PHARMACIA

Pharmacia 69(2): 327–335
DOI 10.3897/pharmacia.69.e77353

Research Article

Peculiar features of bone marrow cell proliferation in Djungarian hamsters with genetic disorders under thiotepa effect

Sergey Kuzin[1], Denis Bogomolov[1], Iza Berechikidze[1], Svetlana Larina[1], Tatyana Sakharova[1]

1 *Department of Biology and General Genetics, I.M. Sechenov First Moscow State Medical University, Moscow, Russia*

Corresponding author: Sergey Kuzin (sergeykuzin44@rambler.ru)

Received 30 October 2021 ♦ **Accepted** 4 February 2022 ♦ **Published** 8 April 2022

Citation: Kuzin S, Bogomolov D, Berechikidze I, Larina S, Sakharova T (2022) Peculiar features of bone marrow cell proliferation in Djungarian hamsters with genetic disorders under thiotepa effect. Pharmacia 69(2): 327–335. https://doi.org/10.3897/pharmacia.69.e77353

Abstract

The paper aims to examine the proliferation of bone marrow cell pool in Djungarian hamsters and the subsequent restoration of their genetic stability after the action of thiotepa (TT). The study involved 36 animals, of which 16 were in the control group (injected with 0.25 ml of physiological solution), and 20 in the experimental group (0.25 ml of thiotepa at a dose of 1.5 mg per 1 kg of body weight). The maximum number of cells with CA amounting to 30.0% was observed 13 hours after TT injection ($p \leq 0.05$ between the control and experimental groups) and rapidly declined to 5.7% over subsequent periods by the 37th hour of the experiment ($p \leq 0.05$). The results suggest that the restoration of cell pool genetic stability is largely associated with the cell selection mechanisms, which confers an advantage over cell proliferation without chromosome anomalies.

Keywords

bone marrow cells, cell cycle, cell proliferation, chromosomal aberrations, genetic homeostasis, thiotepa

Introduction

Studies examining mutagen activity, which can be a variety of chemical compounds, are still highly relevant nowadays (Saxena and Kumar 2020). One of the primary mutagenicity tests is the analysis of chromosomal aberrations in laboratory-preserved chromosomes of simple-toothed rodents (Egoshin et al. 2018; Nin et al. 2020). The advantage of this method is its availability. Similarly, the karyotype of small rodents is dominated by acrocentric chromosomes, complexifying the identification process of such anomalies in quantitative terms (Chen et al. 2020).

In modern medicine, the prevention of inherited or mutagenic diseases is a central focus (Hall 2015). Therefore, studies assessing mutagenesis in cells of small rodents are of great importance, including for medical purposes. Most mutagens are chemicals that can cause changes in both DNA and chromosomes, affecting the hereditary background. In addition to chemical compounds, mutagens also include physical or biological factors, such as ultraviolet light, radioactive radiation, and certain viruses. Among chemical mutagens, drugs used to treat tumors are particularly distinctive (Qin et al. 2019b).

The spectrum of mutations is very broad and can include both somatic and generative cells of the adult body and embryo or fetus (Wei et al. 2018). Mutagenic consequences include infertility, hereditary diseases, various forms of cancer, and birth defects (Deng et al. 2018; Farooqi et al. 2021). Only for Russia each year, according to the Ministry of Statistics, there are about

0.3 to 0.5 million registered cases of cancer and the same volume of disablement due to birth defects. Infertility is observed in one-tenth of all couples in the country. More than half of the embryos fail to implant into the uterus or fully develop through spontaneous abortions (RBC 2019). With the aggravation of environmental conditions caused by exposure to industrial emissions, there is a growing incidence of disease, deaths caused by mutagenic effects. It is important to note that the body has efficient mechanisms that repair genetic disorders in cells or remove cells with such damages. Hence, genetic disturbances occur only when mutant cells persist in the body. Currently, the mechanisms and models of mutant cell elimination, unlike DNA repair, are not well understood.

Bone marrow (BM) is a rapidly renewing tissue based on polypotent stem cells (SCs) that spend most of their lives during the G0 period. Under normal conditions, the reproduction of hematopoietic cells, like other SCs, occurs primarily by asymmetrical mitosis. Afterward, one cell remains a stem cell, and the second – a transient cell – is released from the niche, loses its stem properties, and differentiates in one of the hematopoiesis directions (Wilson et al. 2008; van der Wath et al. 2009; Hérault et al. 2021). In contrast to stem cells, transient cells can actively proliferate, resulting in the replacement of differentiated cells leaving the BM. In various damages, SCs are activated to proliferation for restorative regeneration and replacement of lost cells (Rossi et al. 2007, 2008; Wilson et al. 2008).

The accumulation of mutations in SCs over a lifetime is one of the reasons for their reduction, which diminishes the possibilities of physiological and restorative regeneration and may also cause a variety of diseases, including tumor development (Wilson et al. 2008; Harding et al. 2017). The maintenance of genetic homeostasis in the tissue requires various effective action mechanisms, the main ones being the DNA repair and the processes happening at the control points of the mitotic cycle, which are currently well studied (Beerman et al. 2014; Brown and Geiger 2018; Ovejero et al. 2020).

However, a significant fraction of mutant cells is capable of passing the mitotic cycle and preserving the capacity to proliferate (Kuzin and Stukalov 1998; Pelevina et al. 2009; Neroyev et al. 2013; Li et al. 2020). The laws and selection mechanisms that can eliminate these cells from the pool and replace them with cells without genetic disturbances during proliferation are poorly understood.

Several medications used in tumor chemotherapy are genotoxic. In all SCs retained after treatment by mutagens, the number of genetic abnormalities increases significantly. The proliferation of these cells should not only provide for the reconstitution of cell loss and tissue regeneration but also maintain genetic stability since its further violation may result in disruption of homeostasis and the development of secondary tumors (Brown and Geiger 2018; Li et al. 2011). Under these conditions, the selection mechanisms which eliminate the most damaged mutant cells from the cell pool become especially important.

