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Introduction of antineoplastic drug NSC631570 in an inpatient and outpatient setting: Comparative evaluation of biological effects

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ARTICLE INFO

Article history:
Available online 10 February 2016

Keywords:
NSC631570
Physical exercise
Blood biochemical parameters
Phagocytes functional polarization

ABSTRACT

The aim of this study is to evaluate the effect of moderate physical exercise and treatment time on the organism's response to NSC631570. The sensitivity of circulating phagocytes to the drug at different times of day was estimated in *in vitro* experiments. NSC631570 was administered intravenously to healthy volunteers (eleven men, 23 ± 2 years) in a single therapeutic dose in an inpatient and an outpatient setting. Blood samples were obtained before the drug administration, 30 min after the drug injection and every fourth hour throughout the 24 hour period. Biochemical parameters were determined using the hematological analyzer. Flow cytometry was used to evaluate phagocyte metabolism. Treatment of circulating phagocytes with NSC631570 *in vitro* resulted in an increase in ROS production along with a decrease in their phagocytic activity, most expressed in the morning time. Drug injection to sedentary persons resulted in pro-inflammatory metabolic polarization of circulating phagocytes. Introduction of NSC631570 to active persons was accompanied by a significant increase in phagocyte endocytosis along with a decrease in the daily mean of ROS generation. Significant oscillation (but in the normal ranges) of urea, creatinine, alanine aminotransferase and aspartate aminotransferase after NSC631570 introduction in the outpatient setting was shown during the day. Physical activity interferes with immunomodulatory action of NSC631570 and abrogates pro-inflammatory shift of circulating phagocytes. Biochemical parameters of blood from patients treated with NSC631570 in the outpatient setting must be interpreted cautiously considering the effect of physical activity on some metabolic biomarkers.

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Peer review under responsibility of Shenyang Pharmaceutical University.

http://dx.doi.org/10.1016/j.ajps.2016.02.004

1. Introduction

NSC631570 is a derivative of the extract of the plant *Chelidonium majus* L. and thiophosphoric acid [1–5]. The drug consists of a mixture of eight *C. majus* alkaloids (chelidonine, sanguinarine, chelerythrine, protopine, allocryptopine, homochelidonie, berberine, and coptisine) that reacted with an alkylating agent (preferably thiotepa) in organic solvent. The preparation has the ability to be selectively accumulated in tumor tissue and activate apoptosis only in tumor cells and not in normal cells, probably due to increased consumption of the drug by tumor cells [5–8]. In addition, the therapeutic effect of NSC631570 is always accompanied by the stimulation of immune responses. The preparation shows an ability to modulate immunologic reactivity *in vivo* and to alter functions of immunocytes including phagocytes *in vitro* [9–11].

In accordance with their functions and metabolism, phagocytes have been classified into two subpopulations. Classically, activated cells (M1 macrophages or N1 neutrophils) exhibit pro-inflammatory phenotype that is characterized by increased production of reactive oxygen species, pro-inflammatory cytokines and chemokines. Alternatively, activated M2 macrophages or N2 neutrophils are described as cells with anti-inflammatory phenotype with increased phagocytic activity and regulatory cytokine expression [12,13]. In our previous investigation, we observed the ability of NSC631570 to cause an influx of macrophages into the tumor growth area after intravenous administration [9]. The previous result from our laboratory also revealed that NSC631570 induces M1 functional polarization of peritoneal macrophages and circulating phagocytes in tumor-bearing mice and can restore pro-inflammatory functions of macrophages, alternatively polarized by hypoxia *in vitro* [14]. Thus, the modulation of phagocyte functions can be considered as an important component of the immunomodulatory effect of NSC631570.

The efficacy of an immunomodulating preparation depends on the reaction of immune cells to the given drug. This reaction of immune cells substantially depends on their initial functional state [15]. The initial functional state of immune cells in turn depends on many factors that are rarely appreciated in the treatment regimen such as treatment time and condition of the patients (inpatient vs outpatient setting). Numerous studies have demonstrated that even moderate physical exercise modulates the functional response of immune cells, including monocytes and granulocytes and affects cytokine generation, reactive oxygen (ROS) and nitrogen species production, and chemotaxis and phagocytosis of these cells [16–20]. It is widely reported that therapeutic effects can be markedly improved by administering a drug taking into account the rhythm of cell functions [21–25]. Treatment with NSC631570 is carried out in the morning in both inpatient (associated with limited excursion) and outpatient settings (associated with moderate physical activity). Clinical observations suggest that the therapeutic efficacy of the drug is lower when treatment is carried out in an outpatient setting.

Traditionally, biochemical parameters have been used as markers to assess the influence of exercise on different systems, organs and tissues [26–28]. Many biochemical parameters also exhibit diurnal variations that must be considered in the clinical setting, when concentration changes in the parameters are evaluated.

The aim of this study was to evaluate the effect of treatment time and moderate physical exercise on the response of circulating phagocytes to NSC631570 as well as on daily oscillations of various biochemical blood parameters after drug administration.

2. Materials and methods

2.1. Subjects

Eleven healthy adult men aged 23 ± 2 years were recruited to participate in the study. Exclusion criteria included a history of somatic disease and a physically active lifestyle. Approval was obtained from the Ethics Committee of the City Clinical Emergency Hospital of Kyiv, and consent was obtained from all subjects before the commencement of the study.

2.2. Study design

The studies were carried out in three stages. The aim of the first stage was to investigate the effect of treatment time on the sensitivity of circulating phagocytes to NSC631570 *in vitro*. For this purpose, blood samples of 5 healthy volunteers in the inpatient setting were collected at different times of day (starting at 8:00 h then every 4 h over the next 24 h). Blood samples from each point in time were immediately treated with NSC631570 (Nowicky Pharma, Austria) at a concentration of 20 μg/ml. Phagocytic activity and ROS generation of circulating monocytes and granulocytes were analyzed in treated and untreated samples. The aim of the second stage was to imitate administration of the drug in the inpatient setting (inpatient setting model). For this purpose, 11 volunteers spent most of their time in bed. NSC631570 was administered i.v. at 8:00 h at a volume of 20 ml (a single therapeutic dose in clinical practice). Blood samples were collected before and 30 min after drug administration (at 8:30 h), as well as every 4 h over the next 16–24 h. The aim of the third stage was to imitate administration of the drug in the outpatient setting (outpatient setting model). For this purpose, 11 volunteers were allowed to move and in addition they were asked to take part in one session of standardized moderate physical exercise. NSC631570 was administered i.v. at 8:00 h at a volume of 20 ml. The physical exercise program (at 12:00 h) included 50 slow squats. Blood samples were collected before and 30 minutes after drug administration, before standardized moderate physical exercise, immediately after it, and every 4 h over the next 16–24 h. Phagocytic activity and ROS generation of circulating monocytes and granulocytes as well as biochemical parameters were analyzed in all collected blood samples.

2.3. Intracellular ROS assay

ROS levels were measured using 2′7′-dichlorodihydro-fluorescein diacetate (carboxy-H2DCFDA, Invitrogen), which is converted into a non-fluorescent derivative (carboxy-H2DCF) by intracellular esterases as described earlier [29]. Carboxy-H2DCF is