Journal of Pharmacy and Pharmacology 6 (2018) 1-9
doi: 10.17265/2328-2150/2018.01.001

Protective Effect of Taurine in the Induction of Genotoxicity by Mutagenic Drugs

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Abstract: Genotoxicity is a toxic action of several drugs, such as anti-neoplastic and nitro-related compounds. TAU (Taurine, 2-aminoetano sulfonic acid) is an important and potent antioxidant amino acid involved in the maintenance of homeostasis in many systems like leukocytes, skeletal muscle, the heart, the kidney, the liver, the bowel, the brain and the retina. The aim of this work was evaluated by the protective effect of TAU co-administered with mutagenic drugs such as benznidazole, cyclophosphamide, hydroxyurea, metronidazole, and nitrofurazone in an in vivo model using a micronucleus assay, evaluatation for the ability of different substances to promote DNA injury. The results showed that TAU decreased genotoxic activity of all drugs suggesting that the protective action of TAU supplementation can be beneficial to reduce genotoxicity of drugs. In this context, we propose one possible mechanism for how TAU blocks the chromosomal aberrations caused by the action of genotoxic substances.

Key words: Battery TAU, genotoxicity, micronucleus, protective effect.

1. Introduction

Several drugs in therapeutics and anticancer therapies can show serious adverse reactions, including death [1, 2]. Other compounds, such as nitrocompounds, are known to promote mutagenic activities [3]. Sometimes, these compounds are the only drugs available in the market, so the patients have no choice in pharmacotherapy. For example, this applies to BZN (benznidazole), and HU (hydroxyurea). Nitrocompounds, such as BZN, MTZ (metronidazole) and NF (nitrofurazone) present antimicrobial activity. They are related to present mutagenic and carcinogenic activity due to the formation of active N-derivatives that react with DNA [4-6].

HU is useful in the treatment of sickle cell disease by increasing fetal hemoglobin. It is an inhibitor of ribonucleotide reductase synthesis, which is the enzyme that converts ribonucleotides to deoxy-ribonucleotide, and thus it restricts the synthesis of DNA [7-9]. However, HU also has deleterious effects, promoting myelosuppression, pro-inflammatory activity, and in some cases, cancer development. Researchers showed that HU exhibited genotoxic potential in mammalian cells at all the concentrations tested [9, 10].

CP (cyclophosphamide) is a chemotherapeutic drug used in cancer treatment. Its mechanism induces DNA-DNA and DNA-protein crosslinks, and it has shown to promote chromosome damage, sister chromatid exchange, as well as other genotoxic effects. It is a widely used and well-documented genotoxic compound reference, and it is used as a positive control in micronucleus assay [11, 12] (Fig. 1).

TAU (Taurine, 2-aminoethanesulfonic acid) is a semi-essential amino acid involved in several physiological functions, including: trophic factor in the development of the central nervous system, maintaining the structural integrity of the membrane, anti-platelets, osmoregulation, regulation of transport and binding of calcium, antioxidation, and immunomodulation [13-16].

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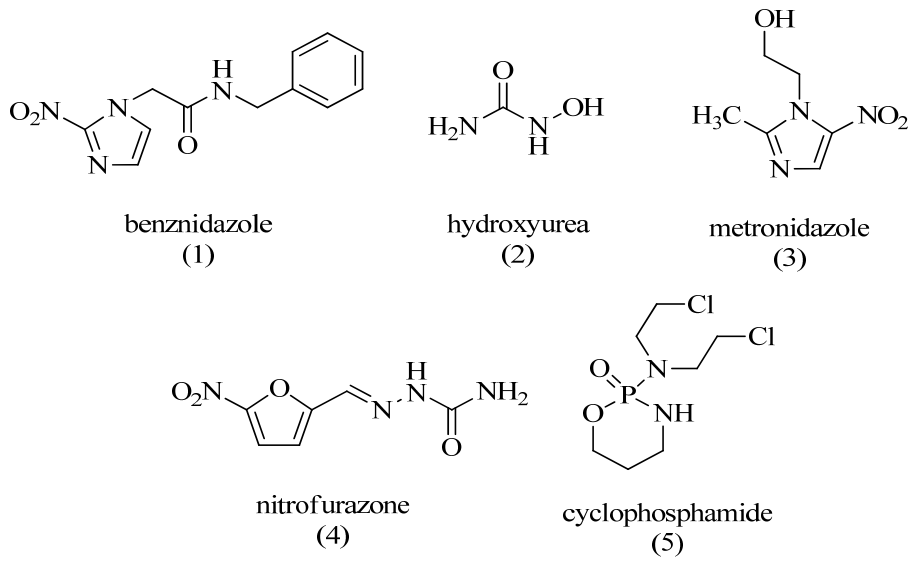

