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Lithium chloride inhibits the coronavirus infectious bronchitis virus in cell culture

Sally M. Harrison[1]*, Ian Tarpey[2], Lisa Rothwell[3], Pete Kaiser[3] and Julian A. Hiscox[1,4]

[1]*Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK,* [2]*Intervet UK Ltd, Milton Keynes, UK,* [3]*Institute for Animal Health, Compton, UK, and* [4]*Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK.*

The avian coronavirus infectious bronchitis virus (IBV) is a major economic pathogen of domestic poultry that, despite vaccination, causes mortality and significant losses in production. During replication of the RNA genome there is a high frequency of mutation and recombination, which has given rise to many strains of IBV and results in the potential for new and emerging strains. Currently the live-attenuated vaccine gives poor cross-strain immunity. Effective antiviral agents may therefore be advantageous in the treatment of IBV. Lithium chloride (LiCl) is a potent inhibitor of the DNA virus herpes simplex virus but not RNA viruses. The effect of LiCl on the replication of IBV was examined in cell culture using two model cell types; Vero cells, an African Green monkey kidney-derived epithelial cell line; and DF-1 cells, an immortalized chicken embryo fibroblast cell line. When treated with a range of LiCl concentrations, IBV RNA and protein levels and viral progeny production were reduced in a dose-dependent manner in both cell types, and the data indicated that inhibition was a cellular rather than a virucidal effect. Host cell protein synthesis still took place in LiCl-treated cells and the level of a standard cellular housekeeping protein remained unchanged, indicating that the effect of LiCl was specifically against IBV.

Introduction

Coronaviruses are a family of positive-sense, single-stranded RNA viruses that replicate in the cytoplasm of infected cells. Infectious bronchitis virus (IBV) is a highly pathogenic respiratory pathogen of chickens that can also affect the kidneys and reproductive systems (Raj & Jones, 1996; Cavanagh, 2005), therefore resulting in both bird mortality and decreased reproductivity (Ignjatovic & Sapats, 2000). As with all coronaviruses, the IBV input genomic RNA is translated by host cell ribosomes to generate an RNA-dependent RNA polymerase (Rep1a, Rep1ab) (Lai & Cavanagh, 1997). This complex is responsible for the transcription of viral subgenomic mRNAs via a discontinuous mechanism (Pasternak *et al*., 2006) and the generation of new genomic RNA. Both the genomic and subgenomic mRNA are 3′ co-terminal and share a common 3′ untranslated region (UTR), whereas the 5′ end of the genomic mRNA has a unique 5′ UTR.

While live attenuated vaccines are used to prevent infection with IBV, these give little cross-strain immunity (Cavanagh, 2005; Gelb *et al*., 2005; Liu *et al*., 2006). The problem of vaccination efficiency against IBV is compounded due to the extensive antigenic variation between different strains (Jackwood *et al*., 2005; Bochkov *et al*., 2006), caused by the high frequency of mutations due to error-prone replication and also recombination (Wang *et al*., 1993; Kottier *et al*., 1995; Lee & Jackwood, 2000). Other important steps that are taken to control IBV infection are serological monitoring to determine virus exposure, reverse transcriptase-polymerase chain reaction (RT-PCR) to detect viral RNA, and enzyme-linked immunosorbent assay and haemagglutination inhibition to detect IBV antibodies (Adzhar *et al*., 1996; Chen *et al*., 2003). Due to the high probability of new and emerging strains of IBV and other coronaviruses in general, such as severe acute respiratory syndrome coronavirus (Peiris *et al*., 2004), the need to develop alternative strategies to vaccination is paramount (Cavanagh, 2003, 2005; Weiss & Navas-Martin, 2005).

Our understanding of the action of lithium chloride (LiCl) on the replication of a range of DNA and RNA viruses is limited. LiCl inhibits the replication of the DNA virus herpes simplex (Skinner *et al*., 1980), whereas with the RNA viruses, encephalomyocarditis virus and influenza virus, there was no apparent effect on virus biology (Skinner *et al*., 1980). Previous studies have also determined that inhibition of virus is specific to the presence of lithium ions, as no reduction in virus replication was seen in cells treated with potassium or sodium chloride (Skinner *et al*., 1980).