Test systems capable of detecting mutagenic activity are important in preventing genetically determined conditions (Hall 2015). The choice of a suitable biomodel for the experimental design remains one of the major links determining the effectiveness of the test system used. In current methods of evaluating mutagenic activity, laboratory mice, less commonly rats, dominate as experimental animals (Khan et al. 2019). Moreover, Djungarian hamsters are more appropriate biomodels for studying chromosomal aberrations because they have a convenient set of chromosomes for karyotyping (Sokova and Pogosiants 1974). However, they are not used as frequently as other rodents in laboratory studies. Another important point is the use of chemicals that have a well-known action mechanism. Thiotepa's formula is SP (NC2H4)3; it is an analog of N,N′,N″-triethylenephosphoramide. This substance, which belongs to the class of alkylating antineoplastic drugs, does not occur in nature. The history of alkylating drugs application goes back to the times of World War I, when the so-called mustard gas was actively used, on the basis of which the drugs of this group are synthesized. In medicine, nitrogenous analogues of mustard gas began to be used (in oncology). Thus, thiotepa contains three groups of ethylene (Saxena and Kumar 2020). The mutagenic effect of this compound has been well studied in mice, fibroblasts, and human white blood cells. For all of the aforementioned biomodels, a high frequency of chromosomal aberrations under exposure to TT was reported (Saxena and Kumar 2020). Meanwhile, such studies have not been conducted on hamsters. In this paper, the authors examined the proliferation of bone marrow cells in Djungarian hamsters at different times after exposure to TT. The limits of maximum TT effect on the induction of chromosomal aberrations were established, showing the kinetic of cells with various chromosomal damages. Besides, the authors hypothesize that the restoration of cell pool genetic stability is largely specified by the mechanisms of cell selection, which confers an advantage on cell proliferation without chromosome anomalies.

The study aimed to examine peculiar features of proliferation in bone marrow cells of Djungarian hamsters with different levels of chromosomal abnormalities after exposure to TT, i.e., a cytostatic agent with an alkylating mutagenic effect.

Materials and methods

Materials

The work was carried out in 2020 in Moscow (Russian Federation), based at the Scientific Research Institute of Oncology of the Russian Academy of Sciences, on Dungarian *Phodopus sungorus* hamsters, which have 28 chromosomes in the practical diploid set for cytogenetics analysis. A total of 36 animals were divided into two groups: 16 hamsters in the control group and 20 hamsters in the experimental group.

Study design

In the control group, the animals received a 0.25 ml intraperitoneal injection of physiological solution, and animals in the experimental group received 0.25 ml of TT at a dose of 1.5 mg/kg weight. Together with the administration of TT or saline solution, all animals were given a 25 mg pill of 5-bromodeoxyuridine (BrdU) under the skin on the back to determine the number of mitotic cycles the cells passed through since the beginning of the experiment. Two-thirds of the pill was coated with biological glue to slow down the absorption of BrdU and create the necessary concentration in the animals during all 37 hours of the experiment. The cutaneous incision of the back after the introduction of the pill was sealed with biological glue. Hamsters were mortified (by cervical dislocation, respecting bio-ethical requirements) after 13, 19, 25, 31 hours, and only for the experimental group – after 37 hours. Two hours before the procedure, the animals were given an intraperitoneal injection of colchicine solution at a dose of 1.5 mg/kg weight to cease mitosis and metaphase buildup.

Research methods

Bone marrow was collected from the femur bones and suspended in Eagle's medium. After centrifugation, cells were treated with a 6.0 g/L KCl hypotonic solution for 7 minutes, centrifuged and bound with a 3:1 solution of methanol and glacial acetic acid. The cells were centrifuged again to remove excess fixative, and the cell suspension was spun on slides to prepare cytogenetic medications. In all instances, centrifugation was conducted at 1000 rpm for 8 minutes.

To obtain differential chromosome staining with BrdU, the preparations were treated with aqueous acridine orange solution (10 min 10-5M), irradiated for 15 min with a UV lamp, incubated for 5 min in saturated Ba(OH)2 solution, washed, dried and stained with 2% Gimz solution in phosphate buffer. Fig. 1 demonstrates the experimental scheme and the differences in chromosomal staining, which allowed determining the number of mitotic cycles the cells passed through for a certain period after the beginning of the experiment. All chromosomes of the first mitosis, i.e., cells that have undergone a mitotic cycle in the presence of BrdU, are uniformly dark in color.

Upper part: chromosome staining: after one mitotic cycle passed by cells in the presence of BrdU, all chromosomes are dark; after two cycles – all chromosomes have one dark and one light chromatid (harlequin staining); after three cycles – half of the chromosomes have harlequin staining, and another half – light staining of both chromatids. In the middle of the diagram: M1, M2, M3, M4 are the numbers of mitoses corresponding to the number of mitotic cycles passed by the cells. M1-2 is the mitosis of cells that were in the S-period of the first mitotic cycle at the beginning of the experiment; these cells passed a part of the first and full second mitotic cycle. Bottom part: the action of TT in the experiment, limited mainly to the first mitotic cycle; and BrdU being active during the whole experiment. Injection of saline in the control group of animals is not indicated on the diagram.

After two cycles, all chromosomes have one dark and one light color chromatid, the so-called harlequin coloration. In the third mitosis, half of the chromosomes (14) and in the fourth, a quarter (7) of the 28 diploids have a harlequin coloration, while the rest have a light coloration. The ratio of chromosomes of different colors in the third and fourth mitoses may be affected by an accidental divergence of chromatids towards the cell poles. However, the chance of mixing the third and fourth mitoses is low and would not significantly affect the results. Sister chromatid exchanges alter the chromosome color but do not affect the determination of the number of mitoses. When the cells were in the replication during S-period at the time BrdU was introduced, its partial incorporation into the daughter's DNA strand also changed the color of the chromosome. These cells can be identified by alternating dark and light segments in the chromatids; they have been enumerated separately.

