Oxidative Stress and Pharmacokinetics of Pendimethalin in Female Rats

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Abstract

The propensity of a dinitroaniline pre-emergence herbicide, pendimethalin, to induce oxidative stress, changes in biochemical parameters, pharmacokinetics, tissue distribution and excretion in serum, kidney, liver and brain of female rats following four oral doses of 109.4 mg/kg b.wt every other day were studied. When rats exposed to pendimethalin, significant increases in tissue malondialdehyde, lactic dehydrogenase and alkaline phosphatase levels (p<0.05) were noticed compared to controls. The activities of catalase in all the tested tissues except brain were significantly increased (p<0.05) after pendimethalin treatment. Pharmacokinetic studies illustrated that when rats were treated with a single dose of 109.4 mg/kg b.wt of pendimethalin, the percentages of pendimethalin excretion in the urine and faeces were 8.72 and 14.31 after 24 h, whereas the peak concentration (245.92 ng/g) reached at 24 h after administration. The Peak concentrations of pendimethalin were reached at 12 h in serum (3229.14 ng/mL), liver (10162.32 ng/g) and kidneys (1969.17 ng/g), whereas the peak concentration (245.92 ng/g) reached at 24 h after dosing. In all the tested tissues the compound declined in all tissues as time passed after 72 h in serum or after 120 h in liver, kidneys and brain following administration. The disappearance of pendimethalin was found to be biexponentially from the tested tissues with half-life) t½ (of 14.0, 15.0, 2.5, and 29.0 h for the serum, liver, brain and kidneys, respectively. It can be concluded that when female rats are exposed to pendimethalin, it is readily absorbed and subsequently distributed throughout the whole body influencing on the biomarkers oxidative stress and enzyme activities.

Keywords: Pendimethalin; Rat; Oxidative stress; Pharmacokinetics; Biomarkers

Introduction

Pesticides are ubiquitous in the environment and have significant economic, environmental and public health impacts. Their usage helps to improve human nutrition through greater availability, longer storage life and lower costs of foods. However, the indiscrimination of its use has aroused a great concern among the environmental and health scientists due to their adverse effects in both targets as well as non-target species.

Agricultural workers and their families as well as general population may expose to pesticides through the application of pesticides or via their residues in food [1,2]. Due to the hydrophobic nature of pesticides, it may largely accumulate in the biological membrane, especially in the phospholipid bilayers [3] and in lipid-rich internal tissues, including body fat, skin, liver, kidney, ovaries and nervous system [4,5].

Biological systems are continually exposed to oxidative stress, which is a causative factor in the etiology of several human degenerative diseases, as well as the aging process [6,7] due to a disruption in redox signaling and oxidative stress control [8]. Many pesticides can produce toxic effects in biological systems of experimental animals through their mode of action and/or by producing free radicals in organs, resulting in cell damage [9,10]. These free radicals are reactive as biological products [11] and produce oxidative stress causing pathological lesions in neurons [12]. These damages affect membrane fluidity, protein, lipid, DNA and functions of platelet [13-15], as well as induce apoptosis [16] which linked with many chronic diseases such as cancers, inflammation, aging and atherosclerosis. Lipid peroxidation [17], enhancement of lactate dehydrogenase (LDH) release, inhibition of superoxide dismutase and catalase [18] are biomarkers of cellular and membrane damage.

Many studies were conducted to examine the pharmacokinetic profile of pesticides in rat [19-23] and mouse [19,24]. The absorption, distribution, metabolism, and elimination of many pesticides were studied to calculate oral equivalent doses (or internal circulating/target organ concentrations) as well as the magnitude and duration of toxicity [25,26].

Pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) is a pre-emergence herbicide used for control of most annual grasses and many annual broad-leaved weeds [27]. It is classified as a 'slightly toxic' compound (toxicity class III) and a possible human carcinogen (group C) by the US Environmental Protection Agency (EPA), with acute oral LD50 values for male and female rats equal to 1250 mg/kg and 1050 mg/kg, respectively [27]. Presently, it is the third largest herbicide and the largest selective herbicide in the world and has been classified as a possible human carcinogen [28]. The toxicological studies are necessary for assessing the sensitivity of animals and their organs to specific toxicants, and for assessing the degree of disorders. Concerning pendimethalin toxicity, few studies have evaluated the in vivo exposure and to our knowledge, no data is available on its effects on antioxidant enzymes in mammalian targets. Therefore, the aim of the present study was to investigate the effect of pendimethalin on producing alteration in the
activities of some antioxidant enzymes and producing cellular oxidative damage as well as demonstrate the pharmacokinetic profile, tissue distribution and excretion of this compound in rats. This study gave a brief insight of possible ill effect of pendimethalin on the targeted tissue, which can be further correlated to its exposure on human beings.