Following the potential application of LiCl to treat DNA virus infection, we investigated the antiviral effects of LiCl on IBV in two cell systems; Vero cells, an African Green monkey kidney-derived epithelial cell line; and DF-1 cells, an immortalized chicken embryo fibroblast cell line. Although Vero cells have been used extensively as a permissible cell line to study virus replication

*To whom correspondence should be addressed. Tel: +44 113 343 5582. Fax: +44 113 343 3167. E-mail j.a.hiscox@leeds.ac.uk
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(Casais *et al*., 2003; Britton *et al*., 2005), cell biology (Dove *et al*., 2006a) and protein targeting (Dove *et al*., 2006b; Reed *et al*., 2006), recent data suggest potential discrepancies in virus biology, in terms of protein trafficking, between avian and mammalian cell lines (Pendleton & Machamer, 2006); therefore, both Vero and chicken cells were used in this study.

Materials and Methods

Cell culture and virus production. Vero cells (an African green monkey kidney-derived epithelial cell line) and DF-1 cells (Doug Foster, a chicken embryo fibroblast-derived epithelial cell line) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum at 37°C in the presence of 5% CO_2, as described previously (Dove *et al*., 2006a). IBV Beaudette US, a strain adapted for growth in Vero cells (Alonso-Caplen *et al*., 1984), was propagated in Vero and DF-1 cells and the virus harvested at 24 h post infection (p.i.). The virus titre was calculated by plaque assay titration in either Vero or DF-1 cells (Dove *et al*., 2006a). All cell culture experiments were conducted in the absence of antibiotic or anti-fungal agents.

Treatment of cells with LiCl. Vero and DF-1 cells were seeded at 2×10^5 in 7 cm^2 tissue culture dishes and grown to 70% confluency prior to mock or infection with IBV at 2×10^6 plaque-forming units/ml. At 8 h p.i. the cells were treated with 0, 5, 10, 25 or 50 mM LiCl, and at 24 h p.i. mock and infected cells were lysed or prepared for subsequent plaque assays.

Preparation of total cellular protein. Mock and IBV-infected Vero and DF-1 cells were harvested 24 h p.i. and lysed using RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, Complete Protease Inhibitor at a dilution of 1/25; Roche). Total protein was quantified by the BCA assay (Promega) and western blot analysis was performed.

Western blot analysis. Ten micrograms of total protein were denatured in Invitrogen NuPage LDS sample reducing buffer containing NuPage reducing agent, and were separated on a NuPage Bis-Tris 10% pre-cast gel in 1 ×MOPS running buffer. Proteins were then electro-transferred onto a polyvinylidene fluoride membrane with transfer buffer that contained 25 mM Bicine, 25 mM Bis–Tris and 10% (v/v) methanol. Western blotting was performed using luminol 3-aminophthalydrazide (Sigma). IBV proteins were detected using a chicken anti-IBV polyclonal antibody (diluted 1:20 000) (Charles Rivers). Mouse anti-GAPDH (6C5) antibody (diluted 1:40 000) (AbCam) was used to detect GAPDH. Horseradish peroxidase-conjugated rabbit anti-chicken and goat anti-mouse secondary antibodies (1:1000 dilution) (Sigma) used as appropriate.

Plaque assay analysis of virus. Tissue culture dishes (7 cm^2) were seeded at 2×10^5 with either Vero or DF-1 cells and grown until confluent. A serial dilution of progeny virus was performed in Dulbecco's modified Eagle's medium with 10% foetal bovine serum ranging from 10^{-1} to 10^{-3} for IBV grown in Vero cells, and from 10^{-1} to 10^{-6} for IBV grown in DF-1 cells. Individual wells were then infected in duplicate with the range of virus dilutions and incubated at 37°C in the presence of 5% CO_2 for 1 h before being over-laid with 1% low melting point agarose in Dulbecco's modified Eagle's medium with 10% foetal bovine serum. The cells were then incubated at 37°C for 72 h before being stained with gentian violet (1% crystal violet, 10% formaldehyde (40%) and 5% EtOH in phosphate-buffered saline). The virus titre was determined by counting the number of plaques formed at a specific dilution (Dove *et al*., 2006a).

Preparation of total cellular RNA. Total cellular RNA was extracted at 0 and 24 h p.i. by the RNeasy method (Qiagen) according to the manufacturer's instructions.

Taqman analysis of IBV genomic and subgenomic mRNA. IBV genomic and subgenomic RNA levels in mock and virus-infected Vero and DF-1 cells treated with 0, 5, 10, 25, 50 mM LiCl were quantified by TaqMan real-time RT-PCR (for other examples, see Kaiser *et al*., 2003; Bicknell *et al*., 2005). Primers and probes for the IBV 5′ UTR to detect genomic RNA and the cellular 28S rRNA were designed using the Primer Express software program (Applied Biosystems). A primer and probe set to detect the IBV 3′ UTR was designed manually as the software did not detect any optimum sequences. The primer and probe sets used in this study are detailed in Table 1 and are based upon the Beaudette US strain (accession number AAA46214).

