THE EFFECTS OF TAURINE ON PERMETHRIN-INDUCED CYTOGENETIC AND OXIDATIVE DAMAGE IN CULTURED HUMAN LYMPHOCYTES

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Permethrin (PM) is a common pyrethroid pesticide used to control pests in agriculture, forestry, horticulture, health care, homes, and textile industry. It is confirmed as a strong mutagen in animals and humans. Taurine (TA) is an amino acid found in mammalian tissues that protects the cell against DNA damage. In this study, we investigated whether supplementation of human lymphocyte cultures with TA (in the concentrations of 25 μg mL⁻¹, 50 μg mL⁻¹ and 100 μg mL⁻¹) provided any protection against PM toxicity applied in the concentration of 200 μg mL⁻¹. Genotoxicity was assessed using the micronucleus (MN) and sister chromatid exchanges (SCE) tests. In addition, we measured the total antioxidant capacity (TAC) and total oxidative stress (TOS) levels in the plasma to determine oxidative effects. PM increased SCE and MN levels and altered TAC and TOS levels. TA alone did not affect SCE and MN levels compared to controls, regardless of the concentration applied. In addition, it increased TAC levels without changing TOS levels. Moreover, it significantly buffered the negative cytogenetic and oxidative effects induced by PM in a clear dose-dependent manner. In conclusion, this study is the first to evidence the beneficial effects of TA against PM-induced DNA and oxidative damages in vitro.

KEY WORDS: antioxidant, micronucleus, pesticide, sister chromatid exchanges

Because of widespread use it is important to keep (re)evaluating the genotoxic properties of pesticides (1, 2). A number of studies (3-6) has suggested that pesticides such as DDT, cypermethrin, and lambda-cyhalothrin produce oxidative stress by generating free radicals and by inducing tissue lipid peroxidation (LPO) in mammals and other organisms. Permethrin (PM), the most popular insecticide among the synthetic pyrethroids, has been used worldwide to control a wide range of insects in agriculture, forestry, public health, and homes (7). Mammalian and non-mammalian bioassays and toxicology studies have confirmed its carcinogenic potential (8, 9). PM exposure leads to DNA damage in humans and experimental animals (1, 10, 11). It induces oxidative damage to purine bases in the heart cells (12) and produces single- and double-strand breaks in striatum cells (13). In addition, PM can cause a genotoxic response through oxidative stress (14). Much of the current research is focused on finding substances capable of countering the genotoxicity of man-made or natural mutagens. These include various vitamins, propolis, sulphhydryl compounds, and plant products (15-19). Taurine (TA) (2-aminoethanesulfonic acid) is found in high concentrations in various mammalian tissues (20). Sung et al. (21) reported that...
TA suppressed oxidant-induced tissue injuries by stabilising the biomembrane and by scavenging free radicals (21). This has been supported by other studies that associate its protective antioxidant properties with direct scavenging of free radicals and indirect preserving of membrane permeability (22, 23). TA has therefore become a promising option to treat chemically induced illnesses (14, 24-27). Recent investigations have shown that TA protects cells from the toxic effects of cadmium (Cd), paracetamol, and arsenic (As) that are mediated by free-radical attack (28-31).

This is why we assumed that TA could provide protection against PM-induced genotoxic and oxidative damages. To test this assumption, we treated human lymphocytes with PM and with TA and determined the frequencies of sister chromatid exchanges (SCEs) and micronuclei (MNs), as both assays are widely used in genotoxicity testing and biomonitoring (32, 33). In addition, we wanted to see how treatment with PM and TA affected the total antioxidant capacity (TAC) and total oxidative stress (TOS) levels in the plasma.

MATERIAL AND METHODS

Blood sampling

Blood was sampled by venipuncture from three healthy non-smoking women aged 22 to 25 years. Blood sampling has been approved by the institutional ethics committee and has been performed according to the Declaration of Helsinki.

