Altered Expression of Cell Cycle Regulators during Repeated Thioacetamide Treatment in Rat Liver

Jin Sook Jeong, Joo In Park, Keung Sook Cho, Mee You, Sook Hee Hong

Department of Pathology and Biochemistry, Dong-A University College of Medicine

Background Thioacetamide (TA) is a weak hepatocarcinogen, ultimately producing malignant transformation in a moderately high dose. With repeated TA administration in a low dose (50 mg/kg), apoptosis or mild necrosis of hepatocytes occurs, and endorses to open cell cycle for regeneration, but arrest in G2. One possible mechanism involved in G2 arrest induced by TA might be engaged by alterations in expression of cell cycle regulators.

Methods TA was administered daily and intraperitoneally to male SD with a dose of 50 mg/kg, till 7 day. Cyclins (D1, E, A, B1), cyclin dependent kinases (CDK4/6, 2, cdc2), cyclin dependent kinase inhibitors (p16, p21, p27) and p53 were studied, using western blot and immunohistochemistry. PCNA- and BrdU-labeling were tried, as well.

Results Cyclin D1, CDK4/6 and p21 showed similar expression pattern, presenting increase from 6 hr, a peak at 2 or 3 day, then decrease and re-increase at 6 day. The expression of p16 remarkably increased from 3 day and sustained till 7 day. Cyclin E and CDK2, cyclin B1 and cdc2, and p53 showed similar pattern, presenting constant expression till 18 hr, decrease at 1 day, re-increase from 2 day and a peak at 3 day. A recycle from 4 day began. Expressions of cyclin A and p21 were similar, showing increase from 12 hr, a peak at 2 day, and subsequent gradual decrease till 7 day. Immunohistochemistry was operated in cyclin D1, p21 and p16. Cyclin D1 and p21 revealed similar expression intensity, corresponding to results of western blot. Noteworthy findings were nuclear translocation of cyclin D1 and p21 started at 1 day, mostly prominent at 2 day. Centrilobular distributions were started and expanded toward perportal area. In situ p16 expression was similar to western blot, and presented as only cytoplasmic localization, started at centrilobular area, finally to entire liver. BrdU-labeled hepatocytic nuclei increased in number from 12 hr, reached a peak at 2 day and decreased. The number of PCNA(+) nuclei increased immediately after TA treatment, peaked at 2 day, slightly decreased from 3 day and showed a second peak at 7 day.

Conclusion Repeated low dose TA treatment permits to enter G1 (cyclin D1 expression) and to progress G1 (by the expression patterns of cyclin D1 and CDK4/6), p21 might play role of a transporter protein to cyclin-CDK complex. p16 expression was limited in cytoplasm, suggesting impossible to function. Through the G1/S transmission, S phase was partly endorsed (by the expression patterns of cyclin E and CDK2, and PCNA and BrdU labeling). And from early S to G2, several steps will be attended to G2 arrest (by the expression patterns of cyclin A-CDK2, p27, and cyclin B1, cdc2 and p53). These overall expression pattern of regulators was once more repeated in the scheme of 7 day accumulation of low dose TA. These results were coincident with incomplete cell division, G2 arrest.