The TaqMan FAST universal PCR Master Mix (Multiscribe) and RNase inhibitor mix (Applied Biosystems) were used to perform real-time quantitative RT-PCR. Detection and amplification of RNA levels using the 28S, 5′ UTR and 3′ UTR probes were carried out using the 7500FAST TaqMan machine (Applied Biosystems). The following cycle profile was used: one cycle of 48°C for 30 min (RT step) and 95°C for 20 sec (Taq activation), then 40 cycles of 95°C for 3 sec (melting step) and 60°C for 30 sec (anneal and extension step). Quantification was based on increased fluorescence detected due to the 5′ exonuclease activity of the Taq DNA polymerase during PCR amplification hydrolysing the target specific probes. The reporter signal was normalized by the reference dye 6-carboxy-*c*-rhodamine, which was not actually involved in amplification. Results are expressed in terms of threshold cycle (Ct) values; the cycle at which the change in reporter dye passes a significance threshold (ΔRn).

Variation in sampling and RNA preparation was accounted for by standardizing the Ct values for the IBV 5′ UTR-specific and 3′ UTR-specific products for each sample to the Ct value of the 28S rRNA product for the same sample. RNA levels between samples in the same experiment were normalized by pooling values from all samples in that experiment and calculating the mean Ct value for the 28S rRNA-specific gene product. Variations in each individual 28S rRNA sample compared with the mean were then calculated. Differences in the input of total RNA were calculated by determining the slope of the 28S rRNA $\log_{10}$ dilution series regression line. Using the slopes of the respective IBV 5′ UTR, IBV 3′ UTR or 28S rRNA $\log_{10}$ dilution series regression lines, the difference in input total RNA, as represented by the 28S

Table 1. *Primer and probe sets used for the Taqman RT-PCR in this study*

Target	Forward primer	Reverse primer	Probe[a]
Vero 28S	GGCGAAAGACTA ATCGAACCAT	CGAGAGCGCCAG CTATCCT	TAGTAGCTGGTT CCCTCCGAAGTT TCCCT
Chicken 28S	GGCGAAGCC AGAGGAAACT	GACGACCGATTT GCACGTC	AGGACCGCTACG GACCT CCACCA
IBV 5′ UTR	CGTACCGGTTCT GTTGTGTGA	GCCCAACGCTAG GCTCAA	TCACCTCCCCCC ACATACC TCTAA GGG
IBV 3′ UTR	ACGAACGGTAGA CCCTTAGA TTTT AATT	TGGGCGTCCTAG TGCTGTACTAA	TACTCAGCGTGG CCCCGGCA

[a]At the 5′ end of each probe was the fluorescent reporter dye 5-carboxyfluorescein, and at the 3′ end was the quencher *N*,*N*,*N*,*N*′-tetramethyl-6-carboxyrhodamine.

