Effect Of Permethrin, Pirimiphos Methyl And Bendiocarb On The Osmotic Resistance Of Rat Erythrocytes

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Abstract

Environmental pollution by insecticides is one of the most important problems in the world. Some of the pesticides were found to exert carcinogenic, teratogenic and/or mutagenic effects even following normal agricultural use (U.S.Geological Survey, 1997). On the other hand residues from insecticides, herbicides and insect growth hormones are known to represent the most common food contaminants particularly in developing countries. Thus the wide spread use of insecticides in agriculture stimulated our interest for studying their possible toxic effect(s) in man and animals.

The aim of the present work was to study the effect of short term (for 2 weeks) oral daily feeding with diet containing 10 times the human maximum acceptable daily intake (a concentration that may be faced by human) of either permethrin, pirimiphos-methyl or bendiocarb on osmotic fragility of rat erythrocytes.

Sprague Dawley male rats were divided into four groups, each consists of 10 animals. Animals of each group were fed either normal diet (control group), permethrin (21.7 ppm), pirimiphos-methyl (4.4 ppm) or bendiocarb (2.0 ppm) for 2 weeks. Twenty four hours later, blood samples were withdrawn and osmotic fragility was determined. The obtained results indicated that permethrin, pirimiphos-methyl and bendiocarb increase the osmotic fragility.

Introduction

The use of pesticides has been largely expanded during the last fifty years. The WHO (1992), reported that 3 million pesticide poisoning cases occurred annually and resulted in 220 000 deaths allover the world. In the developing countries the situation is worse, since higher proportions of pesticides poisoning and deaths occurred. The reasons behind this include, inadequate occupational safety standards and insufficient knowledge of pesticide hazards. Some pesticides are carcinogenic, most are teratogenic, and others are mutagenic. All are attributed to normal agriculture use (U.S.Geological Survey, 1997). So, it is safe to assume that sooner or later higher percentages of our people (especially in developing countries) will suffer from some serious forms of diseases like cancer and kidney failure (Cheraskin, 2000). These diseases will be resulted from toxins in air we breathe, food we eat and water we drink.

Among the potent synthetic insecticides that have been increasingly employed in recent years are synthetic pyrethroids, organophosphates and carbamates. Pyrethroids are known to increase relative liver weight, to change haematocrite and mean corpuscular volume, to induce chromosomal aberrations (Ismael and Lithfield, 1988 and Institoris et al., 1999a and b), to suppress erythropiosis and hemoglobin synthesis and to increase number of leukocytes (Tos-Luty, et al., 2001). Pyrethroids were also reported to cause slight activation of cytochrome P 450 1A and 2B mediated reactions (Kostka et al., 1997 and Moresseau et al., 1999) and to act as a tumor promotor at non-hepatotoxic doses (Hemming et al., 1993). They may inhibit the G2 phase in the cell cycle and
consequently may suppress the cell entering into the stage of mitosis (Kostka, et al., 2000). Pyrethroids were also found to affect male and female reproductive system (Eil and Nisula, 1990).

Organophosphate insecticides were in existence since 1854, but were not recognized as having toxic potentials until 1930 (Marrs, 1993). These compounds induce significant fall of body weight (Gajewski and katkiewicz, 1981), and reduce glycogen content in liver and kidney (Awasthi, et al., 1984). Pirimiphos methyl is known to affect the proteolytic enzyme activities in rat heart, kidney, brain and liver (Mantle et al., 1997). It induces significant inhibition of brain and erythrocytes acetyl cholinesterase, plasma pseudo cholinesterase and non-specific carboxyl esterase of brain, plasma and kidney (Rajini et al., 1989).

Carbamates may represent a class of chemicals which function through a mechanism separate from ligand binding, as they may act as general endocrine modulators in mammalian cells (Klotz et al., 1997). They induce dose dependent decrease in body weight (Pant et al., 1995a and b and Kackar et al., 1997) and significant change in the weight of testes, epididymis and accessory sex organs (Pant et al., 1995b). Carbamate insecticides were found to inhibit both aggregation and arachidonic acid metabolism in human blood platelets (Krug et al., 1988), to inhibit brain and blood acetylcholinesterase, liver glucose 6 phosphatase and succinic acid dehydrogenase (Fayez and Kilgore 1992).

The wide spread use of the above-mentioned insecticides in agriculture and in public health drew our attention for studying their possible toxic action(s) in man and animals.

The aim of the present work is to study the influence of 14 days daily ingestion of diet containing permethrin, pirimiphos-methyl or bendiocarb at a concentration equivalent to 10 times the human maximal acceptable daily intake (a concentration that may represent the real life dose) on the osmotic resistance of red blood corpuscles in rats.

Materials And Methods
1 – Animals :
Male Sprague Dawley rats of 110-120 g body weight were obtained from the breeding colony maintained at the animal house of the National Organization for Drug Control and Research (NODCAR), Cairo. They were housed as 10 animals /cage where food and water were given ad libitum.

2 - Insecticides :
- Permethrin represents pyrethroid insecticides.
- Pirimiphos-methyl represents organophosphorus insecticides.
- Bendiocarb represents carbamates insecticides.

3-Experimental design:
Rats were divided into 4 equal groups (each consists of 10 rats).
- First group: animals were fed normal diet and serves as a control.
- Second group: animals were fed diet containing 10 times the human maximal acceptable daily intake of permethrin (21.7 ppm) for 2 weeks [about 3.6×10^{-3} of the oral determined LD50].
- Third group: animals were fed diet containing 10 times the human maximal acceptable daily intake of pirimiphos methyl (4.4 ppm) for 2 weeks [about 5.5×10^{-3} of the oral determined LD50].
- Fourth group: animals were fed diet containing 10 times the human maximal acceptable daily intake of bendiocarb (2.0 ppm) for 2 weeks [about 3.3×10^{-3} of the oral determined LD50].

