Effectiveness of Trino-IB on rat infected with respiratory syncytial virus

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1. Introduction

Respiratory syncytial virus (RSV) is a pneumovirus of the paramyxovirus family. It is an enveloped non-segmented negative strand RNA virus (Collins et al. 2001). The 15.2-kb genome contains 10 mRNA species encoding 11 distinct proteins. The genome is encapsidated by the nucleocapsid (N) protein, which forms a helical nucleocapsid and protects the RNA from ribonucleases. The N protein is also associated with the viral polymerase, phosphoprotein and M2-1 protein, which together forms the transcriptase complex. The ribonucleoprotein is essential for transcription, and naked RNA does not provide a template for the viral polymerase. As with all single-stranded RNA viruses, this virus does not have a proofreading mechanism during replication, which results in a relatively high error rate and frequent mutations, and the promising inhibitors targeting the fusion event were unsuccessful (Cianci et al. 2004).

RSV, a lower respiratory tract infection, is the most common cause of viral bronchiolitis and pneumonia in infants, and is also evident that this virus is also important in respiratory disease of young animals. It is a virus that causes cold-like symptoms that can trigger chronic breathing difficulties if the lungs are involved. RS virus typically begins with a mild fever, runny nose, sore throat, mild cough, blocked nose and ear infection. Signs of more serious disease include wheezing, difficulty breathing and trouble eating, drinking or sleeping (Paul et al. 2003). Trino-IB is an example of immune modulator often used to bring under control different immune-compromised conditions. It is an immune modulatory agent containing a mixture of oleuropein and alpha lipoic acid, which has been proven to be effective in animal models with viral infections (Ziegler et al. 1995; Merin et al. 1996; Coni et al. 2000; Oloke 2008).

2. Materials and methods

2.1. Rats

Thirty specific pathogen-free Wister rats (male and female) of about 120 g each purchased from the Animal House of Ladoke Akintola University, Ogbomoso and were allowed to acclimatise for seven days before the commencement of the experiment. All procedures performed on the animals in this study were in accordance with the ethical standards of the institution or practice at which the study was conducted. The rats were grouped into three (A, B and C), 10 rats in a group.

2.2. Administration of Trino-IB

Group A was pre-treated with 1 ml Trino-IB daily for a week and treatment continued after inoculation with RS virus until when the experiment was terminated.

2.3. Virus strain

RSV with number FR-294, VR-1540™ HRSV strain A-2 LOT: 58978263, IRR ATCC obtained from Center for Disease and...
Control was used in the study. A single pool of virus prepared in HeLa cell cultures that contained approximately $10^6$ PFU/ml was used in the study. Each rat in Groups A and B was injected intranasally with the virus with a volume of 0.05 ml.

### 2.4. Clinical signs

Observations were made on clinical signs after inoculation and treatment through the period of the experiment. At intervals of 2 days after inoculation mice were sacrificed by cervical dislo- cation and lungs were also removed for histologic testing.

Group C which comprises the control mice likewise undergoes the same experimental procedures for comparison with groups A and B.

### 2.5. Histology

Lungs were preserved in 10% Formosaline and transported to Histopathology Department of University Teaching Hospital (UCH), Ibadan for histologic analysis. Histological sections were stained with haematoxylin and eosin.

**Figure 1.** Histogram showing the clinical signs for rats in each group.

**Figure 2.** Small lymphocytes infiltrating the peribronchiolar tissue in the lung of the control.
Figure 3. Mild infiltration of the peribronchiolar (blue arrow) and perivascular tissue in the lung of the rat inoculated intranasally 2 days previously with RS virus.

Figure 4. Mild infiltration of the intra alveolar spaces (slender arrow) in the lung of the rat pre-treated with Trino-IB, inoculated 2 days previously with RS virus.
Figure 5. Moderate dilation and oedema of the alveolar ducts in the lung of a rat inoculated intranasally 4 days previously with RS virus.

Figure 6. Moderate infiltration of the perivascular and intra alveolar spaces in the lung of the rat pre-treated with Trino-IB, inoculated 4 days previously with RS virus.
Figure 7. Moderate infiltration of intra-alveolar spaces by inflammatory cells chiefly consisting of polymorphonuclear cells (slender arrow) in the lung of a rat inoculated intranasally 6 days previously with RS virus.

Figure 8. Intra-alveolar spaces containing an area of mild haemorrhage (black arrow) in the lung of a rat pre-treated with Trino-IB, inoculated 6 days previously with RS virus.
3. Results and discussion

The rats were examined daily after inoculation for various clinical signs. The rats in groups A and B showed different degree of clinical signs such as coughing, wheezing, runny nose and fever (Figure 1). Group C did not show any clinical sign as they were not inoculated with the virus.