In addition to determining the number of mitoses (the number of mitotic cycles the cells passed through), chromosome aberrations (CA) were determined in the metaphases of all preparations. The analysis of chromosomal aberration type was conducted visually using a Leica DM2500 microscope (Germany). All CAs that can be recognized without karyotyping were analyzed: paired and unique fragments, ring and dicentric type chromosomes, chromosome exchanges, and chromatids (Nadirov et al. 2013, 2016; Qin et al. 2018a, b). In cells with aberrations, the total number of chromosomal ruptures resulted from these aberrations was determined. The CA rates (percentage of mitosis) for each study term were calculated by averaging the results for four animals in an analysis of 600 to 1100 metaphases. The selection of chromosomal aberrations is related to the fact that these disorders are one of the most sensitive tests for genetic damage caused by chemicals, radiation, and other environmental mutagens (Qin et al. 2019a).

Statistical analysis

The obtained data were entered into Excel 2016 application (Microsoft Inc., USA). Further, the database was processed using a compatible program Statistica v.7.0 (StatSoft Inc., USA). Throughout the text, data is given in percentages as a more convenient method to display the number of cells that have undergone mitotic reproduction after TT administration. Statistical comparison of difference significance between control and experimental groups was performed using the Student's t-criterion. A minimum level of significance was $p \leq 0.05$.

Results

TT is a cytostatic agent with an effect related to DNA alkylation. The maximum concentration of the drug and its active derivatives is reached immediately after

administration and is progressively reduced with metabolism and excretion. The data presented in Table 1 show that TT causes a significant delay in the duration of the mitotic cycle in cells that resisted the drug effect and retained the ability to proliferate. While in the controls, 9% of cells managed to undergo two cycles of replication already by 13 hours. For the experimental group, such cells appeared 6 hours later, by the 19th hour after TT injection. A similar delay of approximately six hours is observed when cells undergo the third and fourth mitotic cycles. These data indicate that the main inhibition of proliferation occurs in the first cell cycle, i.e., when the drug and its active metabolites are involved. Afterward, the rate of proliferative processes is largely re-established and becomes comparable to the control parameters.

Table 1. Percentage of bone marrow cells in Djungarian hamsters that have undergone various mitotic cycles following administration of thiotepa (experiment) or physiological solution (control).

Animal Group	Time after injection (hour)	M1	M1-2	M2	M3	M4
Control	**11–13**	74.4	16.6	9.0	0	0
	17–19	61.1	16.1	21.6	1.2	0
	23–25	21.4	14.5	47.4	16.2	0.5
	29–31	3.9	0.9	29.8	47.7	17.7
Experimental	**11–13**	98.1	1.9	0	0	0
	17–19	67.7	19.7	12.6	0	0
	23–25	54.8	20.8	23.3	1.1	0
	29–31	25.3	13.7	43.2	18.7	0.7
	35–37	9.1	2.8	25.4	33.1	29.6

M1, M2, M3, M4 are cells that entered mitosis after passing one, two, three, or four mitotic cycles, respectively. M1-2 are cells that were in the S-period at the beginning of the experiment, passed the rest of the first cycle, and completed the second mitotic cycles. The 2-hour interval for each study term reflects the accumulation of cells in the metaphase during that time with colchicine.

Based on the data obtained, the average time of the proliferation cycle of the bone marrow cells in the control is 10 to 12 hours in the experiment – 13 to 15 hours ($p \leq 0.05$ between control and experiment). In animals with no TT injection, 17.7% of the fastest cells were able to complete four mitotic cycles in 31 hours. Therefore, the average cycle lasted about 8 hours ($p \leq 0.01$ with the control). After TT injection, 29.6% of the cells with the highest proliferation rate entered their fourth reproduction only by the 37th hour, i.e., each mitotic cycle took on average 9–10 hours ($p \leq 0.05$).

Given the delay of about 6 hours in the first cycle, their passage rate over the next three cycles was roughly the same as in controls ($p \geq 0.05$). In both controls and experiments, there are a very small fraction of cells (about 1%) with an even higher proliferation rate, and the duration of each of the four passed cycles was about 1–2 hours shorter. In contrast, the fraction of cells that entered into 1 or 2 reproduction cycles by the 25th-31st hour in the control and 25th – 37th hour in the experiment ($p \leq 0.05$) have significantly longer cycle durations. However, at least some of them are in the G0 period for a while, so it is difficult to determine by how much is their mitotic cycle prolonged.

It should be noted that the use of BrdU does not make it possible to determine, in what part of the G1 period the cells were at the time of its introduction. Consequently, the exact total duration of the first mitotic cycle can be determined only for those cells that were at the beginning of such period. For cells that have already passed some G1 at the moment of injection, an upward correction is required. For cells in the G0 period, the time to reach the first mitosis is longer than the duration of the mitotic cycle by the amount necessary to activate proliferation and pass from G0 to G1 period.

The data presented in Fig. 2 show that a selected dose of TT has a pronounced mutagenic effect on Djungarian hamsters, leading to CAs in a large proportion of bone marrow cells. The maximum effect is reached 13 hours after injecting TT. The mean number of cells with CA in the control group ranges from 1% for all phases of the experiment. In the experimental group, the number of aberrant cells 13 hours after the drug injection exceeded the control values by more than 30 times ($p \leq 0.001$). In subsequent terms, the cell fraction with CA decreases and reaches 5.7% by 37 hours of the experiment, which is more than 5 times less than the values at 13 hours (30.0%, $p \leq 0.05$). The data of this study show that the decline in chromosomal abnormalities is associated with the preferential proliferation of CA-free cells. After each mitotic cycle passed, the proportion of cells with CA decreases significantly, with less than 4% in the third or fourth mitosis. At the same time, the number of abnormal cells entering the first mitosis increases even after 13 hours and reaches 39.2% by the 31st hour ($p \leq 0.05$). The proportion of complex chromosome aberrations caused by multiple chromosomes ruptures increases in them (Fig. 2b). A significant decrease in chromosomal abnormalities in cells entering the first mitosis after TT injection begins only after 37 hours. The number of CA decreases to 16.0%, with cells with multiple abnormalities of more than 5 chromosome ruptures per cell completely disappearing ($p \leq 0.05$).

