# DISRUPTIVE EFFECTS OF 5-FLUOROURACIL ON THE CIRCADIAN CLOCK GENES: A POSSIBLE MOLECULAR MECHANISM OF ANTI-CANCER CHEMOTHERAPEUTIC-INDUCED CIRCADIAN RHYTHMIC DISTURBANCES

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Introduction: Circadian clock system is necessary to adapt endogenous physiological functions to daily variations in environmental conditions. Recent molecular studies have revealed that oscillation in the transcription of specific clock genes plays a central role in the generation of 24 h cycles of physiology and behavior. Abnormality in circadian rhythms, such as the sleep/wake cycle, is implicated in various physiological and psychiatrical disorders such as cancer. It was reported that shift workers are highly susceptible to cancer and showed very high morbidity indicating the effect of alteration of circadian rhythm of normal cells on cancer development. Moreover, cancer chronotherapy is attracting attention as a novel and logical therapy in which anti-cancer drugs are administered with optimal timing according to circadian rhythms of anti-cancer action and those of adverse effects on normal cells. 5-Fluorouracil (5-FU) is widely used for several types of cancer. The principal mechanism of 5-FU cytotoxicity is the inhibition of thymidylate synthase. Furthermore, it is incorporated into RNA as well as DNA accounting for the reduction of mRNA level of many genes. Background: It has been noticed that patients receiving chemotherapeutic agents experience disturbances in their behavioral and physical performances, including circadian rhythms. Anti-cancer chemotherapeutic medication in either curative or palliative settings is associated with various undesirable side effects, and the patient's quality of life is remarkably decreased. These side effects may include acute and delayed nausea, vomiting, and anorexia. Decreased whole body weight, disrupted gastrointestinal tract function, including dyspepsia and diarrhea, and also fatigue are accompanied in many cases. Cancer chemotherapeutic drug-associated fatigue has subjective (self-reported) and objective (reduced physical activity or capacity to undertake physical and mental tasks) dimensions. It differs from body tiredness and the feeling of fatigue by sleep deprivation or excessive physical exercise in that this fatigue is not relieved by rest or sleep. Measuring the amount of physical activity using ''Actigraphy'' in patients receiving chemotherapeutic drugs and comparing with the results of normal people showed that physical activity is not only decreased but also that there is a disturbance of the circadian rhythm. However, the detailed mechanism of this disturbance of the circadian rhythm has not been clarified. Purpose: Explore the underlying mechanism of chemotherapeutic agent-induced disturbance of these rhythms. Method: We investigated the influence of 5-FU on the expression of clock genes in NIH3T3 cells and in mouse liver and SCN cells. Then we examined how 5-FU affected overt rhythms in physiology and behavior through the energy uptake and protein synthesis of mouse SCN and the locomotor activity. Result: Treatment of cultured NIH3T3 cells with 5-FU for 48 h resulted in a significant reduction of mRNA levels of Period1 (Per1) and Period2 (Per2) in a dose dependent manner without affecting cell viability. Following treatment with 10 nM Dexamethasone for 2 h, Per2-Luc C6 cells showed bioluminescence oscillation over a period close to 24 h. This circadian oscillation was generally sustained for more than 5 days. On the other hand, no obvious bioluminescence rhythm was observed for Per2-Luc C6 cells treated with 10 mM of 5-FU. The amplitude of the rhythm was decreased by treatment. The mRNA levels of Per1 and Per2 in the SCN of saline-treated mice showed significant circadian oscillations. Their

mRNA levels increased during the light phase and decreased during the dark period. On the other hand, continuous administration of 5-FU severely decreased the amplitude of the rhythms in the expression of Per1 and Per2 in the SCN, although their mRNA levels still exhibited circadian oscillations. Similar decreased amplitude of the rhythms was also observed in the expression of Per1 and Per2 in the liver of mice continuously administered 5-FU. The amounts of [3H]-2-DG uptake, an index of energy metabolism, into the SCN of saline-treated mice showed a significant circadian oscillation, with a high value during the light phase and a low value during the dark phase. In marked contrast, the continuous administration of 5-FU completely abolished the rhythm of [3H]-2-DG uptake in SCN. In addition, the amount of [14C]-Leu uptake, an index of protein synthesis, into the SCN of the saline-treated group was high in the early light phase, while the nadir was observed during the dark phase. In contrast, continuous administration of 5-FU induced an altered rhythm of [14C]-Leu uptake into the SCN. Consistent with its inhibitory actions, in the behavioral study, control mice clearly exhibited an entrained locomotor activity rhythm, and thus hyperactivity was observed during the dark phase. In contrast, continuous administration of 5-FU (2 mg/kg/h) substantially decreased the amplitude of locomotor rhythm. Locomotor activities during the dark phase were significantly decreased, resulting in a reduction of total daily activity. Conclusion: These results reveal a possible pharmacological action by the chemotherapeutic agent 5-FU on the circadian clock mechanism, which is the underlying cause of its adverse effects on 24-h rhythms of physiology and behavior.