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Original Article

Synergic effect of curcumin and its structural analogue (Monoacetylcurcumin) on anti-influenza virus infection

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ABSTRACT

Curcumin (Cur), a polyphenolic compound extracted from spice and common food colourant turmeric, contains versatile bio-activities. Monoacetylcurcumin (MAC), a structural analogue of Cur, differs from Cur by acetyl modification, but retains enone groups. Comparative analysis revealed MAC effectively inhibited influenza virus infection (IAV) to a similar extent as, if not superior to, curcumin. Both compounds mildly reduced viral NA activity. Surprisingly, unlike Cur, the MAC inhibition of IAV did not occur through the blocking of HA activity. However, MAC strongly dampened Akt phosphorylation, the prerequisite signalling for efficient IAV propagation. A much stronger inhibition effect on IAV infection was observed when MAC treatment was in combination with Cur. Collectively, MAC demonstrated clear antiviral activity, and likely inhibited IAV via multiple mechanisms that were not identical to Cur. Importantly, Cur and MAC in combination synergistically inhibited IAV infection.

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1. Introduction

Influenza A virus (IAV), an enveloped virus belonging to the family *Orthomyxoviridae*, is responsible for causing the human-to-human transmission of pandemic influenza, as well as cases of seasonal influenza. The World Health Organization (WHO) reports that of the estimated 5–10% of adults and 20–30% of children infected annually, 3–5 million cases of severe disease and 250,000–500,000 deaths occur from influenza each year worldwide. Recently, there has been a rise in the number of cases of bird-to-human transmission of avian influenza A viruses, including different HA subtypes (H5, and H7). H5N1 IAV first infected people in Hong Kong in 1997 and has subsequently infected more than 800 people with an approximate death rate of 60% [1]. In March 2013, a novel subtype of avian influenza A virus, identified as subtype H7N9, was transmitted from birds to human in China [2], and while H7N9 has lower mortality rate than H5N1, cases of H7N9 IAV continue to be reported in China. The most common antiviral drugs used to treat severe influenza target the viral neuraminidase (NA), despite the frequent emergence of viral resistance to these drugs [3]. In light of this, there is a clear need to develop drugs with more varied targets to treat influenza virus infections.

Natural products are potential sources of bioactive compounds, including some with antimicrobial activity [4,5]. Curcumin (Cur), a polyphenolic component of the spice and common food colourant turmeric (from the root of *Curcuma longa*), has gained much attention for its anti-oxidant and anti-inflammatory [6] activities, along with its anti-proliferative and anti-tumour activities [7]. It has been shown that the regulation of cellular signalling pathways, such as the activation of the transcription factor NF-κB, is central to providing curcumin with its versatile biological properties. Recently, Cur has also been shown to exhibit antiviral activity at non-cytotoxic concentrations against IAV [8–10], as well as against several enveloped viruses, including Japanese encephalitis virus, pseudorabies virus, dengue virus type 2, and vaccinia virus, but not against the non-enveloped enterovirus-71 [9]. The initial investigation into Cur's anti-influenza activity was based on the importance of NF-κB activation in IAV replication [11] and Cur's ability to act as a potent NF-κB inhibitor [12]. In addition to NF-κB, Cur also inhibits the phosphatidylinositol-3-kinase (PI3K)/AKT cellular pathway that leads to apoptosis in many tumour cells [13], while influenza virus infection activates PI3K/AKT [14], presumably in an attempt to prevent the cell from undergoing apoptosis and to benefit viral propagation at the initial stage of infection.

The anti-influenza activity of Cur was previously shown to be independent of one or both of the methoxyl groups found on its phenols [8] but is now thought to be dependent on the presence of two enone groups (α,β-unsaturated carbonyl groups) in the seven-carbon chain attached to its phenol rings (see Fig. 1A, dashed boxes). The enone groups of Cur could spontaneously form Michael adducts with sulfhydral (SH) groups [15], and the presence of glutathione (GSH), consisting of amino acids with an SH group, diminished Cur's ability to inhibit IAV plaque formation [10].

It has been reported that application of Cur is limited by its intrinsic properties such as low aqueous solubility and poor stability, leading to poor bioavailability [16]. To circumvent this drawback, several strategies have been proposed, for instance, generation of curcumin structure analogues with bioactivity [17], and development of new formulation [18–20]. Several curcumin structural analogues have been reported and characterised. Among those analogues, tetrahydrocurcumin (THC), a Cur analogue that lacks the enones of Cur, markedly reduced anti-influenza activity compared with Cur [10]. Because THC is a major early metabolite of Cur *in vivo* [21], it is possible to render Cur less effective as an influenza therapeutic in patients. Monoacetylcurcumin (MAC) has identical enone groups and differs from Cur by only one acetyl group (Fig. 1A, grey box). MAC was first identified as an *in vitro* inhibitor of mammalian DNA polymerase λ [22], a polymerase involved in DNA repair; Cur also inhibits DNA polymerase λ [23]. More recently, MAC was shown to reduce NF-κB nuclear translocation induced by lipopolysaccharide (LPS) more effectively than curcumin [24], further strengthening its potential as an anti-influenza agent.

Hence, in this study, as MAC and Cur share similar structure, a series of experiments were performed to initially define whether MAC possess anti-influenza activity. Moreover, the underlying mechanisms were further investigated. Our results indicated that as with Cur, MAC acts as a potent inhibitor of influenza virus infection. However, MAC has no impact on viral HA function. As the treatment of cells with both MAC and Cur together demonstrated a synergistic effect, this implies that these two drugs may have different affinities for their targets and could be used in combination as an alternative influenza antiviral therapeutic.

2. Materials and methods

2.1. Cells and virus

Madin-Darby canine kidney (MDCK) cells were grown in minimal–essential medium (MEM) with 10% fetal bovine serum (FBS), and antibiotics (penicillin 100 U/ml, streptomycin 10 μg/ml). For infection, virus was diluted in infection medium (MEM without FBS) supplemented with antibiotics and 1 μg/ml of trypsin (Worthington, Freehold, NJ, USA) and inoculated to cells that have been washed with PBS.

Influenza Type A virus (IAV) strain PR8, A/Puerto Rico/8/34 (PR8, H1N1), was kindly provided by Paul Digard (University of Cambridge, UK).

2.2. Compounds

Curcumin and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich. MAC was synthesized as described in one previous study [22]. Reduced glutathione (GSH), purchased from Sigma-Aldrich, was dissolved in 10 mM of Tris (pH 7.5, adjusted using 5N KOH).

2.3. Cytotoxicity test

The cytotoxicity effect of curcumin and MAC was determined on the basis of cell survival rate [8]. Briefly, 7.5×10^4 MDCK

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