Discussion

TT has been used in cancer medicine for over 60 years. Pharmacokinetics study of the drug showed that the highest concentration of TT and its metabolites is achieved in the blood of mice within one hour after administration and decreases thereafter. The majority of TT and its metabolites are excreted into the urine within 10 hours (Kuzin et al. 1989). Studies on the kinetics of alkylating agents in the blood of monkeys and rabbits have also shown that they

reach their maximum concentration in one hour, and their principal effect manifests within the first hours after administration of the medication (Stukalov 1988; Sinai and Kerem 2018). Thus, the effects of TT observed in this study are associated with the action on the cells only during one first mitotic cycle, which is reflected in the scheme of Fig. 1. The exception is the "fastest" cells, which were in S-period at the time of injection and, having completed the first cycle, could have had time to enter the second cycle, when a small amount of alkylating agents was still active. However, according to data of this study, only 1.9% of such cells existed (Table: experiment, M1-2, 11–13 hours).

Bone marrow is a heterogeneous cell system that consists of cell sub-pools, whose degree of maturity and sense of differentiation vary significantly. CAs constitute a very small fraction of cells that seldom replicate (Schneider et al. 1977; Hérault et al. 2021). Consequently, among the mitoses in the series of control, there could only be simple cells. As a result, the findings observed in this study on cell kinetics and cell cycle parameters do not refer to them but to different groups of actively proliferating transient cells of various differentiation directions. Among these heterogeneous cell groups, the duration of the mitotic cycle can be precisely calculated only for those that underwent 3–4 cycles in 31 hours of the experiment. For actively proliferating transient cells, it varies from 8 to 12 hours in monitoring. For cells that enter into first or second mitosis at 25 to 31 hours, there is a high probability that they spent some part of their time in the G0 period. Therefore, the precise duration of their mitotic cycle cannot be determined. For example, cells that have undergone one replicative cycle in the presence of BrdU and entered the first mitosis at 31 hours may have a mitotic cycle duration of only 8 hours, and the remaining 23 hours is the time spent in the G0 period.

However, the data of this study allow determining fairly precise cycle parameters for the slower cells as well. The specifics of chromosomal coloration identified the cells that were in the S-period at the time of BrdU administration. These cells entered the second mitosis at 25 hours of the experiment (M1-2 in the Table), passed a part of the first cycle(½S, G2, M), and then full second mitotic cycle during this time. As a result, the duration of the mitotic cycle for them is approximately 16–18 hours.

The variation in the duration of a mitotic cycle of bone marrow cells between 8 and 18 hours reflects the heterogeneity of its constituent cells. For a small part (1% of the fastest or slowest cells), these values may be lowered or increased by several hours. The mean value of 12 hours is the data known from available literature sources (Sokova and Pogosiants 1974). Significantly higher values characterize the cell cycle rather than mitotic, i.e., include more G0 period time.

Introducing TT significantly alters the kinetics of bone marrow cells. The data obtained shows that these changes primarily affect the first mitotic cycle, i.e., there is a delay in its passage of around 6 hours. Over the next three cycles, the proliferation rate has recovered. Based on the pharmacokinetics of the drug it can be concluded that TT and its metabolites affect cell proliferation at the moment they are in the body. Their action affects mainly the cells that continue to be in the first cycle and insignificantly affects the proliferative processes of the majority of cells that manage to overcome the first mitotic cycle. Obviously, much of this is due to the action of the control point mechanism, which slows down the cells presenting genetic abnormalities for DNA repair.

It should be noted that TT has a significant cytotoxic effect, and the data in this study were obtained only for the fraction of cells that managed to survive and retain the ability to proliferate. Djungarian hamsters are very

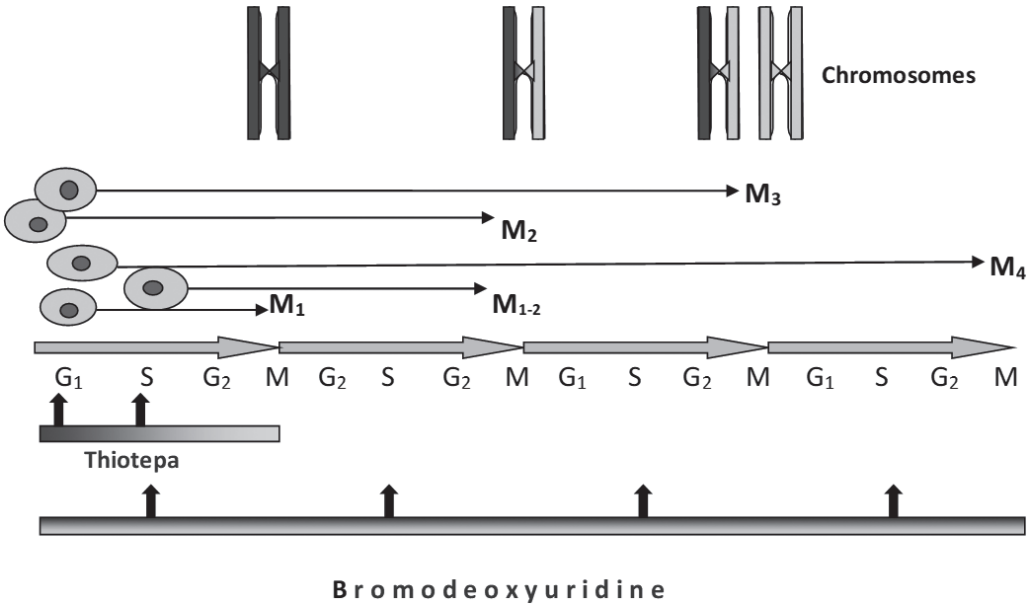

Figure 1. Design of the experiment.

sensitive to cytostatic and mutagenic agents (Kuzin et al. 1987). The dose of 1.5 mg/kg is comparatively high for these animals. However, it maintains sufficient cell viability to determine cell kinetics and chromosomal anomalies in several successive cycles of division. Preliminary experiments performed specifically to choose the dosage of the drug have shown that the increase to 2–7 mg/kg significantly reduced the number of dividing cells, making it difficult to obtain the number of metaphases necessary for analysis.

