In-vitro NMR studies of Echovirus 11 infection in RD human cells

S. N. Akhtar[1], R. K. Singh[2], Y. Jadegoud[1], G. A. Nagana Gowda[1], T. N. Dhole[2], A. Ayyagari[2], M. Bhandari[3], C. L. Khetrapal[1]

[1]Centre of Biomedical Magnetic Resonance, LUCKNOW, UTTAR PRADESH, India, [2]Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, LUCKNOW, UTTAR PRADESH, India, [3]King George's Medical University, LUCKNOW, UTTAR PRADESH, India

SYNOPSIS: The effects of Echovirus 11 infection on RD human cell lines (derived from rhabdomyosarcoma) were studied using ^{1}H NMR spectroscopy and optical microscopy. Both uninfected and infected cells, consumed glucose and produced lactate and acetate as extracellular metabolites. In infected whole cells, glycerophosphocholine and uridinediphosphoglucose were observed in addition to the metabolites observed in uninfected cells. Intracellular metabolites of infected cells showed glutamine, glycerophosphocholine and glycine which were not observed in uninfected cells. The uninfected and infected cells did not show any difference in their lipid components. Unlike optical microscopy, NMR spectroscopy identified early stages of infection through metabolic changes.

INTRODUCTION: Studies on the virus-cell infection have proven valuable in elucidating viral cellular processes. The delivery of the viral nucleic acid into the host cell involves a complex series of tightly regulated events where the entering virus utilizes the host cells biosynthetic machineries to reach its goal and alter cell metabolism. The Echovirus 11 (genus: Enterovirus; family, Picornaviridae) can cause various diseases e.g. Paralysis, Encephalities, Ataxia or Guillain-Barre syndrome, Respiratory diseases and Exanthema. Understanding the molecular mechanism of viral infection may be useful for the study of pathophysiology of virus- cell interactions. In this study in-vitro infection of Echovirus 11 in RD human cells derived from rhabdomyosarcoma was studied using ^{1}H NMR spectroscopy and optical microscopy to understand metabolic alterations and cell morphological changes, respectively.

MATERIAL AND METHODS: RD cells obtained from Center for Disease Control and Prevention, Atlanta, USA. Echovirus 11 prototype Gregory strain was obtained from Dr. Kenji Sakai, Aichi Institute of Public Health, Nagoya, Japan. Minimum essential medium Egale's (MEM) obtained from Sigma Aldrich, USA. RD cells grown under sterile condition in 75 cm^2 flask at 37^o C, in 5% CO_2 - 95% air incubator, in MEM media supplemented with 10% Fetal Bovine serum. Dynamic studies of Echovirus 11 infection on RD human cell line was performed at different intervals of time of infection (12, 24, 36 and 48 hrs). Cell morphological changes at different stages of viral infection were monitored through Nikon Inverted microscope with 40X and 10 X magnifications. At each stage of viral infection, extracellular and whole cells metabolites were monitored using ^{1}H NMR spectroscopy. Subsequently, intracellular metabolites were determined after cell extraction into deuterated water and the residual cell pellet was subjected to lipid extraction using chloroform and methanol to monitor lipid components. All ^{1}H NMR experiments were performed on a Bruker Biospin 400 MHz spectrometer at 25^o C by suppressing the residual water signal by presaturation. Typical parameters used were, spectral width: 8000 Hz; data points: 32K; flip angle: 45^o; number of scans 128; relaxation delay 5s and FT size: 32 K. A reusable co-axial capillary containing calibrated quantity of sodium salt of trimethylsilylpropionate (TSP) dissolved in deuterium oxide was used for each NMR experiment to serve as a reference.

