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**EFFECTS OF THE CARBON MONOXIDE RELEASING MOLECULE-2 ON HUMAN
ERYTHROCYTE MEMBRANES**

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Lecture 2

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Carbon monoxide (CO) is the third most common agent that causes poisoning. Nevertheless it known that CO produced endogenously and can have a beneficial effect, especially at lower concentrations. The first reports have shown that CO is actively involved in the regulation of key intracellular functions. At physiological concentrations, CO can affect processes of signal transmission in various organs and in various cells, also including endothelial cells. For example, CO may regulate aspects of the cardiovascular system, such as platelet activation, inflammation or blood pressure, demonstrate neuroprotective and neurotherapeutic properties.

CORMs (Carbon monoxide releasing molecule-2) or molecules that release carbon monoxide make up a recently classified group of chemicals. These are compounds capable of releasing a controlled amount of CO into cells and tissues to induce biological activity. CORM molecules consist of carbonyl groups linked to metals, such as ruthenium (CORM-2 and CORM-3), and can act as drugs. The potential of a CORM lies in its ability to release a CO molecule bound to a metal when it reaches its destination.

We used washed and "packed" red blood cells of donors 28-30 years old. In 3.150 ml of medium (with different osmotic strength), 0.35 ml of erythrocytes was added and light scattering was measured. After this, the tricarbonyldichlororuthenium (II)-dimer carbon monoxide donor (CORM-2 was dissolved in DMSO (<1%)) was added and the light scattering was measured again. After the measurement, was adding the blocker of calcium-dependent K⁺ channels of the membrane clotrimazole.

In environments with different osmotic strengths after adding CORM-2, decrease in the intensity of light transmission was observed. In the environment of 220 μmol (hypoosmotic), the light scattering index changed from 2.898 ± 0.144 to 2.759 ± 0.138 , in the medium of 320 μmol (isoosmotic) from 2.984 ± 0.142 to 2.887 ± 0.144 . After adding erythrocytes to the environment of 420 μmol (hyperosmotic), the index changed from $3,000 \pm 0,15$ to $2,887 \pm 0,144$, and in medium 520 μmol from $3,000 \pm 0,14$ to $2,912 \pm 0,146$ (4.8, 3.2, 3.2 and 2.9%, respectively). In this case, the addition of clotrimazole did not significantly affect the light transmittance.

Thus, CORM-2 at a dose of 200 μM caused an increase in the volume of erythrocytes in hypertonic, isotonic and hyposmotic solutions. After melting the spectrin after the calcium-dependent potassium channel blocker was added, clotrimazole and CORM-2 erythrocytes swelled. The exception was the compression of erythrocytes in isoosmotic solution.

Thus, CORM-2 affects on the properties of the erythrocyte membrane, their ion channels. This is due to the erythrocyte cytoskeleton protein - spectrin. Thus, this should influence the micro-rheological properties of erythrocytes in vivo after consuming CORM-2 medication.