The lungs from the control mice showed mild lymphocytes follicle with mild peribronchiolar infiltration. The intra alveolar spaces and alveolar ducts appeared normal with moderate vascular dilation and moderate congestion (Figure 2). Figure 3 shows mild infiltration of the peribronchiolar (blue arrow) and perivascular tissue in the lung of the rat inoculated intranasally 2 days previously with RS virus while Figure 4 shows mild infiltration of the intra alveolar spaces (slender arrow) in the lung of the rat pre-treated with Trino-IB, inoculated 2 days previously with RS virus. The rats developed histological lung lesions which were maximum at days 4 (Figures 5 and 6) and 6 post-inoculation (Figures 7 and 8). These changes in lung histology were resolving at days 8 (Figures 9 and 10) and 10 after injection. The predominant lesion consisted of infiltrations of the perivascular and peribronchiolar tissue, dilation of vessels with mild congestion, as well as thickening of the wall vessels. The alveolar duct appear bloated and showed moderate oedema. The infiltrating alveolar spaces were predominantly filled with polymorphonuclear cells. In some areas the alveolar ducts showed hyperplasia with moderate dilation of vessels and trachea (Figure 11). There were no changes in the lungs of the rats pre-treated with Trino-IB as compared with the control except day 10 with mild haemorrhage within the intra alveolar spaces (Figure 12).

Infection of rats with RS virus was highly reproducible. The histopathological effects observed in the rats were similar to that reported by Johnson et al. (2007). The histological changes are composed of mild to moderate perivascular and peribronchiolar infiltration, moderate dilation of vessels and trachea, hyperplasia of alveolar ducts which is similar to those observed in infants leading to brochiolitis and pneumonia. Similarly, the immodulatory effect observed in the pre-treated rats was similar to that reported by Oloke (2008). The study on RS pathogenesis and vaccine development by Johnson et al. (2007) using rat models reveal that epithelial cells and macrophages are also affected by RSV showing histopathological effects such as peribronchiolar and periarteriolar inflammation which are characterised by mononuclear and lymphocytic infiltration, pulmonary parenchyma and interstitial inflammation with marked vascular congestion. A study by Auais et al. (2003) on the immunomodulatory effect of sensory nerve during RS infection in rats revealed a markedly increased influx of lymphocytes and
Figure 10. Alveolar spaces mildly infiltrated by inflammatory cells (slender arrow) in the lung of a rat pre-treated with Trino-I8, inoculated 8 days previously with RS virus.

Figure 11. Alveolar ducts showing hyperplasia and mild infiltration of inflammatory cells (slender arrow) in the lung of a rat inoculated 10 days previously with RS virus.
monocytes in the airways. This is associated with an upregulation of the mRNA encoding the high-affinity substance P receptor, neurokinin 1 (NK1), which translates into a large increase of substance P binding sites expressed by the airway epithelium and vascular endothelium causing strong appearances of neurogenic-mediated inflammation (Piedemonte et al. 1999; King et al. 2001). In addition to the bronchoconstructive and pro-inflammatory effects of NK1, it has also shown to have important immunomodulatory properties specifically regulating the functions of T and B lymphocytes, monocytes and macrophages by affecting their migration to the airways, mitogens and allergens (Maggi 1997; Goetzel et al. 2004). These effects of NK1 are generally mediated via the NK1 receptor subtype, and several studies have confirmed expression of this receptor on immunocytes from rodents (Bost et al. 1992; McCormack et al. 1996) and humans (Ho et al. 1997; Lai et al. 1998).

The active compounds present in Trino-IB were identified as anti-oxidants that help to boost the immune system activating macrophages and neutrophils (Ziegler et al. 1995; Merin et al. 1996; Coni et al. 2000). Trino-IB, a mixture of oleuropein and alpha lipoic acid, has been found to be effective in reducing the deleterious effects of sodium arsenite on sperm count and motility in rats. Therefore, it is quite conceivable that the immune boosting effect of Trino-IB may be largely due to the antioxidant properties of the major constituents (Oloke 2008). The drug has also been found to be effective in inactivating HIV virus in HIV-infected patients by maintaining the health of the infected persons and stopping the progression of the disease to AIDS (Oloke 2008). It has also been observed by Oloke (2008) that the drug may be useful for patients with diverse immunosuppressive diseases including diabetes mellitus. Trino-IB, as observed in group A of the study, is capable of restoring perfect health to all categories of patients. It helps to neutralise free radicals, destroy cancerous cells, suppress HIV, correct malformation of spermatogenesis and treat low sperm count and ovulation. It boosts insulin metabolism of sugar, reduces sickle cell formation. For preventive use, Trino-IB raises CD4 count sufficiently, if taken regularly, to a level that would inhibit development of diseases (Simoes 2001; Oloke 2008).

4. Conclusion

The use of Trino-IB could improve the immune system of RSV-infected individual, in both infants and immunocompromised individuals, as observed in the Wister rats inoculated with the virus. This is beneficial to strengthening the existing immune defence, enabling it to attack any virus or microbe, at the same time activating stem cells to produce large volume of soldiers to attacking invading diseases thereby overwhelming them.

Figure 12. Mild haemorrhage within the intra alveolar spaces (black arrow) in the lung of a rat pre-treated with Trino-IB, inoculated 10 days previously with RS virus.
Disclosure statement
No potential conflict of interest was reported by the authors.

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