

Sertoli cells (SerW3) are Affected by Linezolid at Therapeutically Relevant Concentrations

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Introduction

Linezolid is an oxazolidinone antibacterial agent for treatment of infections by Gram-positive pathogens. In routinely performed studies during preclinical development of the drug, it was found that linezolid causes infertility which was irreversible if animals were treated during postnatal development. The mechanism of this toxic effect on male fertility is not known. We evaluated the effects of linezolid on Sertoli cells in vitro by analysing the expression of connexin 43 which is the most abundant gap junction protein in the testis.

Materials and Methods

Cells of the rat derived Sertoli cell line SerW3 were incubated with linezolid (Zyvoxid i.v.[®]) at concentrations of 0.1, 1.0, 10.0 and 100.0 mg/l. Cells were cultured for seven days in DMEM supplemented with 5% fetal calf serum. At the end of the culture period cells were trypsinized and immuno-histochemistry was performed with the cells using a monoclonal antibody against connexin 43 (CX 43) and an FITC labelled secondary antibody. Images were analysed semi-quantitatively using the software Scionimage 4.022 (Scion Corp. USA).

Results and Discussion

Figures 1 to 5 are typical examples of control cells and the cells incubated for 7 days with various concentrations of linezolid and stained with the connexin 43 antibody. Visually, there is decrease of staining of the cells exposed to linezolid.

In Figure 6 results of the image analysis data are presented as stained area to total area of the tissue section (%). In each group 5 slides were analysed.

At the lowest concentration tested no significant difference was noticed in comparison to the controls (14.9 ± 0.3 vs. $14.8 \pm 0.3\%$), however, only 69%, 30% and 8.3% of the control value were achieved after exposure to at 1.0, 10.0 and 100.0 mg linezolid /l medium.

During therapy with the antimicrobial agent concentrations up to approx. 20 mg/l are achieved in the plasma of patients. Our experiments indicate that Sertoli cells are a target for linezolid at therapeutically relevant concentrations. Possibly, the irreversible fertility impairment observed in juvenile rats is a sequel of this effect.

Results

Immunohistochemistry & Image Analysis

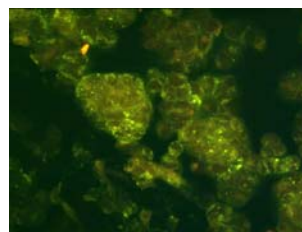


Figure 1: Control
SerW3 incubated for 7 days with plain medium, antibody against CX 43

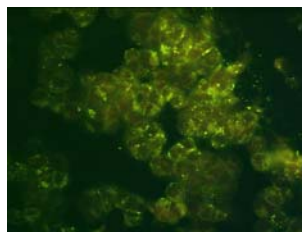


Figure 2: 0.1 mg/l linezolid
SerW3 incubated for 7 days with 0.1 mg linezolid/l medium, antibody against CX 43

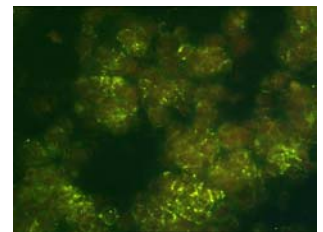


Figure 3: 1.0 mg/l linezolid
SerW3 incubated for 7 days with 1.0 mg linezolid/l medium, antibody against CX 43

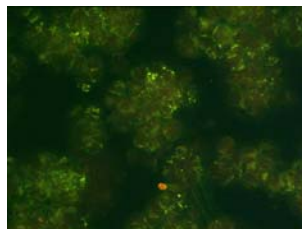


Figure 4: 10.0 mg/l linezolid
SerW3 incubated for 7 days with 10.0 mg linezolid/l medium, antibody against CX 43

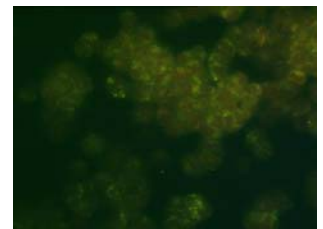


Figure 5: 100.0 mg/l linezolid
SerW3 incubated for 7 days with 100.0 mg linezolid/l medium, antibody against CX 43

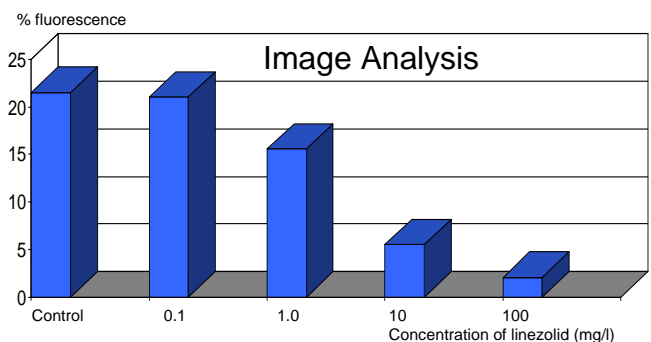


Figure 6: Results of the image analysis of Sertoli cells after staining with an antibody against Connexin 43 (mean values, n=5)