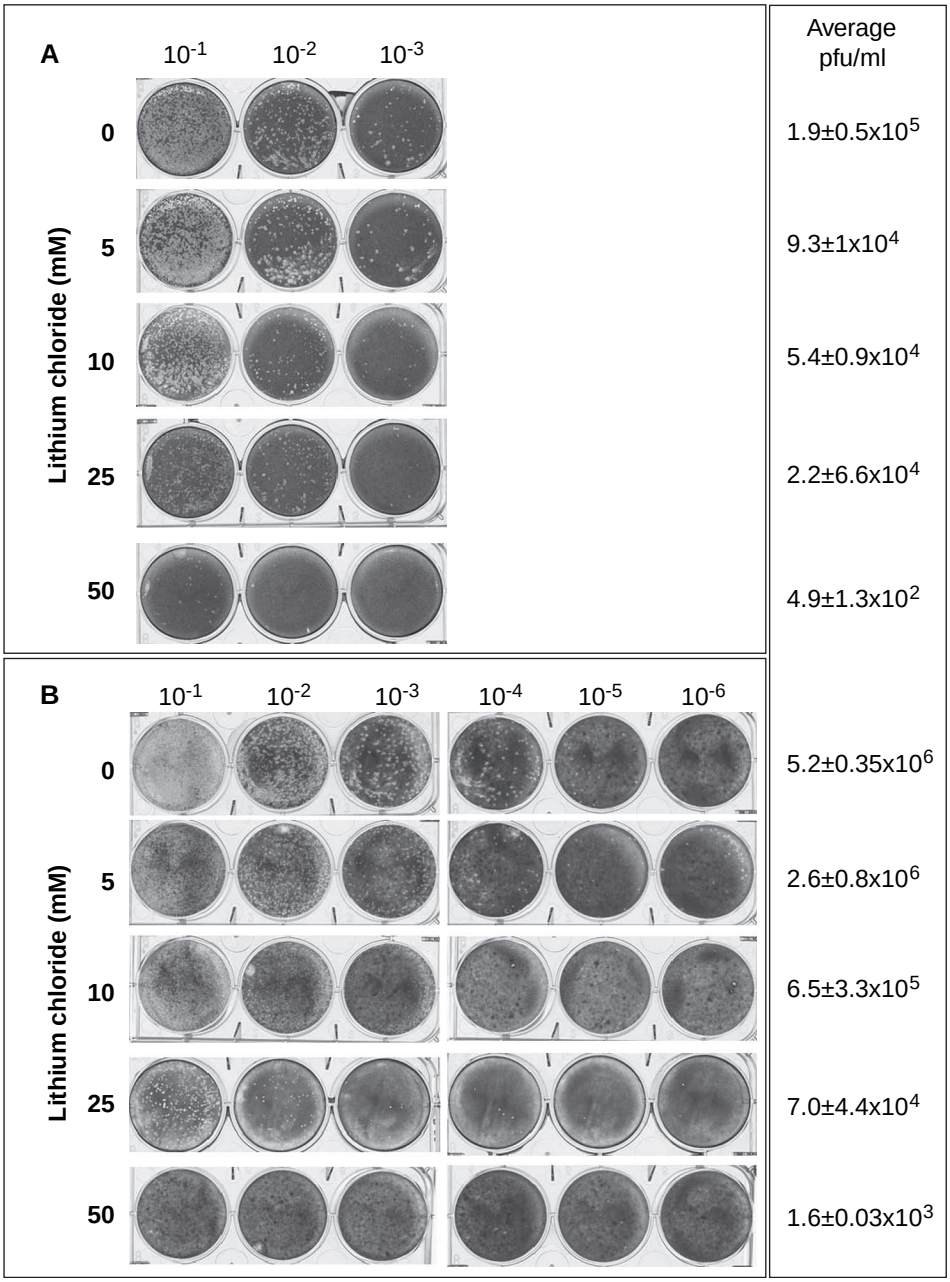

Figure 1. *Plaque assay showing plaque formation in (1a) Vero cells and (1b) DF-1 cells infected with progeny IBV from cells treated with the concentrations of LiCl indicated to the left. Due to IBV replicating less efficiently in Vero cells compared with DF-1 cells, only three-fold to 10-fold serial dilutions of progeny IBV from Vero cells were required compared with six-fold to 10-fold serial dilutions of progeny IBV from DF-1 cells. The experiment was repeated three times and one representative set of data are presented, the average plaque-forming units/ml is indicated to the right.*

rRNA, was then used to adjust the IBV 5′ UTR-specific or IBV 3′ UTR-specific Ct values. This was done as follows:

$$\text{Corrected Ct value} = \text{Ct} + (\text{Nt} - \text{Ct}') \times S/S'$$

where Ct is the mean sample Ct, Nt is the experimental 28S mean, Ct′ is the mean 28S of sample, S is the IBV 5′ UTR/IBV 3′ UTR slope, and S' is the 28S slope.

Results were then expressed as 40 − Ct values.

Results and Discussion

Previously it has been shown that LiCl has an antiviral effect on herpes simplex virus (Cernescu *et al*., 1988; Ziaie & Kefalides, 1989; Ziaie *et al*., 1994) when used at concentrations ranging from 1 to 10 mM. The same trend in reduced viral yield is seen in the DNA viruses pseudorabies and vaccinia. However, inhibition was not observed in the RNA viruses influenza and encephalomyocarditis (Skinner *et al*., 1980). This study investigated the effects of LiCl on IBV replication in cell culture.