Fig. 1 Chemical structure of mutagenic drugs.

Reports showed that TAU could prevent DNA damage by diminishing production of ROS (reactive oxygen species) [17]. Several other publications reported the protective action of TAU to toxic drugs such as CCl_4 (carbon tetrachloride), anticancer drugs such as DOX (doxorrubicin) and cisplatin, and pesticides such as permethrin [18, 19].

TAU preserves the morphology of the hepatocytes and delays the development of fibrosis action of CCl_4 by its antioxidative action [20]. It showed biochemical modulator activity by inhibiting DOX efflux, decreasing tumor weight by 40% compared to DOX-alone, neither increasing DOX concentration in normal tissue nor increasing adverse effects [21]. Ito and co-workers (2009) [22] showed the effect of benefits of TAU treatment against cardiotoxicity caused by DOX. They also showed that it prevents DOX-induced testicular abnormalities, proving it to be an effective cytoprotectant nutrient. Cancer treatment with cisplatin can cause kidney injury that is reversed by TAU [18].

In the present work, we evaluated the protective effect of TAU co-administered with BZN, CP, HU, and MTZ by the micronucleus assay in the peripheral blood of mice.

2. Method and Materials

2.1 Drugs

Benznidazole (BNZ, CAS No. 22994-85-0), cyclophosphamide (CP, CAS No. 6055-19-2), hydroxyurea (HU, CAS No. 127-07-1), metronidazole (MTZ, CAS No. 443-48-1), nitrofurazone (NF, CAS No. 59-87-0), TAU (TAU, CAS No. 107-35-7) carboxymethylcellulose (CMC, CAS No. 9066-07-3), tween-20 (CAS No. 9005-64-5), and acridine orange (CAS No. 65-61-2) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Animals

Swiss albino mice (25-30 g) were housed at a constant temperature (23 ± 1.8 °C) and humidity (55 ± 5%), under a 12/12 h light cycle, with food and water ad libitum. The experiments were conducted during the light phase. The study and all the procedures were approved by the Research Ethics Committee Animal Experimentation of the School of Pharmaceutical Science, UNESP, Araraquara, São Paulo, Brazil.

2.3 Experimental Protocol and Groups

The animals were divided into groups of 10 (5 males and 5 females) and each group received

different concentrations (therapeutic dose) of drugs BZN (100 mg/Kg), CP (50 mg/Kg), HU (100 mg/Kg), MTZ (250 mg/Kg), NF (100 mg/Kg) and, a group received the TAU (37.5 mg/kg), which were administered concomitantly with 300 μM (37.5 mg/kg) of TAU by gavage, except for CP that was administered intraperitoneally (i.p.). In addition, five groups of animals were pretreated with the TAU (37.5 mg/kg) during 15 days, once a day. After the pretreatment, the animals received the same concentrations of the drugs BNZ, CP, HU, NF, and MTZ. The positive control group was treated with CP (50 mg/Kg, i.p.), the negative control group was treated with carboxymethylcellulose (1%) and tween (0.2%, vehicle used to solubilize the drugs), and the white control group received only water. The collect peripheral blood for micronucleus assay, followed by euthanasia of mice, was performed 30 hours after single administration of the drugs. (Fig. 2). The protocol adopted for this work was described by Hayashi et al. [17] which employs pre-stained laminas by acridine orange.