Experimental design

Taurine (TA; C₂H₇NO₃S; CAS No. 107-35-7; Sigma-Aldrich® Chem. Co. St. Louis, MO, USA) was dissolved in distilled water and tested in the concentrations of 25 μg mL⁻¹, 50 μg mL⁻¹, and 100 μg mL⁻¹. Permethrin (PM; C₂₁H₂₀Cl₂O₃; CAS No. 52645-53-1; Riedel-de Haen®, Seelze-Hannover, Germany) was dissolved in a mixture of 95 % ethanol and distilled water and tested in the concentration of 200 μg mL⁻¹. These concentrations are based on earlier reports; specifically by Undeger and Basaran (1), who have evidenced the mutagenic effects of PM at 200 μg mL⁻¹ while Chesney (34) evidenced the protective effects of TA in the concentrations of 25 μg mL⁻¹, 50 μg mL⁻¹, and 100 μg mL⁻¹. The compounds were added to the lymphocyte cultures just before incubation for cytogenetic analysis. Cultures were incubated for 72 h at 37 °C according to standard protocols for SCE and MN tests. The blood used for determining TAC and TOS levels was incubated with the tested compounds for 2 h at 37 °C. Negative control samples were treated with distilled water only. Positive control samples were treated with ascorbic acid (C₆H₈O₆; CAS No. 50-81-7, Sigma®; 10 μmol L⁻¹) in the TAC analysis and with hydrogen peroxide (H₂O₂; CAS No. 8007-30-5, Sigma®; 25 μmol L⁻¹) in the TOS analysis.

SCE assay

We slightly modified the protocol proposed by Evans and O’Riordan (35). Peripheral blood lymphocyte cultures supplemented with 5-bromo-2′-deoxyuridine (Sigma®) were incubated at 37 °C in complete darkness for 72 h. Microscope slides were prepared in triplicate using the standard procedure, air-dried, and stained according to fluorescence plus Giemsa (FPG) method (36). Scorings of SCE were performed under a light microscope on coded slides. To determine the number of SCEs per cell at least, 25 well-spread, second-division metaphases were analysed as described in previous studies (37-39). To ensure the same scoring criteria (40), all microscopy was performed by a single well-trained observer.

MN assay

We followed the protocol proposed by Fenech and Morley (41). Cytochalasin B (Sigma®) was added on hour 44 of cultivation. We used duplicate cultures for each concentration. After incubation, the lymphocytes were fixed and placed directly on slides using a cytospin. Air-dried slides were stained with May Grünwald-Giemsa, coded, and analysed under a light microscope applying the criteria reported by Fenech (42). We examined at least 2000 binucleated lymphocytes per concentration for the presence of one, two, or more micronuclei. Here too the slides were analysed in triplicate.

TAC and TOS analysis

Plasma samples, obtained from the lymphocyte cultures 2 h after incubation with PM and TA, were analysed using commercial kits (Rel Assay Diagnostics®, Gaziantep, Turkey) for automated Trolox-equivalent total antioxidant capacity (TAC) assay and the total oxidant status (TOS) assay (43-46).
The major advantage of the TAC assay is that it measures the antioxidant capacity of all antioxidants in a biological sample and not just of a single compound (43). In this test, antioxidants in the sample reduce dark blue-green coloured 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate (ABTS) radical to its colourless form. The change in absorbance at 660 nm corresponds to the total antioxidant level in a sample. The assay is calibrated with a stable antioxidant standard solution of vitamin E analog, (Trolox-equivalent) (44).

The TOS assay used here is based on the oxidation of the ferrous ion-chelator complex to ferric ion (Fe³⁺), which is mediated by oxidants contained in the tested sample. The reaction is further enhanced by other molecules from the reaction medium. The reaction of Fe³⁺ with chromogen in an acidic medium produces a coloured complex. Its intensity corresponds to the total amount of oxidants in the sample and can be measured spectrophotometrically. The TOS assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (45).

**Statistics**

Statistical analysis was performed using the SPSS software (version 12.0, SPSS, Chicago, IL, USA). The results are expressed as mean ± standard deviation (S.D.). Groups were compared by one-way analysis of variance followed by Duncan’s multiple range test. The level of significance was set at P<0.05.

**RESULTS**

Table 1 shows the effects of TA and PM on oxidant status in lymphocyte cultures that were determined by TAC and TOS analysis. Total antioxidant capacity decreased with the addition of PM. In the positive control (10 μmol L⁻¹ of ascorbic acid) it was twice as high as in the negative control. Similarly, TA caused a significant and dose-dependent increase in TAC compared to negative control. In addition, TA showed dose-dependent inhibitory effects on the oxidative damage caused in lymphocytes by PM. On the other hand, the positive control showed about a threefold increase in TOS compared to the negative control. PM also increased the TOS level, but TA did not.