LiCl treatment reduces progeny virus production in both IBV-infected Vero and DF-1 cells. To determine whether LiCl had an inhibitory effect on IBV growth *in vitro*, Vero and DF-1 cells were infected with IBV and then left untreated or treated with increased concentrations of LiCl ranging from 5 to 50 mM. Virus progeny production was quantified by plaque assay at 24 h p.i. The data indicated that with the lowest concentration of LiCl tested (5 mM) there was an approximately 50%

reduction in virus titre compared with untreated IBV-infected cells, and at the highest concentration (50 mM) virus progeny production was abrogated, as determined by plaque assay (Figures 1 and 2). Although the data indicated that IBV grew better in DF-1 cells compared with Vero cells, as evidenced by plaque formation at 10^{-6} dilution of progeny virus from DF-1 cells compared with 10^{-3} dilution in Vero cells, the equivalent reduction in virus titre with LiCl treatment was observed in both Vero and DF-1 cells. There was no apparent difference in the plaque morphology of IBV between Vero and DF-1 cells, and likewise at any concentration of treatment with LiCl.

LiCl does not have a direct virucidal effect on IBV. In the above experiments, LiCl may have exerted its inhibitory effect either via interfering with viral or cellular processes or through a direct virucidal effect on progeny virus that is present in the supernatant, both of which would result in a drop in progeny virus production. Therefore, to distinguish between these possibilities (i.e. to determine whether LiCl has a direct virucidal effect on IBV), a 0.5 ml preparation of IBV ($\sim 2 \times 10^6$ plaque-forming units/ml) was treated with 5 to 50 mM LiCl. As controls, this concentration of virus was also incubated for 1 h and 16 h at either 4°C or 37°C to assess the effects of temperature in the presence of the different concentrations of LiCl. After these treatments the amount of virus was determined by plaque assay. The data indicated that there was no significant variation in virus titre of IBV treated with the range of LiCl concentrations, and therefore LiCl did not have a direct virucidal effect on IBV (Figure 3). However, temperature was shown to influence the IBV titre; when the virus was incubated at 4°C for 1 h, the titre was approximately 10-fold higher than when the virus was incubated at 4°C for 16 h. When the virus was incubated at 37°C for 16 h, the virus was rendered non-infectious (data not shown).

LiCl treatment causes a dose-dependent decrease in the synthesis of IBV protein in infected Vero and DF-1 cells. To determine the effect of LiCl on virus protein production, the amount of IBV nucleocapsid (N) protein

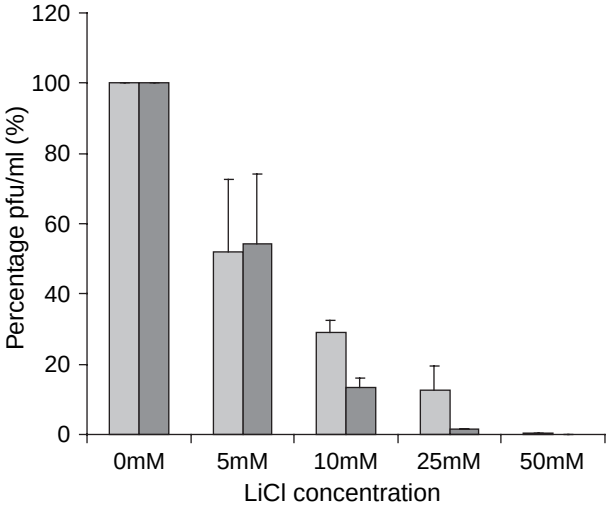

Figure 2. *Histogram showing the relative virus titre of progeny virus from Vero cells (grey) and DF-1 cells (dark grey) treated with the concentrations of LiCl indicated on the x axis as compared with those cells untreated (=100%).*

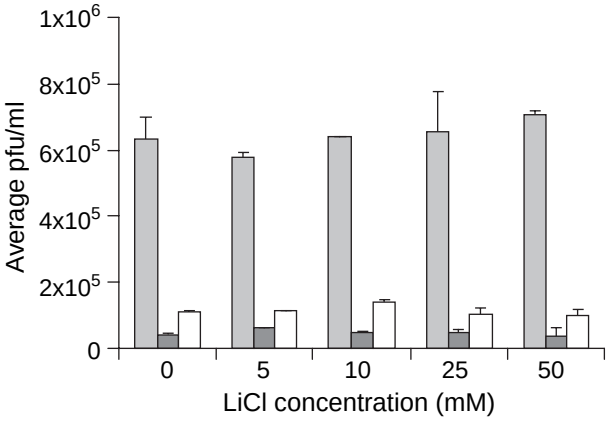

Figure 3. *Histogram showing the relative average virus titre of IBV treated directly with the concentrations of LiCl shown at 4°C (light grey) or 37°C (dark grey) for 1 h or at 4°C for 16 h (white), as assayed in DF-1 cells.*

