted. The results are presented as mean and standard deviation of three independent experiments conducted in duplicate independent wells. Statistical analysis was performed using Microsoft excel (2013 version). NT: cells which were neither stimulated with interferon nor treated with aflatoxin B_1; AFB_1: cells which were treated with only aflatoxin B_1; AFB_1+IFN: cells which were stimulated with interferon and simultaneously treated with aflatoxin B_1: IFN: cells which were stimulated with only interferon.

To examine the effects of AFB_1 on replication of virus, chandipura virus was cultured in the presence or absence of AFB_1 (10 µM) and IFN-α (4, 40 and 400 IU/ml). The concentrations of AFB_1 and IFN-α used in this experiment were those reported previously [1]. Briefly, HepG2 cells were seeded at a density of 5 x 10^5 cells/well in 6-well plates. The cells were then stimulated with increasing concentration of IFN-α (4, 40 & 400 IU/ml) and simultaneously treated with or without AFB_1 (10 µM). Twenty-four hours later the cells were infected with chandipura virus at a Multiplicity of Infection (MOI) of 0.1. After 1 hour of incubation to allow the viruses to adsorb onto the surfaces of the cells, the supernatant was removed and 2 mL growth medium containing neither AFB_1 nor IFN-α were added. Cells were incubated for 24 hours and the supernatant collected and the virus titer determined by plaque assay using L929 cells following standard protocol. After incubation for three (3) days, plaques were stained with 0.05% crystal violet solution and counted. Titer concentrations (in plaque forming unit (PFU) per mL) were calculated by using the equation:

$$PFU/mL = \frac{Average\ number\ of\ plaques}{d\ x\ v}$$

Where, d = dilution

v = volume of diluted virus added to the plate

Data were entered into excel spread sheet and Microsoft excel (2013 version) was used to calculate the averages and the standard deviations of the three independent experiments.

Results and Discussion

To be able to investigate the biological significance of the inhibition process on the anti-viral property of the type I IFN by AFB_1 in HepG2 cells, the survival of the HepG2 cells in the presence of AFB_1 was very critical. The concentration of AFB_1 employed in this study was 10 µM as previously reported [1]. This concentration was found to be non-toxic to the HepG2 cells but was high enough to significantly inhibit the type I IFN response signalling pathway. In addition, the concentration of IFN-α which induced maximally the type I IFN signalling was established as 400 IU/mL as previously reported [1]. Figure 1 shows AFB_1 reduced the replication of chandipura virus and the rate of reduction was exacerbated by activated anti-viral type I IFN response signalling pathway.

The type I IFN signalling pathway is well known for its antiviral property. Therefore, after demonstrating in a previous study that AFB_1 suppressed the type I IFN signalling pathway, the expectation was that AFB_1 would ultimately suppress that antiviral pathway and enhance viral replication to significantly higher titres.

A number of studies have utilized the replication of viruses to study the effects of different inhibitors or chemical groups on the antiviral effects of the type I IFN response signalling pathway. For example [2], utilized the replication of IFN sensitive Encephalomyocarditis Virus (EMCV) in vIRF-2 clone 3-9 and EV clone 5 cells to study the biological significance of vIRF-2 inhibition of the type I IFN signalling pathway and reported that vIRF-2 rescued the EMCV from the antiviral effects of type I IFN (2). In another study, [3] also employed the replication of Enterovirus in human cervical carcinoma (HeLa S3), Vero and human hepatoma (Huh 7) cell lines to determine whether proteinase 2 A^{pro} was essential for Enterovirus replication in type I IFN treated cells and reported that proteinase 2 A^{pro} rescued the Enterovirus from the antiviral effects of the type I IFN. However, proteinase 2 A^{pro} could not rescue the replication of vesicular stomatitis virus and EMCV in type I IFN pre-treated cells [3].

Taken together the above information, the replication of chandipura virus was utilized in this study to investigate the biological significance of the AFB_1 inhibition of type I IFN signalling pathway in HepG2 cells. Contrary to the initial expectation that AFB_1 would rescue the IFN sensitive virus from the antiviral effects of the type I IFN response signalling pathway, our result pointed to the fact that AFB_1 rather significantly reduced the IFN-sensitive chandipura virus replication and that the rate of reduction was exacerbated by activated type I IFN response signalling pathway. The result obtained from this study was similar to that of [4] who reported that AFB_1 decreased the replication of hepatitis B virus (HBV) in HepaRG cells after exposure of HBV to AFB_1 at a concentration of up to 5 µM [4].

However, a study conducted by [5] to investigate the effects of AFB_1 exposure on the replication of duck hepatitis B virus in Pekin duck model during the initial stage of virus-AFB_1 interaction showed a significant increase in the titre of duck hepatitis B virus in the serum and liver of AFB_1 treated-ducks compared with Dimethyl Sulfoxide (DMSO) treated controls [5]. The increase in viral replication

observed in the Pekin duck model was confirmed *in vitro* in primary duck hepatocytes [5]. In addition, [6] investigated the effects of AFB_1 exposure on the replication of influenza virus in $LLC\text{-}MK_2$ cells and reported that the levels of virus concentration attained in AFB_1 treated cells were two to four-fold higher compared with the untreated control. The enhanced influenza viral growth rate observed in AFB_1 treated cells in the study of [6] was attributed to the ability of AFB_1 to inhibit IFN production [6].

There seem to be conflicting information in literature as to the effects of AFB_1 on the replication of viruses. While some studies have reported that AFB_1 enhances the replication of some viruses in the presence of the activated type I IFN response signalling pathway, others have reported that AFB_1 inhibits the replication of some viruses. Findings from this study indicates that AFB_1 reduced the replication of chandipura virus. The mechanism by which AFB_1 kills some viruses and rescues others in the presence of the activated type I IFN signalling pathway is a subject of further research. It is possible that the inability of AFB_1 to rescue some viruses but others could be due to the fact that the replication of some viruses may differ from others in their sensitivity to AFB_1 and the diverse activity of AFB_1 under experimental conditions. It is also possible that AFB_1 may bind and block certain enzymes and substrates that are required for the replication of some viruses hence its ability to kill them.

Conclusion

In conclusion the current study has shown that AFB_1 reduced the replication of chandipura virus and the rate of reduction was exacerbated in the presence of IFN-. Considering finding(s) from this and other studies, further research may be required to unravel the exact mechanism by which AFB_1 kills some viruses and rescues others.

Limitation(s)

The chandipura virus used in this study has tropism for cells of the nervous system and that its use on human liver derived cell line could have affected the result. In place of chandipura virus, hepatitis B virus which has tropism for liver cells could have been used. However, the difficulty in procuring the virus made it impossible for it to be used in the current study.

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