Materials and Methods

Chemical

Pendimethalin (3,4-Dimethyl-2,6-dinitro-N-pentan-3-yl-aniline) was purchased from by Chem Service, West Chester (94.9% purity), while other chemicals were obtained either from Sigma or BDH Companies.

Animals

Female rats, Ratus norvegicus (75 g ± 5) were obtained from the High Institute of Public Health, Alexandria University, housed in stainless steel cages (5 per cage) and left two weeks under the laboratory conditions before starting the experiments. Animals were fed on rodent chow and tap water ad libitum through the study.

Oxidative stress studies

Treatment of animals and tissue preparation: Rats were divided into two groups, five rats each, and orally treated with either four doses of 109.4 mg/kg body weight of pendimethalin or corn oil as vehicle control every other day. Twenty-four hours after the last oral dosing, rats were decapitated and blood samples were collected in non-heparinized tubes. Blood was centrifuged at 3000 rpm for 5 min at 4°C to separate serum, while brain, liver, and kidney were dissected, and stored at -20°C. Selected tissues were homogenized in saline solution (1:10 w/v) using a polytron homogenizer. Homogenates were used for determination of thiobarbituric acid reactive substances (TBARS), lactic dehydrogenase (LDH), catalase (CAT) and alkaline phosphatase (ALP).

Biomarkers of oxidative stress: The determination of lipid peroxidation (LP) was based on the formation of TBARS [29]. Malondialdehyde (MDA) was employed as the standard and the molar absorptivity constant of 1.56 × 10⁻⁵ M cm⁻¹ was used. LP is expressed as nmole MDA/mg protein. The activity LDH was determined according to the method of Moss et al. [30] and expressed as unit/mg protein, while CAT activity was performed following the method of Beers and Sizer [31] and expressed as Bergmeyer units (Bu)/mg protein. ALP activity was estimated according to the method of Bessey et al. [32] using sodium p-nitrophenyl phosphate as a substrate and expressed as unit/mg protein.

Protein contents in the tested tissues were determined by the method of Lowry et al. [33], using bovine serum albumin as standard.

Pharmacokinetics studies

Treatment of animals and sampling: Eleven groups of Animals were individually placed in glass metabolic cages and left at least 24 h prior to dosing for acclimatization. The first ten groups of rats were orally dosed with a single dose of 109.4 mg pendimethalin/kg body weight, while the last group was treated with corn oil as a vehicle control and immediately replaced in their cages. Blood samples from the eyes were collected in non-heparinized tubes after 0.5, 1, 3, 6, 12, 24, 48, 72, 120 and 168 h of treatment, and then centrifuged at 3000 rpm for 5 min at 4°C to separate serum. Urine and faeces were collected after 1, 2, 3, 4, 5, 6, and 7 days. Animals were decapitated 0.5, 1, 3, 6, 12, 24, 48, 72, 120 and 168 h of treatment and then brain, liver, and kidneys were dissected and stored at -20°C until analysis.

Extraction of pendimethalin from tissues and excreta: Pendimethalin was extracted of from serum, liver, kidneys or brain tissue according to the method described by EPA [34] with slight modifications. Tissue sample was placed into a flask and then 2.5 ml of 5% methanolic potassium hydroxide per g (ml) tissue was added. Samples were refluxed for one hour on a hot plate in the presence of porcelain chips and then left to cool at room temperature. Samples were filtered and then 0.75 ml of concentrated sulphuric acid, 10 ml of saturated sodium chloride solution and 30 ml of distilled water per g (ml) tissue were added. Samples were filtered through a bed of florisil (5 g) and then extracted three times with methylene chloride (10 ml per g or ml tissue). The organic layers were taken, combined, dried over anhydrous sodium sulfate and evaporated in rotary at 40°C to 5 ml.

The extraction of pendimethalin from urine and faeces was carried out according to the method of EPA [35] with slight modifications. Urine samples were collected, acidified with 1 ml of hydrochloric acid (4N), mixed very well and then refluxed for 1 h on a water bath. Samples were partitioned three times with hexane (3 ml) and the hexane layers were collected, evaporated to dryness and then dissolved in 2 ml acetonitrile. Faeces samples were homogenized with acetonitrile (5 ml/g sample), filtered and then partitioned three times with dichloromethane (5 ml). Dichloromethane layers were collected and concentrated to 1 ml using a rotary evaporator.