Cellular death after cytostatic action causes the activation of preserved SCs and the transition of a significant portion of them into the mitotic cycle. The transition from G0 to proliferation takes one to two days (Wilson et al. 2008; Saakyan et al. 2011; Brown and Geiger 2018). Therefore, it can be assumed that, unlike the control, in the experiment, the number of SCs entering mitosis at an advanced stage significantly increases. The data obtained in this study support this hypothesis on the increase of the first mitosis fraction in the experiment versus the control at 31–37 hours of the experiment. Undoubtedly, some of them are cells with significant damage that require a long time to overcome the mitotic cycle. However, most of these cells are destroyed by apoptosis by 37 hours. A sharp decrease in the CA cells fraction with entering their first mitosis at 37 hours (Fig. 2) indicates the activation and transition to the proliferation of relatively mutagen-resistant SCs that were in the G0 period during TT injection and had relatively few genetic abnormalities.

It has been shown a pronounced mutagenic effect of TT on bone marrow cells in Djungarian hamsters, leading to chromosome damage. The genotoxic effect of TT has been studied in numerous sources of literature and is well documented (Thiotepa 2011; Sinai and Kerem 2018; Lezaja and Altmeyer 2020). It is important to note that the CA observed represents only a small fraction of the visible mutations. Every single cell receives significant genetic damage as a result of TT injection. Meanwhile, after cytostatic action, SC preserved during multiplication should not only provide replenishment for cell loss but also restore genetic homeostasis. Otherwise, increasing genetic instability can result in different pathologies. This is particularly important for intensive chemotherapy, when SC transplantation is not used after treatment and regeneration is ensured by the own preserved cells.

Under the action of high mutagenic doses, including TT, the recovery begins after the increase in the number of genetic disorders, and the number of cells with CA gradually decreases (Stukalov 1988; Sinai and Kerem 2018). Currently, the processes reducing the number of mutations that occur at the molecular genetic level, i.e., DNA repair and apoptosis of cells, including those associated with the mitotic cycle control points are quite well studied. It is generally believed that cells with large genetic abnormalities that have not undergone effective repair and have entered proliferation are delayed in control points, eliminated, and cannot be reproduced. However, this applies only to some fraction of cells. The data obtained demonstrate that even cells with very significant chromosomal abnormalities can pass through and replicate into one mitotic cycle. And the part of the cells in which CA is caused by 1 or 2 ruptures is capable of passing at least 4 mitotic cycles. A number of studies have shown that DNA disruption may not only persist and continue to be repaired in cells that have passed the control points, but also cause extension of DNA replication beyond S-period, i.e., in G2 and even mitosis (Schneider et al. 1977; Mankouri et al. 2013; Beerman et al. 2014; Kuzin 2019). And the study of sister chromatid exchanges indicates that some of the primary DNA damage is preserved and causes recombinant repair even in the third cycle following the action of TT (Li et al. 2020). Thus, mechanisms for repairing genetic homeostasis are not limited to repairing damage to the DNA or apoptosis of mutant cells in a single cell cycle. They are largely associated with cell selection, which occurs at the cell pool level during proliferation.

Furthermore, the decrease in the number of genetic disorders in the BM pool is associated with the predominant reproduction of cells without CA or with minor chromosome damage. With significant genetic abnormalities, cells are slower to prepare for division, which is related to the preservation and even increase in the proportion of such cells by 25th and especially by 31st hour of the experiment. They are unable to divide more than once and have a limited lifespan, as shown by a significant decrease in their number at 37 hours. Moreover, at this time only cells with less than 5 chromosome ruptures preserve the ability to division, while at 31 hours of the experiment, cells with 5 or more ruptures account for 36.3% of all aberrant metaphasesq associated not only with the death of the most damaged cells delayed in the mitotic cycle but also with the activated proliferation of SCs that were in the G0 period. Resting cells are known to be less sensitive to genotoxic effects than proliferating cells and exhibit therefore fewer chromosomal abnormalities. Besides, the entry of SCs into proliferation involves additional mechanisms of genetic homeostasis, namely, effective postreplicative repair and mitotic cycle checkpoint filter (Pelevina et al. 2009; Brown and Geiger 2018; Kuzin 2019).

