Rapid HPLC analysis of melphalan applied to hyperthermic isolation limb perfusion*

D. Romanová[1], J. Netriová[2], P. Božek[3], Z. Ovesná[4], K. Kroupa[2], E. Valovičová[2], A. Vachálková[1]

[1]Department of Experimental Therapy, Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovak Republic, e-mail: exondrom@savba.sk; [2]Department of Clinical Biochemistry, National Cancer Institute, Bratislava; [3]Department of Clinical Biochemistry, State Hospital, Bratislava; [4]Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

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Hyperthermic isolated limb perfusion (HILP) with melphalan (MH) as a standard cytotoxic drug has been performed in 28 patients suffering from malignant melanoma. MH has been administered by HILP via extracorporeal circulation system. The drug given locoregionally reduces subsequent toxicity of organs. For all that residues can leak into the systemic circulation during HILP. Because of known carcinogenic potential and secondary cancer formation, the main interest of this work is to determine MH concentration profile in the patient plasma during and after HILP and evaluation of its potential toxicity in patients. Reversed-phase HPLC assay, which uses isocratic elution and fluorimetric detection has been shown to be sensitive, reliable and suitable for routine analyses. The assay was validated for the concentration range of 50–2500 ng.ml^{-1} with the limit of detection (LOD) 6.881 ng.ml^{-1}. The samples were treated by methanol precipitation with the recovery more than 80%. The stability of standard solutions and methanolic extracts of MH were also followed. The concentration profile of MH in patient samples has been pursued in three time points during and after chemoperfusion (45 min after application of MH in extracorporeal circulation, 10 min after the joining the extremity to systemic circulation and one hour after the great vessels reconstruction). The concentrations of MH ranged 100–1500 ng.ml^{-1} and varied from patient-to-patient. Some complications were observed after HILP in 11 patients and are correlated with the higher concentrations of MH (over 150 ng.ml^{-1}) found in plasma.

Key words: Melphalan, hyperthermic isolation limb perfusion, malignant melanoma, isocratic HPLC, fluorescence detection.

Melphalan or phenylalanine mustard, MH, a bifunctional alkylating agent, is the standard cytotoxic drug in hyperthermic isolated limb perfusion HILP for melanoma, mainly in recurrent malignant melanoma (MM) of extremities, satellitosis and in-transit metastasis. It exerts a cytotoxic effect through the formation of interstrand or intrastrand DNA cross-links or DNA-protein cross-links via the two chlorethyl groups of the molecule [8, 13, 16]. It is also extensively used in the treatment of ovarian cancer, breast cancer, neuroblastoma, multiple myeloma, advanced malignant melanoma and localized soft tissue sarcoma [1].

The main advantage of HILP is that by isolating a limb from the rest of the body, a high dose of cytotoxic drug can be given locoregionally, which minimizes systemic exposure to the drug and subsequent toxicity to vital organs [12, 17]. HILP has been performed at the National Cancer Institute in Bratislava since 1995.

Although the MH is administrated by HILP via extracorporeal circulation system, the persistance of the drug residues in blood and its potential depositing in the organism after chemoperfusion can affect tissue toxicity and/or its damage. From that reasons attention is being focused on the determination of MH residues in peripheral circulation system during and after HILP.

Some analytical methods have been developed to quantify MH in biological samples. High-performance thin-layer

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