2.4 Evaluation of the Mutagenicity by Micronucleus Test in Peripheral Blood Cells of Mice

After 30 hours of the drugs administration, the blood was collected. We counted 1,000 reticulocytes per animal and recorded the frequencies of micronucleated cells. After the cytological analysis of the laminas containing samples of peripheral blood of mice treated with the drugs, the average cell micronuclei frequency was calculated, as well as the standard deviations for each treatment group.

2.5 Statistical Analysis

From these results, a test of analysis of variance (ANOVA) was applied where $p < 0.05$. The averages collected from treatments were compared using the Tukey method, which calculated the minimum significant difference for $\alpha = 0.05$.

3. Results

The results showed that all drugs BNZ (100 mg/kg), CP (50 mg/kg), HU (100 mg/kg), MTZ (250 mg/kg), and NF (100 mg/kg) induced a significant increase in the formation of micronucleated reticulocytes in the concentrations given. When these drugs were co-administered with the same concentration of 300 μM TAU, there was a decrease in the incidence of micronucleus formation in these animals. The incidence of micronucleus was even lower in animals that were pretreated for 15 days with TAU (PreTau) (Table 1).

The Fig. 3 shows the data obtained from the micronucleus formation in mice exposed to control groups, BNZ, CP, HU, MTZ, and NF, respectively. As

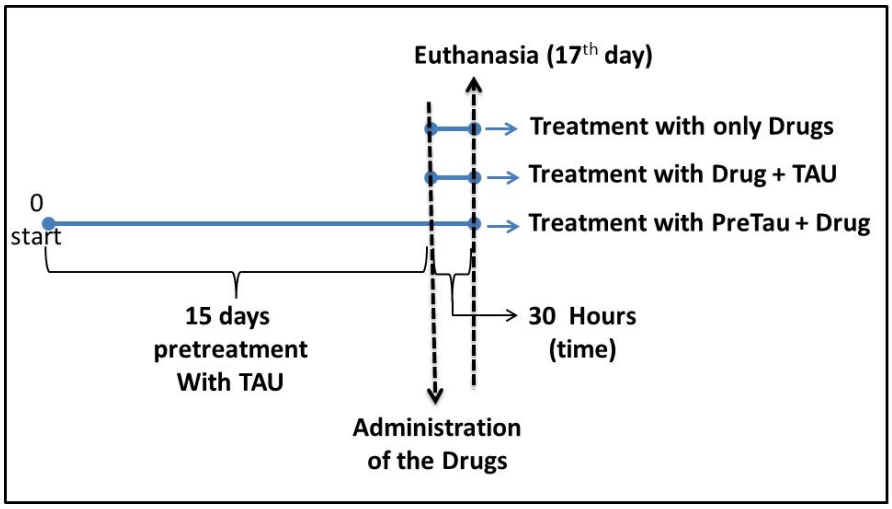

Fig. 2 Experimental protocol.

Table 1 Frequency of micronucleated reticulocytes (MNRET) of 1,000 cells from mice.

	Treatment with only drugs	Treatment with drug + TAU	Treatment with PreTau + drug
TAU	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6
Water*	0.7 ± 0.7	0.7 ± 0.7	0.7 ± 0.7
Negative control**	0.8 ± 0.7	0.8 ± 0.7	0.8 ± 0.7
CP***	47 ± 14.2	34 ± 5.7	18.6 ± 2.8
BNZ	4.8 ± 2.2	2.4 ± 0.9	2.4 ± 1.9
HU	33.7 ± 10.7	20.7 ± 4.7	4.2 ± 4.4
MTZ	4.1 ± 1.1	2.3 ± 1.3	1.4 ± 0.5
NF	4.3 ± 1.9	3.2 ± 1.64	2.1 ± 1.1

CP: Cyclophosphamide; BNZ: benznidazole; HU: hydroxyurea; MTZ: metronidazole; NF: nitrofurazone. TAU: taurine. PreTau, Pretreatment for 15 days with TAU. * White control; ** CMC 1%/Tween 0.2%;*** Positive control.