The data we obtained enables presenting the following scheme of bone marrow cell proliferation following mutagenic exposure to TT. Some of the cells most affected by DNA cannot grow and die. An additional part of these cells can undergo a mitotic cycle and division. Their sustainability is limited to about 30 hours, and they can only pass through one cycle. Less damaged cells with 1–2 chromosomal ruptures remain viable throughout the observation and can pass through at least four mitotic cycles. However, after every division, a part of these cells is eliminated. Loss recovery occurs at the expense of reproduction of cells without CAs, including proliferating activated SCs that were dormant before exposure. Genetic disorders lead to a slower proliferation, apparently associated with delayed cells at first control points after

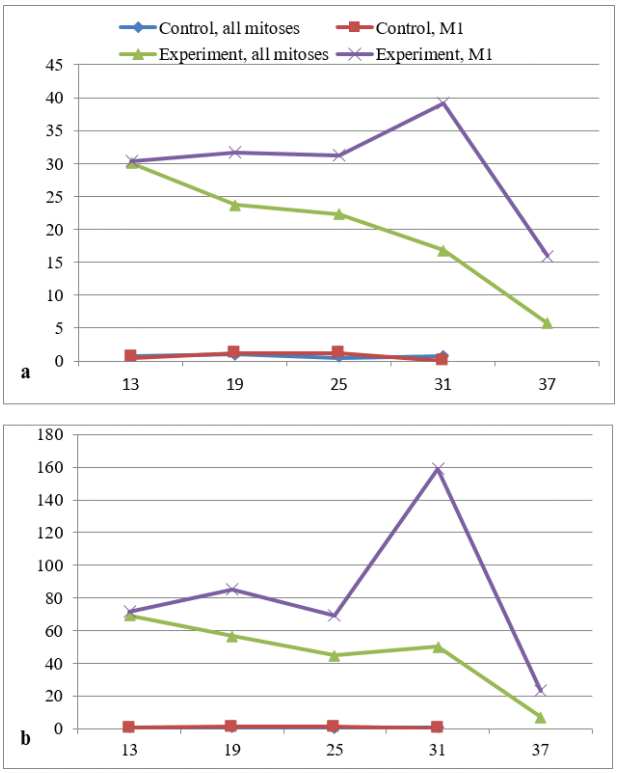

Figure 2. Changes in the number of metaphases with chromosomal aberrations (**a**) or the number of chromosome ruptures (**b**) in the bone marrow of Djungarian hamsters at different periods after the injection of saline (control) or thiophosphamide (experiment). Abscissa axis: time since injection (hour); ordinate axis: number (%) of metaphases with chromosome aberrations (**a**) or number of chromosome ruptures per 100 metaphases (**b**) among all cells under division (all mitoses) or those that passed only one mitotic cycle (M1)

the mitotic cycle of cytostatic action. The mean duration of the delay is 6 hours, which is proportional to the number of genetic abnormalities.

The mechanisms of cell selection, which give an advantage in cell reproduction without genetic disorders, play an essential role in maintaining the genetic homeostasis of the cell pool. The importance of such mechanisms is also indicated by the fact that the rate of CA elimination after TT action in the pool of rapidly proliferating BM cells is significantly higher than in blood lymphocytes (Stukalov 1988; Sinai and Kerem 2018). The mechanisms used to select cells maintaining genetic stability are poorly understood. As suggested earlier, one of these mechanisms could be associated with changes in the spatial and temporal organization of mutant cells (Georgiades et al. 2010). It is well known that SCs that changed their location and left the niche lose their stem properties and start differentiating. Similarly to the spatial niche, the delay of mutant cells with genetic disorders in the control points of the mitotic cycle leads to their falling out of the "temporary window" of circadian proliferation rhythm typical for all renewing tissues. That also causes their differentiation. This mechanism preserves the slightly damaged mutant cells, allows them to exercise their functions in the differentiation process, and only afterward removes them from the tissue. The possibility of such a mechanism is confirmed by the data on the second wave of increase in the number of lymphocytes with CA in the blood of rabbits on day 4 after TT administration (Saakyan et al. 2013; Subarkhan et al. 2019). Such an increase cannot be attributed to the mutagenic effect of TT, which is eliminated during the first 11 hours but is well explained by the displacement of differentiated cells with chromosomal abnormalities from the bone marrow by this time. It should be noted that differentiating mutant cells (except generative cells) are usually not harmful. As opposed to stem cells, they have a limited number of divisions and a limited lifetime.

The results of this work allow concluding that the restoration of genetic stability in the BM cell pool after TT injection is only partially performed by the well-known mechanisms of DNA and control points repair, which act mainly in the first mitotic cycle. Then, less studied selection mechanisms begin to play a decisive role. They work through the selective advantage of cells without chromosomal aberrations, which progressively move mutant cells from the pool during proliferation. Studying cell-pool mechanisms of genetic homeostasis will enable to influence the processes of preserving genetic stability of renewing tissues after mutagenic exposure, which is especially important in the treatment of oncological diseases.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interests

Authors declare that they have no conflict of interests.

Data availability

Data will be available on request.

Ethics approval

Experiments with animals were carried out according to bioethical requirements.

Acknowledgements

None.

References

Beerman I, Seita J, Inlay MA, Weissman IL, Rossi DJ (2014) Quiescent Hematopoietic Stem Cells Accumulate DNA Damage during Aging that Is Repaired upon Entry into Cell Cycle. Cell Stem. Cell 15(1): 37–50. https://doi.org/10.1016/j.stem.2014.04.016

Brown A, Geiger H (2018) Chromosome Integrity Checkpoints in Stem and Progenitor Cells: Transitions upon Differentiation, Pathogenesis, and Aging. Cellular and Molecular Life Sciences 75(20): 3771–3779. https://doi.org/10.1007/s00018-018-2891-z

Chen X, Tang WJ, Shi JB, Liu MM, Liu XH (2020) Therapeutic Strategies for Targeting Telomerase in Cancer. Medicinal Research Reviews 40(2): 532–585. https://doi.org/10.1002/med.21626

Deng J, Yu P, Zhang Z, Wang J, Cai J, Wu N, Sun H, Liang H, Yang F (2018) Designing anticancer Copper(II) Complexes by Optimizing 2- Pyridine-Thiosemicarbazone Ligands. European Journal of Medicinal Chemistry 158: 442–452. https://doi.org/10.1016/j.ejmech.2018.09.020

Egoshin VL, Ivanov SV, Savvina NV, Kapanova GZ, Grjibovski AM (2018) Basic Principles of Biomedical Data Analysis in R. Ekologiya cheloveka (Human Ecology) 7: 55–64. https://doi.org/10.33396/1728-0869-2018-7-55-64

Farooqi AA, Gulnara K, Mukhanbetzhanovna AA, Datkhayev U, Kussainov AZ, Adylova A (2021) Regulation of RUNX proteins by long non-coding RNAs and circular RNAs in different cancers. Non-coding RNA Research 6(2): 100–106. https://doi.org/10.1016/j.ncrna.2021.05.001

Georgiades SN, Abd karim NH, Suntharalingam K, Vilar R (2010) Interaction of Metal Complexes with G-Quadruplex DNA. Angewandte Chemie International Edition 49(24): 4020–4403. https://doi.org/10.1002/anie.200906363

Hall JE (2015) Guyton and Hall Textbook of Medical Physiology e-Book. Elsevier Health Sciences, New York.

