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Comparison of the relative sensitivity of human lymphocytes and mouse splenocytes to two spindle poisons

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Abstract

Aneugenic compounds act on non-DNA targets to exert genotoxicity via an indirect mechanism. In contrast to DNA-binding agents, these compounds are expected to possess threshold levels of activity. Therefore, the risk for adverse effects following human exposure to an aneugen could be minimal, if the threshold of activity has been clearly determined in vivo and in vitro and providing the human exposure level is below this threshold. Thus, the development of a single-cell model to allow comparisons between in vitro and in vivo threshold values for aneugenic compounds is of importance.

The in vivo micronucleus test is one of the main assays used in genetic toxicology, and is often performed in the mouse. Thus, an extensive database is available in the literature. However, there are only few data concerning the in vitro micronucleus assay using mouse cells, as the majority of in vitro micronucleus assays have been performed using human lymphocytes. In addition, there is a lack of data concerning thresholds for any compound using this model.

First, we evaluated whether the use of mouse splenocytes would be an acceptable alternative to that of human lymphocytes to identify aneugens. To allow valid comparisons, the two protocols were first harmonized. Thus, phytohemagglutinin (PHA) and concanavalin A were used as specific mitogens for human lymphocytes and mouse splenocytes, respectively, in order to achieve similar cell-proliferation rates. To achieve similar and sufficient numbers of binucleated cells, cytochalasin B was added 44 and 56 h after culture initiation of the human and mouse cells, respectively.

Second, we compared the sensitivity of the mouse protocol with that of the human protocol by exposing the cells to the aneugens nocodazole and paclitaxel.

There was good reproducibility of the cytotoxic/genotoxic responses of the two cell models following exposure to the aneugens. The sensitivity of the mouse splenocytes to paclitaxel was higher than that of the human lymphocytes. The two cell types were equally sensitive to nocodazole.

Keywords: Micronucleus assay; Mouse splenocyte; Human lymphocyte; Nocodazole; Paclitaxel

1. Introduction

Micronuclei contain either an acentric chromosomal fragment that is formed by an unrepaired double-strand break (due to chromosomal damage), or a lagging

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chromosome. Molecular dysfunctions leading to unstable structural chromosomal damage are quite different from those inducing gain or loss of the whole chromosome. Structural chromosomal damage is thought to be linked mainly to exposure to direct DNA-damaging agents and/or intracellular defects in DNA replication, recombination or repair mechanisms. In contrast, numerical chromosome aberrations are thought to be linked mainly to exposure to compounds that induce intracellular defects of the mitotic spindle, the kinetochore apparatus and/or the centrosome. Thus, as aneugenic compounds act according to an indirect mechanism of genotoxicity, it is likely that these compounds exhibit thresholds for such effects. For example, the two microtubule inhibitors, nocodazole and carbendazim have been shown to have thresholds of activity of 32 and 2850 nM, respectively [1].

Recently, the in vitro and in vivo threshold values for the aneugen, griseofulvin, were determined [1]. An in vivo lowest observed adverse effect level (LOAEL) of 2000 mg/kg and a no observed adverse effect level (NOAEL) of 100 mg/kg in rat peripheral blood erythrocytes were demonstrated. In vitro, a lowest observed effect concentration (LOEC) of 3 μg/ml, and a no observed effect concentration (NOEC) of 2 μg/ml was established for rat splenocytes. Thus, it is possible that a compound found to be aneugenic in vivo and in vitro could be still developed if a threshold could be determined and its pharmacokinetic profile is taken into consideration.

The objective of this paper was to develop an in vitro micronucleus test using cells that would not only allow the characterization of aneugenic compounds, but would also allow a direct comparison of in vitro and in vivo threshold calculations. The in vivo micronucleus test is one of the main assays used in genetic toxicology, and is often performed in the mouse. Thus, an extensive database is available in the literature and data exist regarding the NOAEL or LOAEL for many compounds. Nevertheless, there are only few data available concerning the in vitro micronucleus assay using mouse cells, as the majority of in vitro micronucleus assays are performed with human lymphocytes. In addition, there is a lack of data on thresholds for any compound using this model.

Mouse splenocytes are easy to obtain and have been previously used in both the in vitro and the in vivo micronucleus test [2,3]. As they are primary cells, they were considered to be a good candidate for comparative studies with the human lymphocyte. Thus, we compared the in vitro effects of two aneugens in these two cell types. Nocodazole is a synthetic analog of the benzimidazole family, which is used as a pharmacological tool. In contrast, paclitaxel is a natural compound belonging to the taxoid family, initially extracted from the bark of Yew (*Taxus brevifolia*). Paclitaxel is used as an antineoplastic drug for various advanced, refractory, or metastatic human cancers such as ovarian, breast, non-small cell lung, and head and neck cancers [4,5]. Although nocodazole and paclitaxel have the same target, the microtubule, their modes of action are different. Nocodazole inhibits tubulin polymerization, and therefore, induces mitotic arrest [6], while paclitaxel stabilizes microtubules by preventing depolymerization, and therefore, tubulin dimer formation. This stability inhibits dynamic reorganization of microtubules during the interphase of the cell cycle and induces mitotic inhibition [6].

The first step of our work consisted of standardizing the mouse and human in vitro micronucleus protocols. Using the human cell model as the reference [7], we assessed the proliferation potential of mouse splenocytes using the mitogen phytohemagglutinin (PHA), which is used in the human lymphocytes assay. We also determined the optimal time for cytochalasin B addition in mouse splenocyte cultures, to obtain a percentage of binucleated (BN) cells of between 35 and 60% as recommended by Fenech [8].

The second phase of our work was to compare the sensitivity of mouse splenocytes with that of human lymphocytes following exposure to the two aneugens, paclitaxel and nocodazole.

2. Materials and methods

2.1. Chemicals

Paclitaxel (CAS 33069-62-4, purity 97%) from *T. brevifolia* and nocodazole (CAS 872-50-4, purity 99%) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Paclitaxel and nocodazole were dissolved in dimethyl sulfoxide (CAS 67-68-5; DMSO) at 100 μg/ml and were kept at −20 °C. Cytochalasin B (CAS 14930-96-2, purity 98%, Sigma, France) was dissolved in DMSO (1 mg/ml) and kept at −20 °C. PHA (CAS 9008-97-3) was purchased from Invitrogen (France), dissolved in Milli-Q water (1 mg/ml) and kept at −20 °C. Concanavalin A (ConA, CAS 11028-71-0) was purchased from Sigma (France), dissolved in Milli-Q water (1 mg/ml) and kept at −20 °C.

Paclitaxel and nocodazole were thawed just prior to use and added to the culture after 24 h. The concentrations used were 0.5, 1.0, 2.5, 5.0, 7.5 nM for paclitaxel and 10, 50, 75, 100, 150, 200 nM for nocodazole. Exposure was for 48 h. A negative control was prepared by adding DMSO to the cells at a final concentration of 0.1%.