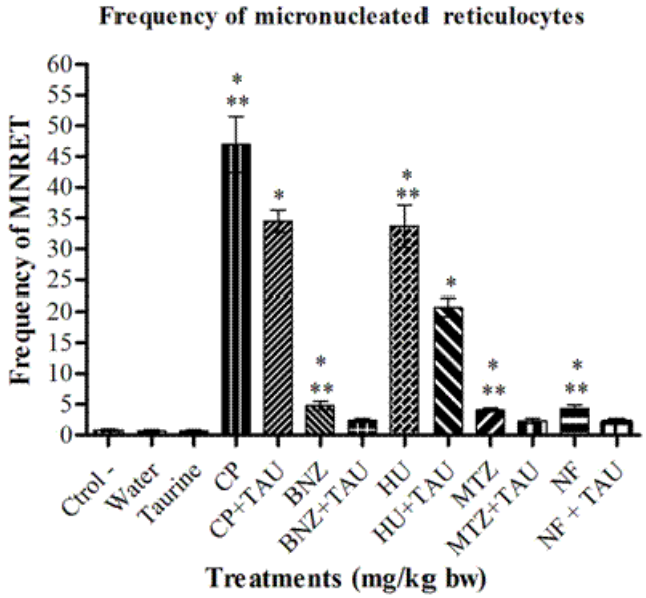

Fig. 3 Average frequency of micronucleated reticulocytes (MNRET) and standard deviation of 1,000 cells from mice treated with TAU (300 µM). CP: Cyclophosphamide; BNZ: benznidazole; HU: hydroxyurea; MTZ: metronidazole; NF: nitrofurazone; TAU: TAU. *p < 0.05 (compared to control negative, water, and TAU); **p < 0.05 (compared to DRUG + TAU).

was expected, the results corroborate the literature data demonstrating the genotoxic effects of these drugs, expressed by the increased incidence of the micronucleus when compared to negative control and water. The results show that all drugs induced an increased frequency of micronucleus in reticulocytes of mice at an administered concentration. CP was considered the positive control (47 ± 14.2); HU showed the highest mutagenic alteration when compared to other studied drugs (BNZ, MTZ, NF). The nitrocompounds showed similar mutagenic activities. After the co-administration of only one dose of TAU, all compounds, including the positive control, showed a decrease in the incidence of micronucleus formation.

The PreTau treatment was able to decrease more than 50% of micronucleated cells in all treated groups (MTZ: < 66%, NF: < 51%, HU: < 88%, CP: < 61%). The concomitant treatment (drug + TAU) also showed a decrease. However, this was lower when compared to pretreatment (MTZ: < 44%, NF: < 26%, HU: < 39%, CP: < 28%). In concomitant treatments (BZN +

TAU) and pre-treatments (PreTau + BZN), a 50% decrease of micronucleated cells was recorded, respectively.

After PreTau, a significant reduction in the number of cells was observed (PreTau + Drug) (Fig. 4). This highlights the pretreatment with TAU for the groups PreTau/CP and PreTau/HU, with a decrease of 45.3% and 79.71 %, respectively, when compared with these drugs co-administered after one day of TAU treatment and to the control.

4. Discussion

Among the mutagenicity evaluation tests recommended by international agencies and government institutions, the in vivo rodent peripheral blood micronucleus test is widely accepted and recommended for the evaluation and registration of new chemical and pharmaceutical products that enter the world market annually [23, 24].

The micronucleus test allows identifying an increase in mutation frequency in cells that are exposed to a varied range of genotoxic agents [24-27]. As expected, the results showed that the frequency of micronuclei in the groups of mice that were exposed to the action of the genotoxic agent CP was significantly higher in comparison to the negative control group and water [24].