Harding SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA (2017) Mitotic Progression Following DNA Damage Enables Pattern Recognition within Micronuclei. Nature 548(7668): 466–470. https://doi.org/10.1038/nature23470

Hérault L, Poplineau M, Mazuel A, Platet N, Remy É, Duprez E (2021) Single-Cell RNA-Seq Reveals a Concomitant Delay in Differentiation and Cell Cycle of Aged Hematopoietic Stem Cells. BMC Biology 19(1): 1–20. https://doi.org/10.1186/s12915-021-00955-z

Khan TM, Gul NS, Lu X, Wei JH, Liu YC, Sun H, Liang H, Orvig C, Chen ZF (2019) In Vitro and in Vivo Anti-Tumor Activity of Two Gold(III) Complexes with Isoquinoline Derivatives as Ligands. European Journal of Medicinal Chemistry 163: 333–343. https://doi.org/10.1016/j.ejmech.2018.11.047

Kuzin SM (2019) Genetic Instability in Disrupted Spatial and Temporal Organization of Stem Cells. Genes and Cells 14(S): e129.

Kuzin SM, Stukalov SV (1998) Relationship between the Duration and Number of Cell Cycles after Mutagenic Exposure and the Incidence of Sister Chromatid Exchanges. Bulletin of Experimental Biology and Medicine 125(2): 165–167. https://doi.org/10.1007/BF02496849

Kuzin SM, Stukalov SV, Popandopulo PG (1987) Quantitative Comparison of the Cytogenetic Effect Ofthiophosphamideon Monkey Lymphocytes in Vivo and in Vitro. Bulletin of Experimental Biology and Medicine 103(3): 394–396. https://doi.org/10.1007/BF00840574

Kuzin SM, Stukalov SV, Viktorov VV, Dshemilev AA, Popandopulo PG (1989) Induction and Elimination of Cytogenetic Disturbances in Lymphocytes of Monkeys Exposed Tothiophosphamide. Bulletin of Experimental Biology and Medicine 107(6): 864–866. https://doi.org/10.1007/BF00840764

Lezaja A, Altmeyer M (2020) Dealing with DNA Lesions: When One Cell Cycle Is Not Enough. Curr. Opin. Cell Biology 70: 27–36. https://doi.org/10.1016/j.ceb.2020.11.001

Li B, Brady SW, Ma X, Shen S, Zhang Y, Li Y, Szlachta K, Dong L, Liu Y, Yang F, Wang N, Flasch DA, Myers MA, Mulder HL, Ding L, Liu Y, Tian L, Hagiwara K, Xu K, Zhou X, Sioson E, Wang T, Yang L, Zhao J, Zhang H, Shao Y, Sun H, Sun L, Cai J, Sun HY, Lin TN, Du L, Li H, Rusch M, Edmonson MN, Easton J, Zhu X, Zhang J, Cheng C, Raphael BJ, Tang J, Downing JR, Alexandrov LB, Zhou BS, Pui CH, Yang JJ, Zhang J (2020) Therapy-Induced Mutations Drive the Genomic Landscape of Relapsed Acute Lymphoblastic Leukemia. Blood 135(1): 41–55. https://doi.org/10.1182/blood.2019002220

Li F, Patterson AD, Höfer CC, Krausz KW, Gonzalez FJ, Idle JR (2011) A Comprehensive Understanding of Thiotepa Metabolism in the Mouse Using UPLC-ESI-QTOFMS-Based Metabolomics. Biochemical Pharmacology 81(8): 1043–1053. https://doi.org/10.1016/j.bcp.2011.01.024

Mankouri HW, Huttner D, Hickson ID (2013) How Unfinished Business from S-Phase Affects Mitosis and Beyond. EMBO Journal 32(20): 2661–2671. https://doi.org/10.1038/emboj.2013.211

Nadirov RK, Nadirov KS, Bimbetova GZ, Nadirova ZK (2016) Synthesis and Cytotoxic Activity of New Flavopiridol Analogs. Chemistry of Natural Compounds 52(3): 499–500. https://doi.org/10.1007/s10600-016-1686-4

Nadirov RK, Nadirov KS, Esimova AM, Nadirova ZK (2013) Electrochemical synthesis of biflavonoids. Chemistry of Natural Compounds 49(1): 108–109. https://doi.org/10.1007/s10600-013-0521-4

Neroyev VV, Saakian SV, Miakoshina EB, Iurovskaia NN, Riabina MV, Zaĭtseva OV, Lysenko VS (2013) Differential Diagnosis of Early Central Choroidal Melanoma and Late Stage Age-Related Macula Degeneration. Vestnik Oftalmologii 129(1): 39–45.