RESULTS: Both uninfected and infected RD cells used glucose and produced extracellular metabolites, lactate and acetate. In ^{1}H NMR spectrum of uninfected whole cells (Fig. 1), lipids, lactate, threonine, alanine, glutamine, creatinine, choline, and fatty acids were observed (Fig. 1a). At 12 hrs of infection glycerophosphocholine and uridinediphosphoglucose were observed in addition to the metabolites observed in uninfected cells (Fig. 1b). These metabolites were gradually decreased and disappeared during 24 hrs to 48 hrs of infection (Fig. 1c-e). Intracellular metabolites of uninfected cells extracted in D_2O showed leucine, isoleucine, lactate, threonine, alanine, glutamate, ctreatinine, choline, glycerophosphoethanolamine, tyrosine, phenylalanine, uridinediphosphoglucose and formate. Whereas in 12 hrs infected cells glutamine; glycerophosphocholine and glycine were observed in addition to the metabolites observed in uninfected cells. Chloroform-methanol extracts of uninfected and infected cells showed fatty acids, cholesterol and phospholipids such as phosphatidylethanolamine and phosphatidylcholine. Optical microscopic studies of the cells showed cytopathic effect after 24 hrs of viral infection (Fig. 2) where as NMR spectroscopy showed molecular changes in early stage 12 hrs of infection.

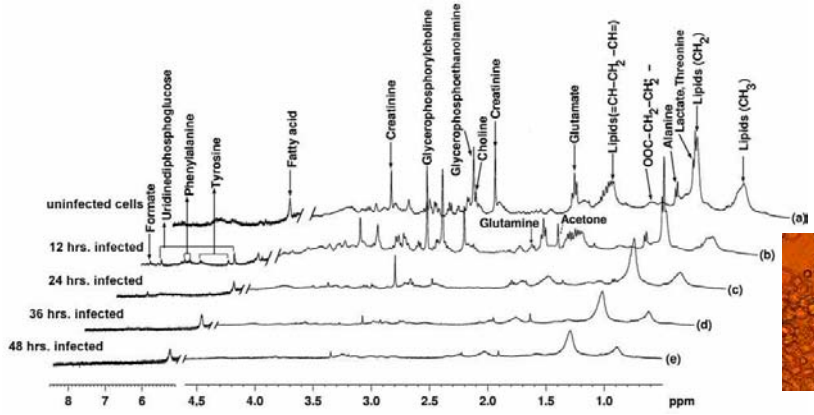

Figure 1: ^{1}H NMR spectra of RD whole cells. (a) Uninfected cells; (b-e) Echovirus 11 infected cells. (Chemical shift scale is shown for only spectrum (e); same scale is maintained for others)

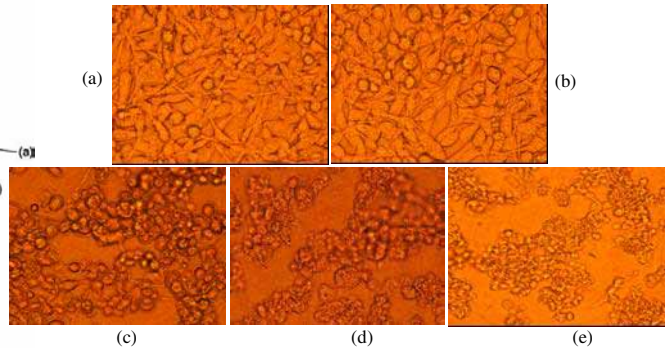

Figure 2: Cells in culture flask at magnification 40X; (a) Uninfected RD cells and (b-e) Echovirus 11 infected RD cells; (b) 12 hrs infected; (c) 24 hrs infected; (d) 36 hrs infected; (e) 48 hrs infected.

DISCUSSION: Dynamic NMR and optical microscopy studies are made here as an attempt to understand molecular level changes in human RD cells before and after infection with Echovirus 11. Striking differences in the metabolites were observed between uninfected and infected cells. Cells utilize glucose through glycolysis and TCA metabolic pathways and excrete metabolic end products in the media. With increasing time of infection, all molecular metabolites of the cells gets used up leaving behind only the cell lipids components. Viral infection does not seem to utilize cell lipids even after 48 hrs of infection where cellular destruction had taken place as observed by optically microscopy (Figure 2e). Echovirus 11 is a non-enveloped single stranded RNA virus which does not posses lipid envelop in its structure. The results of the present study appear to indicate that the virus does not utilize cell lipids during its viral particle synthesis. The fact that effect of viral infection could be observed through metabolic changes at much earlier stages using NMR spectroscopy, it indicates possible application of cell-viral interactions studies using NMR.

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