Currently, HU is the main drug available for the treatment of sickle cell anemia that is authorized by the U.S. Food and Drug Administration. HU is a selective inhibitor of ribonucleotide diphosphate reductase, an enzyme that converts ribonucleotides to deoxy-ribonucleotide diphosphates, preventing cells from leaving the G1/S phase of the cell cycle [28]. The beneficial effects of HU in the treatment of SCD are associated, in part, with its ability to be bioconverted in vivo to NO (nitric oxide). NO contributes significantly to vaso-occlusive disorders, reducing the expression of adhesion molecules and inhibiting platelet aggregation, thus maintaining the vascular homeostatic balance [29].

Compounds with NO generation capacity could generate cell death and DNA damage, resulting in cytotoxic and genotoxic effects, which are associated

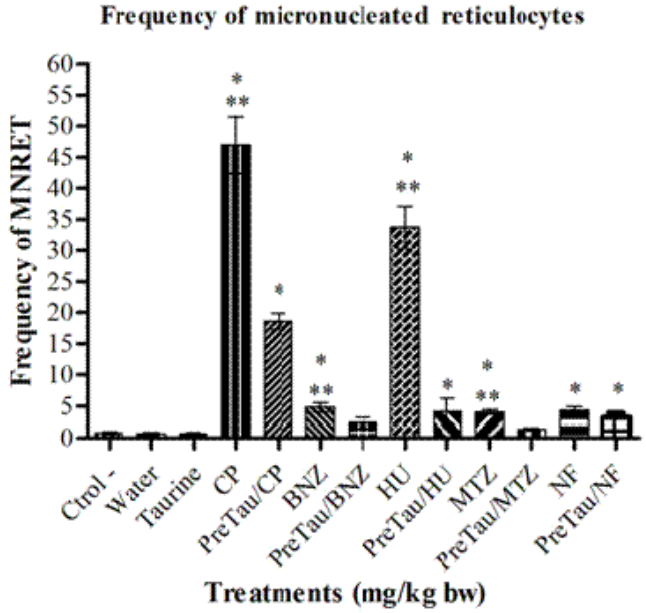

Fig. 4 Average frequency of micronucleated reticulocytes (MNRET) and standard deviation of 1,000 cells from mice pretreated (PreTau) 15 days with TAU (300 µM). CP: Cyclophosphamide; BNZ: benznidazole; HU: hydroxyurea; MTZ: metronidazole; NF: nitrofurazone; TAU： TAU. *p < 0.05 (compared to control negative, water, and TAU); **p < 0.05 (compared to PreTau/drug).

with carcinogenicity. In addition, it is reported that NO can induce chromosomal mutations, and therefore, be mutagenic in a variety of cells, from bacteria, mammalian cells, to in vivo models [30]. NO can react and alter DNA by different pathways, especially those involving the formation of possible reactive species such as $ONOO^-$ (peroxynitrite), through the reaction of NO with superoxide, causing oxidative damages [31].

Another study related the genotoxicity of HU to its capacity to indirectly generate hydrogen peroxide, probably by the inhibition of catalase-mediated hydrogen peroxide decomposition [32]. Flanagan and co-workers (2010) [33] demonstrated that HU had a detectable genotoxic effect on micronucleus production in humans.

According to our experimental data, TAU may be related to the blockade of chromosomals. This may be caused by the action of aneugenic substances, generating aneuploidy (chromosomal non-separation) or the action of clastogenic substances that promote chromosomal breaks, terminal or interstitial, during the cell division. The Fig. 5 shows the probable mechanism.

The results showed the protective action of TAU. It's a potent antioxidant action most likely act as a scavenger for oxidative reactions of these drugs, promoting the decrease of toxicity.

The molecular mechanism of genotoxicity of nitrocompounds is well established. The DNA damaging effect of nitro compounds is promoted by the difference in nitro bioreduction potential that allows the generation of free radicals in intracellular environments with a low-oxygen concentration. The generation of radicals can break the structure of the DNA double helix [28]. The Fig. 6 shows the mechanism of DNA damage by nitrocompounds. According to the mechanism, we suggested one possible mechanism for how TAU blocks the oxidative compounds formed during the metabolism of nitro agents, suggesting that the antioxidant effect of TAU may reduce the genotoxicity of these drugs.

