Spectroscopic signatures of remdesivir and its interaction with synaptosome

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Introduction

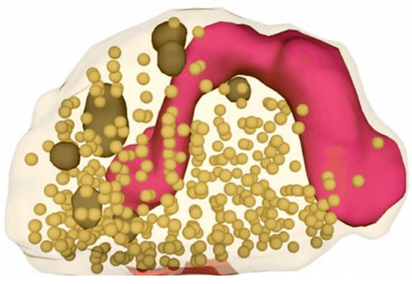

Fig.1. Schematic image of synaptosome. (Коржова В., 2014)

Antiviral drug remdesivir (also known as GS-5734) is a phosphoramidate prodrug of an adenosine C-nucleoside or adenosine nucleotide analogue. The anti-viral drug remdesivir, being effective against RNA viruses, called nucleoside reverse transcriptase inhibitors and works as specifically inhibitor of viral protein RNA-dependent RNA polymerase (RdRp), required for viral replication .

Isolated presynaptic nerve terminals called synaptosomes. They are a subcellular fraction which is formed during the destruction of nerve cells, when the synaptic endings break off from the axons and form vesicles. They are a convenient model system for studying membrane drug influence on cellular membrane.

Materials and methods

Synaptosomes (12 mg of protein/ml) in the phosphate buffer were incubated with remdesivir (5 mkl 10mM) for 5 min and washed 3 times with 10 volumes of buffer and centrifuged. FTIR-ATR spectra were registered using INVENIO-R instrument (Bruker, Germany) at the Bio-ATR attachment with ZnSe crystal. The sample was dried in the sample compartment for 1 hour in a stream of nitrogen at room temperature fixing a state of synaptosomes at the present moment. For FTIR-ATR spectra the baseline correction and band intensity normalization by the Amide I band centered at 1652 cm-1 have been done out on the basis of supposition that a number of protein molecules are constant in the synaptosomes in both states.

Spectroscopic signature of remdesivir

Infrared

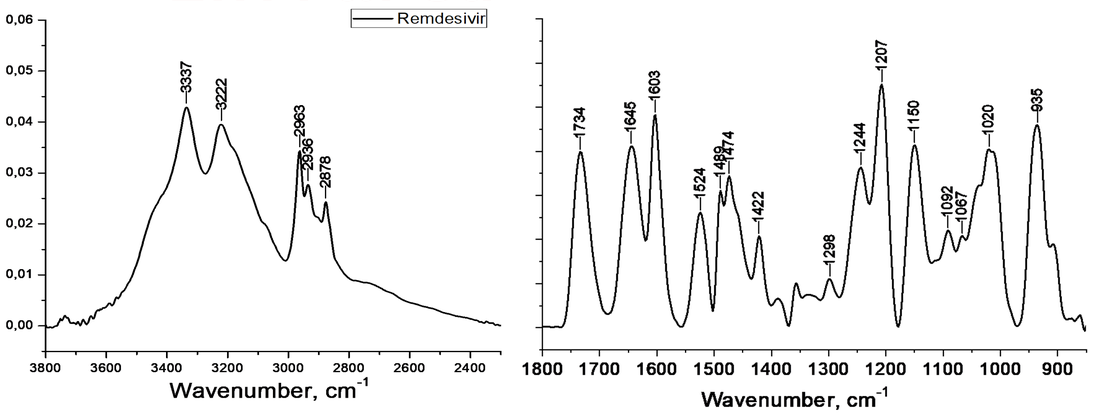

Fig. 2. FTIR spectra of remdesivir on the ATR in the regions (a) 3800-2400 cm − 1 and (b) 1800-800 cm − 1

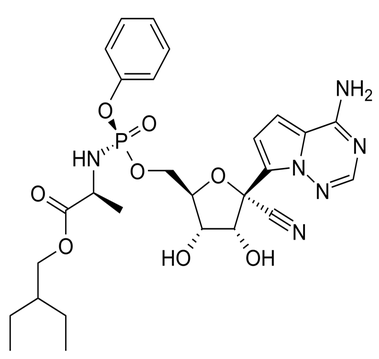

Fig.3. Structure of remdesivir

Raman

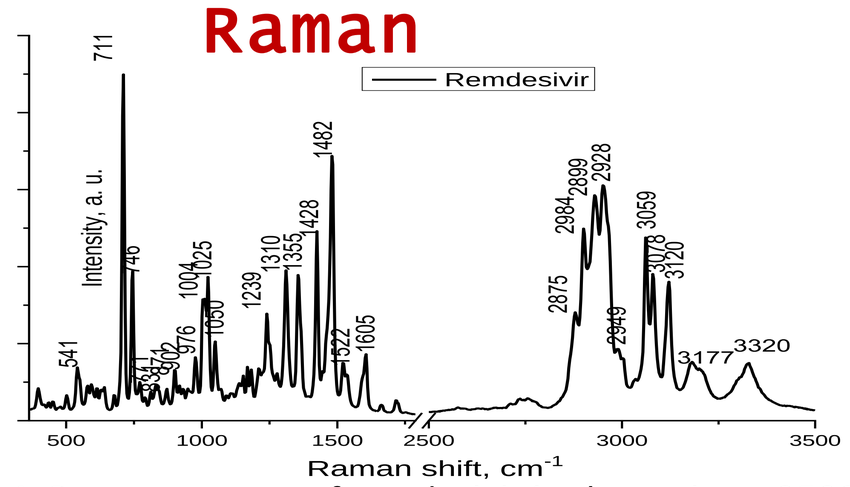

Fig. 4. Raman spectra of remdesivir in the regions 3500-2500 cm − 1 and 1600-800 cm − 1

Results

The Amide I band has a characteristic position for the dominant contribution of the α-helix proteins at 1650 cm-1 (C = O and C-N stretching mode) with band asymmetry in at 1628 cm-1 (corresponding to β-layers). The Amide II band (1544 cm-1) is more complex, the main contribution being made by C-N valence (40%) and N-H deformation, which are directed perpendicular to α-helixes (60)%.