was assayed by western blot. N protein binds viral RNA with high affinity (Chen *et al*., 2005; Spencer & Hiscox, 2006) and is one of the most abundantly expressed viral proteins in an infected cell (Laude & Masters, 1995), and can thus be used as a sensitive marker for viral protein production. The potential effect of LiCl on cellular protein expression was determined by examining the amount of GAPDH, a cellular housekeeping protein, compared with total protein present. Cell lysates were prepared from either mock-infected or IBV-infected Vero and DF-1 cells either untreated or treated with 5 to 50 mM LiCl. The yield of total protein was determined using the BCA assay and equivalent amounts of protein extract from each experimental treatment used for western blot analysis. The data indicated that the amount of N protein decreased in proportion to the amount of LiCl treatment with apparent abrogation in the amount of N protein when either Vero or DF-1 cells are treated with 50 mM LiCl. Western blot analysis indicated that the amount of GAPDH did not vary between LiCl-treated or untreated Vero or DF-1 cells. Thus the effect of LiCl on the amount of protein was specific to IBV (Figure 4).

The reduction in progeny virus production could therefore be due to the decreased amount of virus proteins observed in infected cells treated with LiCl. This may be a result of either a reduction in the translation of viral subgenomic mRNAs or a decrease in the amount of subgenomic mRNAs themselves. With regard to the former possibility, as the translation of both viral and cellular mRNAs is cap dependent, if LiCl affected this then we would predict cellular translation would also be decreased. However, analysis of the amount of GAPDH suggested that this protein was unaffected by LiCl.

Therefore, LiCl may act as an inhibitor at the level of genomic RNA and subgenomic mRNA synthesis (with a corresponding effect on translation). Previous studies on herpes simplex virus have shown that LiCl inhibits DNA synthesis (Skinner *et al*., 1980), and therefore it is possible to tentatively hypothesize that LiCl may inhibit RNA-dependent RNA polymerases, which are characteristic of positive and negative stranded RNA viruses. One possibility is that the activity of components of the IBV-encoded RNA-dependent RNA polymerases may be affected by LiCl. As the activity of the severe acute

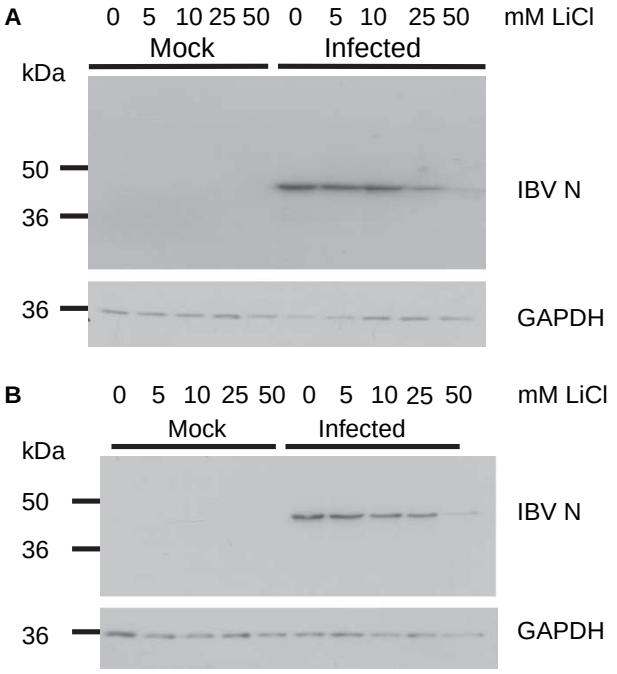

Figure 4. *Western blot analysis of the amount of IBV N protein in mock and IBV-infected (4a) Vero cells and (4b) DF-1 cells treated with the concentrations of LiCl indicated above each blot. GAPDH was used as a marker for cellular protein levels. The migration of molecular weight markers is indicated to the left.*

respiratory syndrome coronavirus helicase is magnesium dependent (Tan *et al*., 2004), and metal ions can also inhibit the activity of the severe acute respiratory syndrome coronavirus 3CL protease (Hsu *et al*., 2004), the same may also be true for IBV. Another precedence for this is the inhibition by LiCl of the activity of certain cellular proteins. For example, LiCl can inhibit glycogen synthase kinase 3 beta by several different mechanisms (Doble & Woodgett, 2003; Jope, 2003), including competition for magnesium ions (Ryves *et al*., 2002). These hypotheses were tested by comparing the levels of viral RNA between infected cells treated and untreated with LiCl.