Our results are in agreement with Alam and co-workers (2011) who evaluated the protective effect

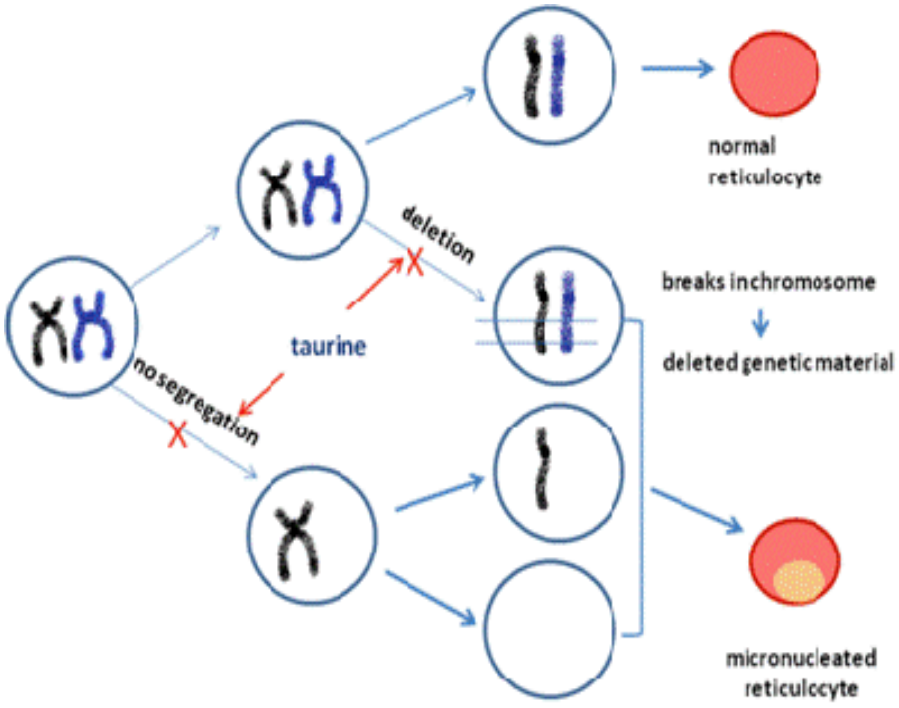

Fig. 5 Mechanism of action of TAU in the process of cell division.

