1-METHYLTRYPTOPHAN ISOMERS INTERFERE WITH TLR-4 SIGNALING AND DECREASE NKG2D EXPRESSION IN NK CELLS AND EXPRESSION OF NKG2D LIGANDS IN TUBULAR EPITHELIAL CELLS AFTER ISCHEMIA-REPERFUSION INJURY

Čepcová D., Mišúth S., Vavrinec P., Vavrincová-Yaghi D.

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, Bratislava, Slovakia

Renal ischemia-reperfusion injury (IRI) leads to the damage of tubular epithelial cells (TECs). Damaged TECs then activate its toll-like receptors 4 (TLR-4), leading to the expression of NKG2D ligands. These ligands are subsequently recognized by natural killer (NK) cells, leading to the apoptosis of TECs.
Previously we observed that 1-MT isomers have protective effect on renal function in rat model of IRI. We found out moreover that expression of NKG2D ligands is increased and was markedly decreased by isomers of 1-methyltryptophan (1-MLT and 1-MDT). Furthermore, it was shown, that 1-M-D-T can interfere with TLR-4 signaling.
To elucidate the mechanism behind the protective effect of 1-MT isomers, we investigated the influence of both 1-MLT and 1-M-D-T treated TECs induced by IRI on NK cells and vice versa.
TECs were treated with 1-MDT or 1-MLT (750µmol/l) 24hr and 1hr before ischemia (30min) and 24hr after. Four groups of cells were used (n=3): control TECs, TECs stimulated by IRI, and 1-MDT or 1-MLT treated TECs stimulated by IRI. Right after the end of ischemia all groups of TECs were co-cultured with NK cells, followed by 48hr reperfusion. At the end of experiment NKG2D and TLR4 expressed by NK cells were analyzed, as well as the expression of TLR4, NLRP3 and NKG2D ligands (RRLT and Raet1d) and Ccl-5 expressed by TECs.
We observed significant increase of NKG2D and TLR-4 expressed by NK cells co-cultured with TECs stimulated by IRI ($p<0.05$) and 1-M-L-T significantly decreased this expression ($p<0.05$).
Additionally, we found out that expression of RRLT, Raet1d, TLR-4, NLRP3 and Ccl-5 was increased significantly in IRI stimulated TECs ($p<0.05$) and that treatment with 1-M-D-T decreased the expression of all assessed molecules ($p<0.05$). Moreover, treatment with 1-MLT decreased above mentioned molecules as well ($p<0.05$), except of RRLT. In conclusion, our data show that both 1-MT isomers decreased NKG2D expressed by NK cells and NKG2D ligands expressed by TECs stimulated by IRI. Moreover, we show that both 1-MT isomers possibly interfere with TLR-4 signaling pathway, showing the molecular mechanism of protective effect of 1-MT isomers in renal IRI.

Supported by the grant VEGA 1/0247/17 and Comenius University grant for young scientists UK/352/2019.