In the region of phosphate groups absorption PO2- we observe the appearance of the shoulder at 1208 cm-1 in the bands of asym. str. mode PO2- at 1226 cm-1 in the sample of synaptosomes after incubation with remdesivir. There is also an increase in the intensity of the bands in the region 1053 cm-1, as well as 989 cm-1. Thus, for functionally active synaptosomes when interacting with remdesivir, we can claim a changes in hydrogen binding in hydrogen-bound groups, and this is especially evident in phosphate groups. The nature of spectral changes is not in frequency shifts, but in changes in the contributions of bands in intensity.

Raman spectra of synaptosomes look different from cell spectra. First, a significant contribution is made by mitochondria (30%) and their components in the form of porphyrins, which is reflected in the presence of lines characteristic of mitochondria. table, secondly, a significant contribution of DNA and peptides, and thirdly, a significant luminescent background from proteins. The action of remdesivite suppressed this luminescent background. This suggests that the drug interacts with the synaptosome.

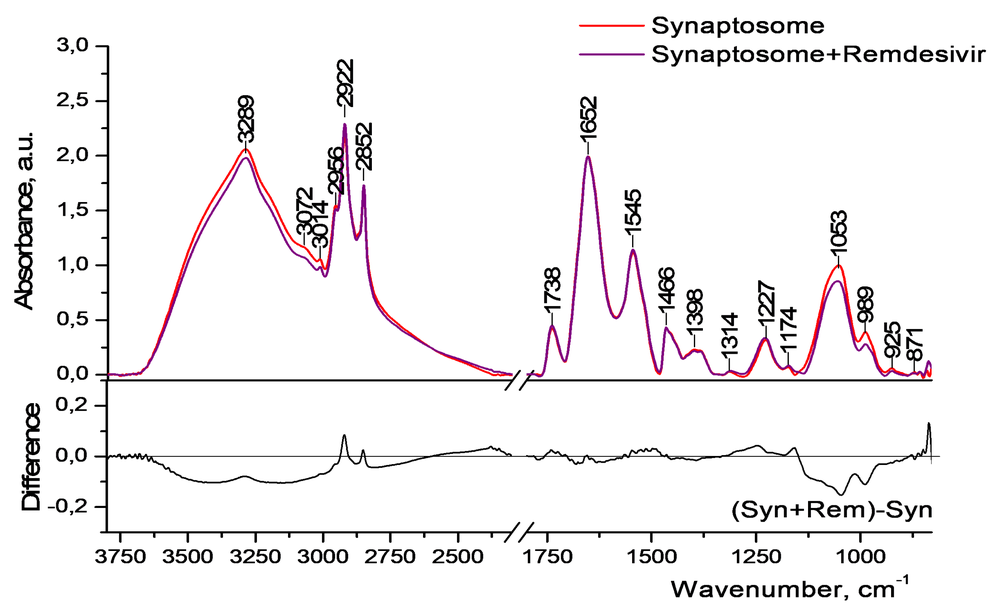

Fig.5. FTIR spectra of fresh isolated synaptosomes and synaptosomes with remdesivirwere deposited on CaF2 substrates and dried at 25 ° C.

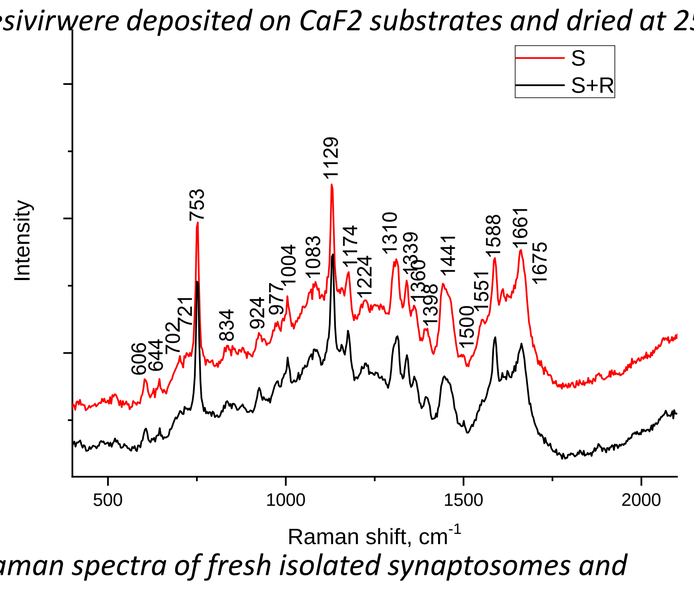

Fig.6. Raman spectra of fresh isolated synaptosomes and synaptosomes with remdesivir were deposited on CaF2 substrates and dried at 25 ° C.

Acknowledgment

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