Figures 1 and 2 show the anti-genotoxic effects of lymphocyte treatment with TA alone and in combination with PM. Permethrin increased SCE frequency compared to the negative control, while TA alone (in the concentrations of 25 μg mL⁻¹, 50 μg mL⁻¹, and 100 μg mL⁻¹) did not change it. When given with PM, it significantly reduced SCE frequency compared to lymphocytes treated with PM alone (Figure 1).

Similar effects were observed with MN frequency. It significantly (p<0.05) increased in lymphocytes treated with PM alone and was unaffected by TA at any of the tested concentrations. In the cells treated with PM and TA together, TA decreased MN frequency compared to the negative control (Figure 2).

**DISCUSSION**

Pyrethroid pesticides have recently been reported to cause apoptosis (47), disrupt the endocrine system (48), change nitrogen metabolism (49), increase mitochondrial membrane permeability (50), and alter constitutive nitric oxide release (51). Higher SCE and MN frequencies and higher TOS plasma levels in our

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TAC (Trolox Equiv. / mmol L⁻¹)</th>
<th>TOS (H₂O₂ Equiv. / μmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>6.5±0.7ₜ</td>
<td>11.5±3.2ₛ</td>
</tr>
<tr>
<td>Positive control</td>
<td>13.71±0.9₄ₙ</td>
<td>39.25±4.6₃₁</td>
</tr>
<tr>
<td>PM</td>
<td>4.4±0.7ₚ</td>
<td>17.2±4.5ₚ</td>
</tr>
<tr>
<td>TA1</td>
<td>7.6±0.8ₚ</td>
<td>11.0±3.9ₚ</td>
</tr>
<tr>
<td>TA2</td>
<td>8.1±0.9ₜₕ</td>
<td>11.6±4.7ₜₕ</td>
</tr>
<tr>
<td>TA3</td>
<td>8.8±1.2ₚₕ</td>
<td>11.6±4.4ₚₕ</td>
</tr>
<tr>
<td>PM + TA1</td>
<td>4.8±0.7ₜₔ</td>
<td>15.4±5.1ₚₔ</td>
</tr>
<tr>
<td>PM + TA2</td>
<td>5.4±0.8ₚₔₕ</td>
<td>14.2±5.3ₚₔₕ</td>
</tr>
<tr>
<td>PM + TA3</td>
<td>5.9±0.9ₙₔₕ</td>
<td>12.1±4.4ₙₔₕ</td>
</tr>
</tbody>
</table>

PM: 200 μg mL⁻¹; TA1: 25 μg mL⁻¹; TA2: 50 μg mL⁻¹; TA3: 100 μg mL⁻¹
study confirm the genotoxic and oxidative action of PM in human lymphocytes. Similarly, earlier studies (1, 52, 53) reported that PM induced MN formation and chromosomal aberrations in human peripheral blood lymphocytes and caused nuclear DNA damage in rats (13). In an experiment with rat adrenal pheochromocytoma (PC12) cells (54), it increased the production of reactive oxygen species (ROS) and malondialdehyde (MDA), which is known as a marker of lipid peroxidation (LPO). At the same time, the authors observed lower antioxidative activity of superoxide dismutase, catalase, and glutathione).

Free radicals damage the cell by, for example, irreversibly damaging lipids in cell membranes and disturbing the structure of proteins. Free radical derivatives damage both the purine-pyrimidine bases and the deoxyribose skeleton in the DNA. The most harmful oxidative lesion of the DNA is 8-hydroxyguanine (8-OH-G), which is involved in mutagenesis, carcinogenesis, and aging (55-60).

The increase in SCE and MN frequency observed in our study could be attributed to the pro-oxidative effects of PM. This is supported by decreased TAC and increased TOS levels in PM-treated cells. Recent studies (12, 13) also associate oxidative stress induced by PM with DNA damage. Moreover, it is known that toxic oxygen metabolites produced by normal human white blood cells cause the formation...
of SCEs and MNs in cultured mammalian cells (61-63). The production of toxic oxygen metabolites in lymphocytes could be set off by a variety of chemicals, including PM.