Nin DS, Idres SB, Song ZJ, Moore PK, Deng LW (2020) Biological Effects of Morpholin-4-Ium 4 Methoxyphenyl (Morpholino) Phosphinodithioate and Other Phosphorothioate-Based Hydrogen Sulfide Donors. Antioxidants & Redox Signaling 32(2): 145–158. https://doi.org/10.1089/ars.2019.7896

Ovejero S, Bueno A, Sacristán MP (2020) Working on Genomic Stability: From the S-Phase To Mitosis. Genes (Basel) 11(2): e225. https://doi.org/10.3390/genes11020225

Pelevina II, Aleshchenko AV, Antoshchina MM, Boeva OV, Gotlib VI, Kudriashova OV, Lizunova EIu, Osipov AN, Riabchenko NI, Semenova LP, Serebrianyĭ AM (2009) Adaptive Response in the Different Mitotic Cycles after Irradiation. Cell Tissue Biology 3(1): 71–77. https://doi.org/10.1134/S1990519X09010106

Qin QP, Meng T, Tan MX, Liu YC, Luo XJ, Zou BQ, Liang H (2018a) Synthesis and in Vitro Biological Evaluation of Three 4′-(4-Methoxyphenyl)-2,2′:6′,2″-Terpyridine Iridium(III) Complexes as New Telomerase Inhibitors. European Journal of Medicinal Chemistry 143: 1387–1395. https://doi.org/10.1016/j.ejmech.2017.10.035

Qin QP, Wang SL, Tan MX, Liu YC, Meng T, Zou BQ, Liang H (2019a) Synthesis of Two Platinum(II) Complexes with 2-Methyl-8-Quinolinol Derivatives as Ligands and Study of Their Antitumor Activities. European Journal of Medicinal Chemistry 161: 334–342. https://doi.org/10.1016/j.ejmech.2018.10.051

Qin QP, Wang SL, Tan MX, Wang ZF, Luo DM, Zou BQ, Liu YC, Yao PF, Liang H (2018b) Novel Tacrine Platinum(II) Complexes Display High Anticancer Activity via Inhibition of Telomerase Activity, Dysfunction of Mitochondria, and Activation of the P53 Signaling Pathway. European Journal of Medicinal Chemistry 158: 106–122. https://doi.org/10.1016/j.ejmech.2018.09.008

Qin QP, Wang ZF, Wang SL, Luo DM, Zou BQ, Yao PF, Tan MX, Liang H (2019b) In Vitro and in Vivo Antitumor Activities of Three Novel Binuclear Platinum(II) Complexes with 4′-Substituted-2,2′:6′,2″-Terpyridine Ligands. European Journal of Medicinal Chemistry 170: 195–202. https://doi.org/10.1016/j.ejmech.2019.03.014

RBC (2019) The Ministry of Health named the number of detected cases of cancer in 2018.

Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL (2007) Deficiencies in DNA Damage Repair Limit the Function of Haematopoietic Stem Cells with Age. Nature 447(7145): 725–729. https://doi.org/10.1038/nature05862

Rossi DJ, Jamieson CH, Weissman IL (2008) Stems Cells and the Pathways to Aging and Cancer. Cell 132: 681–696. https://doi.org/10.1016/j.cell.2008.01.036

Saakyan SV, Myakoshina EB, Yurovskaya NN (2011) Tumor-Associated Distant Maculopathy Caused by Small Uveal Melanoma. Russian Ophthalmological Journal 4(2): 41–45.

Saakyan SV, Zharua AA, Myakoshina EB, Lazareva LA, Yakovlev SB (2013) Superselective Intra-Arterial Chemotherapy Treatment for Resistant and Severe Retinoblastoma. Russian Pediatric Ophthalmology 1: 31–34.

Saxena AK, Kumar A (2020) Effect of Cyclophosphamide On Chromosomes. In: Fish Analysis for Drug and Chemicals Mediated Cellular Toxicity. Springer, Singapore, 7–24. https://doi.org/10.1007/978-981-15-4700-3_2

Schneider EL, Sternberg H, Tice RR (1977) In Vivo Analysis of Cellular Replication. Proceedings of the National Academy of Sciences USA 74(5): 2041–2044. https://doi.org/10.1073/pnas.74.5.2041

Sinai MIT, Kerem B (2018) DNA Replication Stress Drives Fragile Site Instability. Mutation Research 808: 56–61. https://doi.org/10.1016/j.mrfmmm.2017.10.002

Sokova OI, Pogosiants HE (1974) Banding Pattern of the Djungarianhamster's Chromosomes. Tsitologiya 16(10): 1303–1305.

Stukalov SV (1988) Dynamics of the Frequencies of Sister Chromatid Exchange and Chromosome Aberrations in Vivo. Bulletin of Experimental Biology and Medicine 106(8): 220–221. https://doi.org/10.1007/BF00840391

Subarkhan MKM, Ren L, Xie B, Chen C, Wang Y, Wang H (2019) Novel Tetranuclear Ruthenium(II) Arene Complexes Showing Potent Cytotoxic and Antimetastatic Activity as Well as Low Toxicity in Vivo. European Journal of Medicinal Chemistry 179: 246–256. https://doi.org/10.1016/j.ejmech.2019.06.061

Thiotepa (2011) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 100 A. International Agency for Research Cancer, Lyon.

van der Wath RC, Wilson A, Laurenti E, Trumpp A, Liò P (2009) Estimating Dormant and Active Hematopoietic Stem Cell Kinetics through Extensive Modeling of Bromodeoxyuridine Label-Retaining Cell Dynamics. PLoS ONE 4(9): e6972. https://doi.org/10.1371/journal.pone.0006972

Wei ZZ, Qin QP, Meng T, Deng CX, Liang H, Chen ZF (2018) 5-Bromo-Oxoisoaporphine Platinum(II) Complexes Exhibit Tumor Cell Cytotoxcicity via Inhibition of Telomerase Activity and Disruption of C-Myc G-Quadruplex DNA and Mitochondrial Functions. European Journal of Medicinal Chemistry 145: 360–369. https://doi.org/10.1016/j.ejmech.2017.12.092

Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, Lió P, Macdonald HR, Trumpp A (2008) Hematopoietic Stem Cells Reversibly Switch from Dormancy to Self-Renewal during Homeostasis and Repair. Cell 135(6): 1118–1129. https://doi.org/10.1016/j.cell.2008.10.048