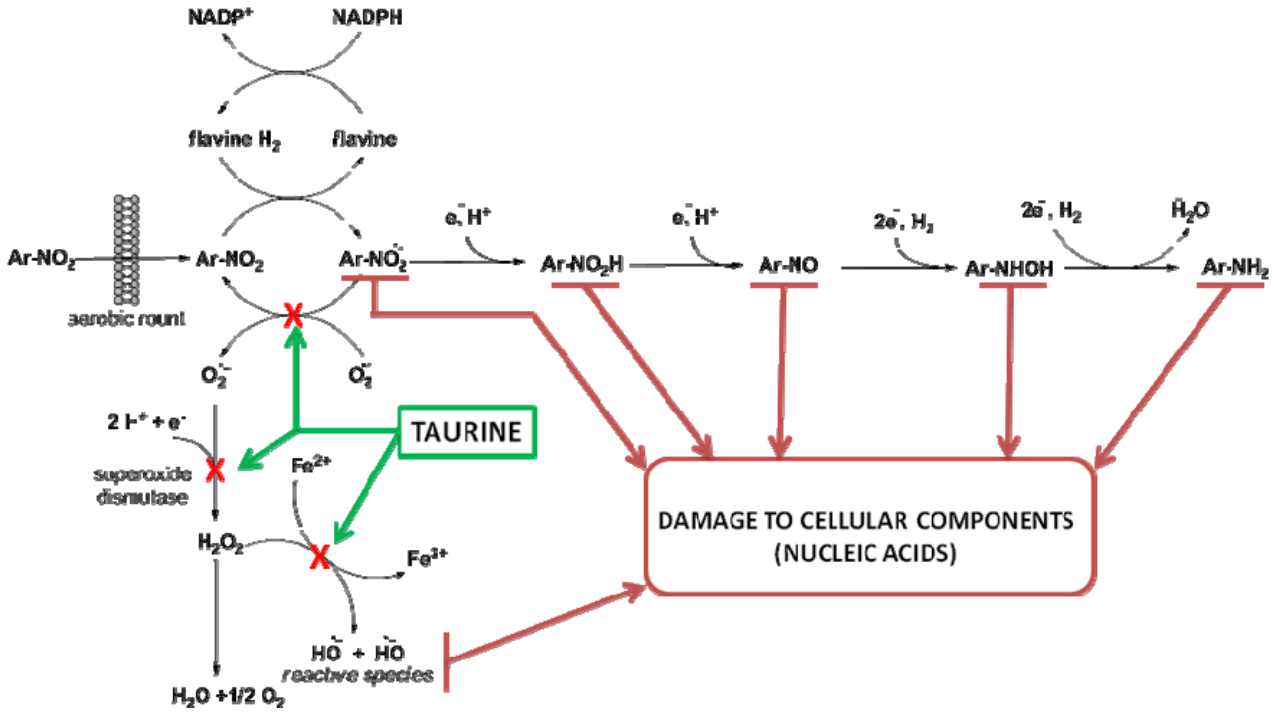

Fig. 6 Mechanism of DNA damage for nitrocompounds and possible TAU actions.

of TAU against genotoxic damage in mice treated with MTX (methotrexate) and TAM (tamoxfine). The authors [34] reported significant increment in the levels of GSH content, reduction in DNA fragmentation, and ladder formation in hepatic tissue, suggesting the antioxidant activity of TAU may reduce the toxic effects of MTX and TAM.

In another study in women with breast cancer, there was a decrease in the serum level of TAU, which may be the result of oxidative stress, indicating the beginning of transformation into malignancy of the breast tissue. In this case, the authors concluded that TAU did not act as an antitumor, but as an antioxidant, an antiangiogenic, and as an apoptotic factor, suggesting that TAU can be dosed in the serum of patients who have a high risk of developing breast cancer. This may be an early form of diagnosis and could act as a biomarker. It was carried out in women with endometrial cancer, also suggesting the dosage of TAU as an early biomarker in the precancerous condition of endometrial cancer [35].

TAU may also be considered as an adjuvant in chemotherapy. Wang and co-workers [36] observed that administration of TAU in mice with lung carcinoma increased not only the number, but the function of leukocytes after chemotherapy with CP, attenuating myelosuppression and immunosuppression in these animals and, consequently, increasing the role of CP in inhibiting tumors.

TAU was associated with agents capable of suppressing tumors, such as cisplatin and curcumin. Kim and Kim (2013) verified the anticancer effects of TAU therapy and TAU combined with cisplatin in human cervical cancer cells. TAU, alone, decreased cell proliferation (time and dose dependent). During co-treatment with TAU-cisplatin, the decrease in cell proliferation was more pronounced, compared to when only with cisplatin. The reduction of cell proliferation was caused by the induction of apoptosis by expression of p53 and activation of caspase -3, -6, -7, and -9. Thus, TAU-cisplatin co-treatment is more effective than cisplatin alone [37].

5. Conclusions

The results of this study show the chromosome protection by TAU against the mutagenic action of

drugs such as HU, BZN, MTZ, NF and, CP. Additionally, with the literature data, this strongly suggests that the supplementation of TAU is beneficial in drug treatments, mainly with chemotherapy and or oxidant compounds. Therefore, we recommend TAU co-administered as a DNA protective compound for the continuation of research pre-clinical studies.

Acknowledgments

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo.

Conflict of Interest

The authors declare no conflict of interest.

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