Summary

Detection of the embryotoxicity of N-Methyl-pyrrolidone and its metabolites by using the whole embryo culture

Introduction: Using the whole embryo culture (WEC), N-Methyl-2-pyrrolidone (NMP) and its three metabolites were tested for their embryotoxic potential. The WEC is suited to scrutinise the mode of action and the specific embryotoxicity. In addition the whole-immuno-staining (WIS) of the neural crest cells (NCCs) and the cranial nerves was performed to understand defects observed in the WEC on a cellular level.
Materials and methods: For the WEC 9.5-days-old rat embryos were incubated as control embryos and as test groups of increasing concentrations of NMP and its metabolites. They were cultured under defined conditions in a rotating system for 48 hours. For the WIS embryos of the WEC were treated with primary antibodies: the CRABP-I-antibody (cellular retinoic acid binding protein 1) was used to detect the NCCs, the 2H3-neurofilament-antibody to detect the cranial nerves. Naphthol was transformed into a blue precipitate by the peroxidase of the secondary antibody.
Results: The substances induced concentration-dependent effects in the WEC. NMP caused abnormalities particularly in the head region. In proportion to the body the head was smaller, the eye seemed to be flattened in its dorsoventral axis and the second branchial arch was adhered to the first and to its surroundings. Beside these abnormalities there was an alteration which occurred as a prominent node in the area of the trigeminal ganglion. This observation could not be judged with the scoring system used and therefore it was impossible to classify it as a abnormality. The WIS of the 2H3-neurofilament showed retardations and anomalies in the development of cranial nerves in embryos exposed to NMP. Using the WIS with CRABP-I it was not possible to determine an altered mirgration of NCCs after treatment with NMP in comparison to the situation in the control group.
Discussion: In the WEC, a weak embryotoxic potential of NMP and the metabolite 5-Hydroxy-N-Methyl-2-pyrrolidone (5H-NMP) was determined which was manifested in specific morphological malformations of the embryos. The metabolites N-Methylsuccinimid (MSI) and 2-Hydroxy-N-Methylsuccinimid (2H-MSI) were classified as non embryotoxic, because they caused only general embryotoxic effects at very high non physiological concentrations. The observed effects on the cranial nerves after exposition to NMP were not seen in the control situation nor in *ex-vivo*-embryos. So the WIS provides an indication of a specific neurotoxicity of NMP *in vitro.*
Conclusion: NMP seemed to be the responsible substance for the embryotoxicity observed in *in-vivo*-studies. The results of the WIS provided amendments on morphological changes seen in the WEC and were linked with them.

Keywords: CRABP-I, cranial nerves, embryotoxicity, 2H3-neurofilament, 5H-NMP, 2H-MSI, neural crest cells, NMP, MSI, whole-immuno-staining, whole-embryo culture