LiCl treatment causes a reduction in IBV genomic and subgenomic RNA levels in infected Vero and DF-1 cells. To determine the effect of LiCl on virus genomic and subgenomic RNA levels, TaqMan RT-PCR analysis was performed on RNA extracted from mock and infected LiCl-treated cells at 16 h p.i., using primer and probe sets designed against the IBV 5′ UTR and 3′ UTR, respectively. These would detect the genomic RNA (5′ UTR sets) and both the genomic RNA and subgenomic mRNAs (3′ UTR sets). The data indicated that in both Vero and DF-1 cells treated with LiCl, there was an overall reduction in viral RNA levels as the concentration of LiCl was increased (Figure 5a,b, respectively). For example, there was an approximately 20-fold decrease in subgenomic mRNA levels between untreated cells and those treated with 5 mM LiCl in Vero and DF-1 cells (each 40 – Ct value represents a two-fold difference). The RNA levels indicated that the amount of genomic RNA decreased and was not significantly different between 5 and 50 mM LiCl treatment. However, the total positive-sense RNA (subgenomic and genomic) in DF-1 cells generally decreased in a dose-dependent manner (except not between 5 and 10 mM LiCl) as the concentration of LiCl was increased. This may account for the general reduction in the amount of N protein observed in infected cells treated with increasing concentrations of LiCl.

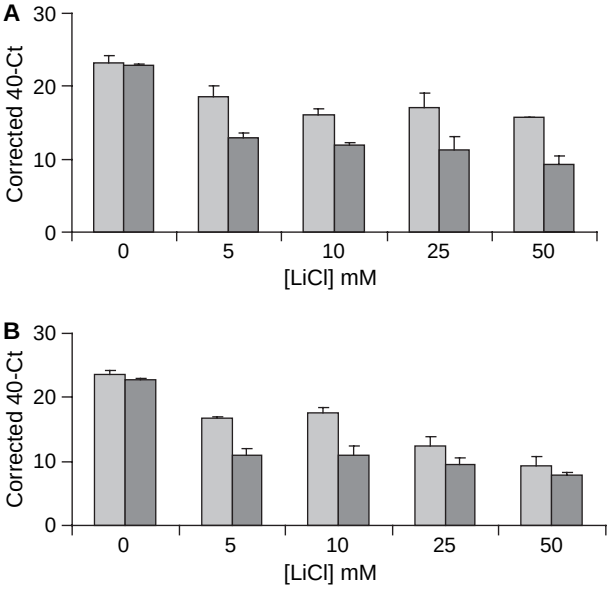

Figure 5. *Real-time RT-PCR analysis of the levels of IBV genomic RNA as well as genomic and subgenomic mRNAs, as determined by analysis of the IBV 5′ UTR (light grey) and 3′ UTR (dark grey), respectively, in infected (5a) Vero cells and (5b) DF-1 cells.*

These data suggest the potential use of LiCl as an antiviral agent against IBV and, by inference, in terms of having common genome and replication strategies, other coronaviruses. Whether or not LiCl could be used in the field against IBV remains to be determined, but certainly it would have application in the laboratory for studying the molecular biology of IBV.

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Non-English Abstracts

Lithium chloride inhibits the coronavirus infectious bronchitis virus in cell culture

Sally M. Harrison[1], Ian Tarpey[2], Lisa Rothwell[3], Pete Kaiser[3] and Julian A. Hiscox[1,4]

[1]*Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK,* [2]*Intervet UK Ltd, Milton Keynes, UK,* [3]*Institute for Animal Health, Compton, UK, and* [4]*Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK.*

Le chlorure de lithium inhibe le coronavirus de la bronchite infectieuse aviaire dans les cultures cellulaires
Le coronavirus de la bronchite infectieuse aviaire (IBV) est un agent pathogène économique majeur pour l'industrie avicole qui, malgré la vaccination, entraîne de la mortalité et des pertes importantes en production. Lors de la réplication de l'ARN génomique, il y a une fréquence élevée de mutations et de recombinaisons qui ont donné naissance à de nombreuses souches d'IBV et qui rend possible l'émergence de nouvelles souches. Actuellement les vaccins vivants atténués confèrent une immunité croisée faible entre souches. Les agents antiviraux efficaces peuvent alors être avantageux dans le traitement de l'IBV. Le chlorure de lithium (LiCl) est un inhibiteur puissant de l'herpesvirus simplex, virus à ADN, mais pas des virus à ARN. L'effet du LiCl sur la réplication de l'IBV a été examiné en culture cellulaire en utilisant deux modèles de type cellulaire, les cellules Vero, une lignée cellulaire épithéliale dérivée de rein de singe vert africain, et des cellules DF-1, une lignée cellulaire immortalisée de fibroblastes d'embryon de poulet. Lors du traitement avec différentes concentrations de LiCl, l'ARN de l'IBV, le taux de protéines et la production de virus ont été diminués de façon dose-dépendante pour les deux types de cellule et les données ont montré que l'inhibition était un effet cellulaire plutôt qu'un effet virucide. La synthèse des protéines de la cellule hôte avait toujours bien lieu dans les cellules traitées au LiCl et le niveau d'une protéine "de ménage" utilisée comme témoin d'expression cellulaire est resté inchangé, indiquant que l'effet du LiCl était spécifiquement dirigé contre l'IBV.