Clean up procedure

Samples were cleaned up according to the method of Bogus et al. [36] by using columns (packed with 2 g florisil, 60-80 mesh, and 2 g of anhydrous sodium sulfate), preconditioned by passing 60 ml of petroleum ether. Sample was loaded onto the column and then eluted with 100 ml of 5% ethyl ether: petroleum ether (40-60°C) in case of tissue or with 10 ml dichloromethane in case of urine and faeces. The eluate was evaporated to dryness under nitrogen, dissolved in 2 ml acetonitrile and subjected to high pressure liquid chromatographic (HPLC) determination.

Fortification: Recovery was carried out at two levels of 10 and 20 µg of pendimethalin and the fortified samples were extracted and cleaned up as described before. The percentage of pendimethalin recovery from serum, liver, kidney, brain, urine and faeces were 93.83, 92.18, 90.22, 85.24, 97.31 and 94.21%, respectively.

Chromatographic analysis

Perkin-Elmer 200 HPLC apparatus, equipped with UV/VIS detector set at 220 nm with an inertsil ph-3 C18 stainless steel column (150 × 4.6 mm I.D.) was used for pendimethalin analysis. Acetonitrile: water (3:2 V/V) at a flow rate of 1 ml/min was used as a mobile phase and identification of pendimethalin was accomplished by retention times and compared with standard at the same conditions. The quantities were calculated on the peak height basis, except for broad peaks which were calculated from area under the peak.
Statistical analysis

Data were calculated as mean ± S.D and analyzed using analysis of variance technique (ANOVA) followed by Least Significant Difference (LSD). A probability of 0.05 or less was considered significant. All statistical analysis was done with Costat Program [37].

Results

Oxidative stress studies

Oxidative stress usually refers to the dysfunction of cell components (e.g. enzymes, nucleic acids, membranes, and proteins) by the reactive oxygen species which initiate oxidative damage leading to cytotoxic effects.

Effect of pendimethalin on oxidative biomarkers

The in vivo effect of 4 repetitive doses of 109.4 mg/kg b.wt (0.1 LD50) of pendimethalin was determined in different tissues of female rats (Figures 1A-D). The concentrations of the degradation product of lipid peroxidation, malondialdehyde (MDA), and the activities of LDH, CAT and ALP were significantly enhanced in all the tested tissues, except brain CAT activity was significantly reduced. The levels of MDA reached approximately 3.76, 4.70, 4.73 and 4.43 (Figure 1A), while LDH activities, were 1.36, 1.40, 1.60 and 1.31 fold the control values in serum, liver, kidney and brain, respectively (Figure 1B). The enhancement of CAT activities in serum, liver and kidney of female rats following pendimethalin administration were 119%, 135% and 138% of control (Figure 1C), while the activity in brain was 92% of control. In case of ALP, the obtained percentages of activity were 133%, 145%, 140% and 133% in serum, liver, kidney and brain, respectively (Figure 1D).

Pharmacokinetics studies

Tissue distribution of pendimethalin: After a single oral administration of pendimethalin (109.4 mg/kg body weight), it was absorbed and subsequently distributed throughout the body. Data in Table 1 illustrate that within 0.5 h of pendimethalin administration, 120.12 ng/ml was observed only in serum, while after 1h and 3 h of dosing, the compound was detected in liver, kidney and serum. After 6 h, pendimethalin was found in all tissues and increased in serum as the time passed. The peak concentrations of pendimethalin in serum, liver and kidneys were observed at the time interval 12 h with mean values of 3229.14, 10162.32 and 1969.17 ng/g, respectively. However, the highest amount of pendimethalin in brain (245.92 ng/g) was observed at 24 h following administration. Pendimethalin was found to decline in all tissues as time passed to reach 75.36 ng/ml in serum after 72 h, while it decreased after 120 h to 33.21 ng/g in liver, 147.52 ng/g in kidneys and 13.11 ng/g in brain. Pendimethalin disappeared after 120 h in serum and 168 h in liver, kidneys and brain following administration.