The results of our study also suggest that TA plays an important protective role. Two hypotheses could explain the modulating effect of TA against PM-induced genotoxicity. The first is that TA acts as an antioxidant in a dose-dependent manner (24). Through antioxidative pathways it seems to modify both target and receptor cell homeostasis (64-66). Plasma and extracellular fluid TA concentrations typically range from 10 μmol L⁻¹ to 100 μmol L⁻¹ (67). Although this amino acid is not incorporated into proteins, TA intracellular concentrations can reach up to 80 mmol L⁻¹, depending on the tissue type (68). Adding TA does not make it toxic in cells where it is already present in high concentration. Moreover, supplementation with TA (150 mg kg⁻¹ or ~1.87 g L⁻¹) in a recent study (20) prevented oxidative stress induced by doxorubicin. However, concentrations this high may lead to saturation. Another evidence in favour of the antioxidative action of TA is that the activities of SOD, CAT, and glutathione peroxidase (GSH-Px) normalise in TA-supplemented cultures, and this normalisation reduces LPO levels (69). The second hypothesis is that TA has a cytoprotective role. It is based on the study by Eppler and Dawson (70), who suggest that TA stabilises membrane receptor proteins, reduces protein carbonyl formation, and inhibits oxidative damage to DNA. Both hypotheses have been supported by Cozzi et al. (71), who found that TA protected the DNA by scavenging ROS. Similarly, Du et al. (72) found that TA supplementation had protective effects in high-fat diet-induced obese rats due to its antioxidant nature. In addition, TA showed anti-mutagenic properties in the Salmonella Ames tester strain assay and against aluminium sulphate (73, 74).

CONCLUSION

We have demonstrated for the first time that TA protects human lymphocytes against PM-induced toxicity. TA significantly attenuated DNA damage related to the overproduction of ROS in PM-treated lymphocytes. Supplementation with TA may eventually be useful in individuals who are exposed to synthetic pyrethroids, but the mechanisms of its beneficial effects remain to be elucidated by future studies.

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Sažetak

DJELOVANJE TAURINA PROTIV CITOGENETIČKOG I OXSIDATIVNOG OŠTEĆENJA U KULTURAMA LJUDSKIH LIMFOCITA UZROKOV ANIH PERMETRINOM

Permetrin je piretroidni pesticid koji se često rabi za suzbijanje nametnika u poljoprivredi, šumarstvu, povrtlarstvu, zdravstvenoj zaštiti, domovima i tekstilnoj industriji. Poznat je kao snažan mutagen u životinja i ljudi. Taurin je aminokiselina koja se nalazi u tkivu sisavaca i štiti stanicu od oštećenja DNA. Svrha je ovog istraživanja bila saznati hoće li taurin (u koncentracijama od 25 μg mL⁻¹, 50 μg mL⁻¹ te 100 μg mL⁻¹) zaštiti ljudske limfocite od toksičnoga djelovanja permetrina koji je dodan kulturama u koncentraciji od 200 μg mL⁻¹. Genotoksično djelovanje ocijenili smo s pomoću mikronukleus (MN)-testa i testa izmjena sestrinskih kromatida (engl. sister chromatid exchanges, krat. SCE). Da utvrdimo oksidativno djelovanje, izmjerili smo ukupni antioksidativni kapacitet (engl. total antioxidant capacity, krat. TAC) i ukupni oksidativni stres (engl. total oxidative stress, krat. TOS) u plazmi. Permetrin je povećao učestalost SCE i MN te promijenio TAC i TOS. Sam taurin, bez obzira na koncentraciju, nije utjecao na učestalost SCE i MN u odnosu prema kontroli. Usto je podigao TAC, a da pritom nije utjecao na TOS. Štoviše, značajno je ublažio štetno citogenetičko i oksidativno djelovanje permetrina, a učinkovitost mu je bila izravno povezana s primijenjenom koncentracijom. Ovo je prvo in vitro istraživanje koje je pokazalo povoljno djelovanje taurina protiv oksidacijskoga djelovanja permetrina i oštećenja DNA koje on uzrokuje.

KLJUČNE RIJEČI: antioksidans, izmjene sestrinskih kromatida, mikronukleus-test, pesticidi, TAC, TOS

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