Lithiumchlorid inhibiert das Coronavirus infektiöse Bronchitis Virus in der Zellkultur
Das aviäre Coronavirus infektiöse Bronchitis Virus (IBV) ist ein ökonomisch bedeutsamer Krankheitserreger für das Wirtschaftsgeflügel, der trotz Vakzination Mortalität und Produktionseinbußen verursacht. Während der Replikation des RNS-Genoms kommt es häufig zu Mutationen und Rekombinationen, die eine Vielzahl von IBV-Stämmen entstehen ließen und die ein Potential für neu entstehende Stämme sind. Die gegenwärtig eingesetzten Lebendvakzinen geben gegen diese neuen Stämme nur eine geringe Kreuzimmunität. Aus diesem Grund wären wirksame antivirale Agentien von großem Vorteil für die Bekämpfung des IBV. Lithiumchlorid (LiCl) ist ein potenter Inhibitor des DNS-Virus Herpes simplex-Virus, aber nicht von RNS-Viren. Der Effekt von LiCl auf die IBV-Replikation wurde in der Zellkultur unter Verwendung von zwei Zelltypvarianten untersucht: Verozellen, eine von der Niere einer afrikanischen Grünen Meerkatze abstammenden Epithelzelllinie, sowie DF-1-Zellen, eine immortalisierte Hühnerembryofibroblastenzelllinie. Durch die Behandlung mit verschiedenen LiCl-Konzentrationen wurden die IBV-RNS und –Proteingehalte und die Virusvermehrung in beiden Zelltypen dosisabhängig reduziert. Die Ergebnisse zeigten, dass die Inhibition eher ein zellulärer als eine viruzider Effekt war. Die Proteinsynthese in der Wirtszelle fand in den LiCl-behandelten Zellen weiter statt und der Gehalt eines zellulären Standard-Organisationsproteins blieb unverändert, was darauf hin weist, dass der Effekt des LiCl spezifisch gegen das IBV gerichtet ist.

El cloruro de litio inhibe el coronavirus de la bronquitis infecciosa en cultivos celulares
El coronavirus aviar de la bronquitis infecciosa (IBV) es uno de los patógenos con mayor impacto económico en avicultura, el cual, pese a la vacunación, causa mortalidad y pérdidas económicas significativas en la producción. Durante la replicación del genoma RNA se produce una gran frecuencia de mutación y recombinación que da lugar a una gran variedad de cepas de IBV e implica un potencial para la generación de cepas nuevas y emergentes. Actualmente las vacunas vivas atenuadas producen una inmunidad cruzada

*To whom correspondence should be addressed. Tel: +44 113 343 5582. Fax: +44 113 343 3167. E-mail j.a.hiscox@leeds.ac.uk
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pobre. Por lo tanto, moléculas antivíricas efectivas podrían ser ventajosas en el tratamiento de IBV. El cloruro de litio (LiCl) es un potente inhibidor del virus herpes simple DNA pero no de virus RNA. Se estudió el efecto del LiCl en la replicación de IBV en dos tipos de cultivos celulares: células Vero, una línea celular epitelial derivada de riñón de mono Verde Africano, y células DF-1, una línea celular de fibroblastos de pollo inmortalizados. Cuando se trataron con un rango de concentraciones de LiCl, los niveles de proteínas y RNA de IBV y la producción de progenie se redujeron de manera dosis-dependiente en ambos tipos celulares, y los datos indicaron que la fue una inhibición más de tipo celular que un efecto viricida. La síntesis de proteínas celulares todavía tuvo lugar en las células tratadas con LiCl y el nivel de una proteína constitutiva estándar se mantuvo, indicando que el efecto del LiCl era específico frente a IBV.