| Time in h | Pendimethalin levels, ng/g(ml) tissue |
|-----------|--------------------------------------|
|           | Serum | Liver | Kidney | Brain |
| 0.5       | 120.12 ± 29.35 | ND | ND | ND |
| 1         | 345.56 ± 9.27 | 180.31 ± 5.19 | 105.13 ± 2.21 | ND |
| 3         | 982.36 ± 92.69 | 346.23 ± 53.23 | 279.02 ± 2.36 | ND |
| 6         | 1419.85 ± 534.36 | 3113.36 ± 318.25 | 770.21 ± 12.36 | 36.12 ± 3.14 |
| 12        | 3229.14 ± 262.54 | 10162.32 ± 450.6 | 1969.17 ± 91.36 | 119.21 ± 3.1 |
| 24        | 689.36 ± 48.32 | 3030.36 ± 145.36 | 1129.32 ± 8.25 | 245.92 ± 10.2 |
| 48        | 130.56 ± 29.25 | 905.98 ± 10.25 | 340.36 ± 1.30 | 64.26 ± 1.32 |
| 72        | 75.36 ± 8.62 | 328.12 ± 16.34 | 273.21 ± 4.30 | 28.81 ± 9.60 |
| 120       | ND | 33.21 ± 8.5 | 147.52 ± 3.45 | 13.11 ± 1.10 |
Table 1: Pendimethalin levels in different tissues of albino rat, Ratus norvegicus following a single oral administration of 109 mg/kg b.wt (Each value represents the mean of three treated animals’ ±S.E; ND means not detected amounts.).

| Time after dosing (h) | Urinary cumulative excretion % | Faecal cumulative excretion % | Total excretion % |
|-----------------------|---------------------------------|-------------------------------|-------------------|
| 24                    | 8.72 ± 0.20                     | 14.31 ± 2.50                  | 23.01             |
| 48                    | 9.72 ± 0.80                     | 34.84 ± 2.80                  | 44.56             |
| 72                    | 10.83 ± 1.10                    | 58.34 ± 3.40                  | 69.14             |
| 96                    | 20.84 ± 0.90                    | 70.21 ± 3.90                  | 91.05             |
| 120                   | 20.81 ± 2.10                    | 70.81 ± 4.10                  | 92.06             |

Table 2: Cumulative excretion % of pendimethalin in urine and faeces.

| Time after dosing (h) | Urinary cumulative excretion % | Faecal cumulative excretion % | Total excretion % |
|-----------------------|---------------------------------|-------------------------------|-------------------|
| 168                   | 23.81 ± 1.30                    | 71.21 ± 4.70                  | 95.02             |

Pharmacokinetics of pendimethalin: A bi-exponential curve according to a two-compartment open model system was used to carry out the statistical study of pendimethalin elimination [38] using the formula:

\[ C_t = A_0 \exp(-\alpha t) + B_0 \exp(-\beta t) \]

where \( C_t \) is the amount of pesticide in the tissue at time \( t \) (h.), and \( \alpha \) and \( \beta \) are the rate constants for the first and second phases, respectively. The half-life (t½) of exponential elimination in tissues was calculated using the formula:

\[ t_{\frac{1}{2}} = \frac{0.693}{\text{rate constant}} \]

Kinetic parameters

| Tissue          | Serum                  | Liver                   | Kidney                  | Brain                   |
|-----------------|------------------------|-------------------------|-------------------------|-------------------------|
| Peak concs, ng/g(ml) | 3229.14                | 10162.32                | 1969.17                 | 245.92                  |
| \( A_0 \) ng/g(ml)   | 3400                   | 14000                   | 680                     | 1000                    |
| \( B_0 \) ng/g/ml    | 2100                   | 9100                    | 580                     | 190                     |
| \( \alpha \), hr⁻¹   | 0.35                   | 0.17                    | 0.46                    | 0.35                    |
| \( \beta \), hr⁻¹   | 0.05                   | 0.05                    | 0.28                    | 0.02                    |
| \( t_{\frac{1}{2}} \), hr | 2                     | 4                      | 1.5                     | 2                       |
| \( t_{\frac{1}{2}} \), hr | 14                    | 15                     | 2.5                     | 29                      |
| \( K_{12} \), hr⁻¹   | 168.04                 | 646.65                  | 187.97                  | 23.58                   |
| \( K_{21} \), hr⁻¹   | 168.43                 | 648.7                   | 188.71                  | 23.95                   |
| \( K_{10} \), hr⁻¹   | 0.0001                 | 0.00001                 | 0.0007                  | 0.0004                  |
| \( K_{21} / K_{12} \) | 1                     | 1                      | 1                       | 1.02                    |
| \( AUC_{0→∞} \), µg.hr/g(ml) | 52.23                 | 277.77                  | 3.56                    | 10.84                   |
| \( V_d(\text{area}) \), ml/kg | 0.04                  | 0.01                    | 0.1                     | 0.39                    |
| \( C_l \), ml/kg/hr | 0.002                  | 0.001                   | 0.028                   | 0.009                   |
| \( R_l \) | 1                     | 5.32                    | 0.07                    | 0.21                    |

Table 3: Pharmacokinetic parameters in different tissues of albino rat given a single oral dose of pendimethalin (109 mg/kg b.wt).

Total area under the pendimethalin concentration versus time curves for serum AUC serum, and the various tissues, AUC organ, were calculated by the trapezoidal rule. The kinetic parameters for pendimethalin in different tissues are listed in (Table 3). The longest elimination \( t_{\frac{1}{2}} \) was associated with the brain (29.0 h) followed by the liver (15.0 h), while the shortest elimination was associated with the kidneys (2.5 h). AUC values were 52.23, 277.77, 3.56, and 10.84 µg.hr/g (ml) for serum, liver, kidneys and brain, respectively. This finding indicates that this pesticide did tend to be retained in animal tissue.
Discussion

Herbicides, such as pendimethalin, may induce oxidative stress, thus leading to the generation of free radicals as well as causing LPO and carbonylation of proteins and increased MDA in tissues [39] which may be the cause of oxidative stress. The present findings implicate a role of oxidative stress and free radical formation in these effects. Evidence from in vivo studies with many toxicants including pesticides supports the concept that free radicals e.g. hydroxyl radicals •OH, H₂O₂ and others, are important mediators of tissue injury and the formation of these radicals result in increased LP [17,15] in some organs and elements of the central and peripheral nervous system that are rich in polyunsaturated highly oxidizable fatty acid [4,40,41]. Luckily, the cell has several ways of alleviating the effects of oxidative stress, either by repairing the damage or directly by diminishing the occurrence of oxidative damage by means of enzymatic and non-enzymatic antioxidants [42]. Enzymatic and non-enzymatic antioxidants have also been shown to scavenge free radicals and ROS [43-46], CAT is a primary antioxidant defense component along with glutathione peroxidase catalyzes the decomposition of H₂O₂ to H₂O [47], has high turnover number and can convert millions of molecules of hydrogen peroxide to water and oxygen each second [48]. Several studies have been reported significant changes in CAT activities in different rat organs exposed to insecticides [49,50]. In the present study, CAT activity was induced in rat exposed to pendimethalin and as a second line of enzymatic defense against H₂O₂ generation.

The present findings are in parallel with many investigators who found that the enhancement of LDH release is indicative of cellular and membrane damage [43-46,51,52], can be used as a biomarker for cellular damage, clinical practice and cytotoxicity of pollutants [52]. Moreover, the significant increase of LDH activity in kidney suggested the change in glycolytic process due to the lower metabolic rate as a result of insecticide exposure [53]. This implies that pendimethalin induced oxidative stress.

The increase in activity of ALP in the present study was relatively higher in liver than other organ; and this is expected because liver is the active organ for dephosphorylation of xenobiotics by phosphatases in the alkaline media. These increases could be related to the influence of glucocorticoids [54], or could be attributed to its release from ruptured cells due to the effect of pesticide [55].

The tissue distribution and excretion of a single oral dose of pendimethalin in female rat illustrated that the compound was absorbed from the intestine and then rapidly distributed to the animal tissues with levels peaking within 12 h in the serum, liver, kidneys and 24 h in brain. Apparently, the compound was distributed in the different tissues via the blood soon after application, then metabolism was taking place and the amount of the compound dropped to a minimum 72 h in serum and 120 h in liver, kidneys and brain after treatment. By comparing the concentration of the compound at various times after administration, it was possible to determine which of these organs had a greatest affinity to the compound [56]). Our findings suggested that the liver had a greater affinity for pendimethalin than for serum, kidneys and brain. Also, the data showed that the faecal excretion was higher than the urinary excretion at the end of the experiment (168 h). These findings were attributed by the higher concentrations in the liver than kidneys. This may be due to the nonpolar properties of the compound.

Conclusion

The observed highest levels of MDA and LDH, CAT and ALP activities in female rat exposed to pendimethalin, means that pendimethalin stress affects the cellular response of facing reactive oxygen species. Oxidative damage or stress usually refers to the dysfunction of cellular components by reactive oxygen species. A major form of cellular oxidation damage is lipid peroxidation, as well as increase in the activity of antioxidant enzymes. It can be concluded that, pendimethalin absorbed from the intestine and then rapidly distributed to the animal tissues, producing significant alteration in some biomarkers mediated oxidative stress.

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