Twenty- four hours after last day of feeding with insecticides, blood samples were collected from the retro-orbital plexus of animals of each group.

The osmotic fragility test was performed as adopted for the rat RBCs. by Boelsterli et al., (1983) on the bases of the procedure described by Beutler (1977) for the human R.B.C.: The RBCs were spun down (5 min at 2000 x g) and washed twice with phosphate-buffered (0.01 M) isotonic
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(0.15 M) saline (PBS), PH 7.4, containing 0.25% bovine serum albumin. The RBCs were suspended in PBS-BSA at a haematocrit of 40%. Fifty μl of the RBCs suspension were added to 5 ml of a series of NaCl solutions (with graded concentrations) made up from a stock solution of PBS. After incubation with the RBCs suspension for 60 min. at room temperature, the tubes were centrifuged (5 min at 2000 x g) to sediment unlysed cells, and the hemoglobin in the supernatant was measured spectrophotometrically at 540nm. The MFC (mean corpuscular fragility) was determined graphically and expressed as NaCl concentration at which 50% of the RBCs haemolyse.

Results

Figure 1 showed the changes in the % haemolysis of red blood corpuscles versus saline concentration due to daily oral feeding with diet containing either permethrin, pirimiphos-methyl or bendiocarb. The curves were sigmoid in shape reflecting a heterogeneous erythrocyte population where the tail representing a small number of cells with osmotic fragility higher (Fig. 1a, b and c) or lower (Fig a and c). Figure 1b showed that pirimiphos-methyl containing diet induced shifting of the curve to the right side.

Table 1 illustrated the mean corpuscular fragility (MCF) expressed by the concentration of saline solution (saline concentration at which 50% of the red blood corpuscles are haemolysed). This table showed that the highest value of MCF (89% saline solution) was obtained due to prefeeding with pirimiphos-methyl containing diet.

Table 2 showed the concentration of saline solution at which haemolysis was first seen and the concentration of saline solution at which all erythrocytes were completely haemolysed. The difference between these two concentrations of saline solution was highest (49%) in case of prefeeding with diet containing pirimiphos-methyl.

Table1: Effect of daily oral feeding by a diet containing either permethrin, pirimiphos-methyl or bendiocarb on the mean corpuscular fragility (MCF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean corpuscular fragility (MCF) ( % of saline solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>83</td>
</tr>
<tr>
<td>Permethrin containing diet</td>
<td>84</td>
</tr>
<tr>
<td>Pirimiphos-methyl containing diet</td>
<td>89</td>
</tr>
<tr>
<td>Bendiocarb containing diet</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 2: % saline solution inducing initial and complete haemolysis of red blood corpuscles due to daily oral feeding for 14 days by a diet containing either permethrin, pirimiphos-methyl or bendiocarb.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial haemolysis ( % saline solution)</th>
<th>Complete haemolysis ( % saline solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>97 ± 1.7</td>
<td>51 ± 6.23</td>
</tr>
<tr>
<td>Permethrin containing diet</td>
<td>99 ± 1.0</td>
<td>54 ± 6.62</td>
</tr>
<tr>
<td>Pirimiphos-methyl containing diet</td>
<td>100 ± 0</td>
<td>51 ± 6.53</td>
</tr>
<tr>
<td>Bendiocarb containing diet</td>
<td>99.2 ± 0.83</td>
<td>58.8 ± 3.95</td>
</tr>
</tbody>
</table>
Figure 1: Changes in % haemolysis of red blood cells due to daily oral feeding for 14 days with diet containing either permethrin (a), pirimiphos-methyl (b) or bendiocarb (c)
Discussion

The osmotic fragility test is known as a tool for testing the haemolytic effect of substances and drugs (Simmons, 1968 and 1997, Mial, 1967 & Bachmann and Zbinden 1973). The utilization of this test as a toxicological screening model was reassessed and highly emphasized by Boelsterli et al., 1983. In the present study very small doses (10 times the human maximal acceptable daily intake) of some commonly used insecticides were used to study the potential of these small doses on the osmotic fragility of red blood cells of male rats.

Figure 1(a) showed the % haemolysis of red blood cells versus % of saline solution due to pre-feeding with diet containing permethrin [21.7ppm] for 14 days. This Figure showed that at saline concentration from 50 to 70%, the osmotic fragility due to permethrin pre-feeding was reduced while there was an increase in the osmotic resistance at concentrations from 85 to 100 %. This result was confirmed by the obtained results in table 1 and 2, where table 1 showed that the MCF was increased from 83% (control value) to 89% while table 2 revealed that the initial haemolysis started at concentration of 99% saline solution and complete haemolysis ended at concentration of 54% saline solution (control values were 97% and 51%).

The obtained changes in the osmotic fragility throughout the whole curve due to permethrin pre-feeding were in agreement with works of Nasuti et al., 2003, Gabbianelli et al., 2002, Moya-Quiles et al., 1995. Where Nasuti et al., 2003 found that permethrin produced an increase in the polarity of the hydrophilic – hydrophobic region of the erythrocyte bi-layer even at low dose. Cypermethrin (pyrethroid insecticide) was preferably localized in the hydrophobic core of the membrane inducing an increase in the lipid packing (Gabbianelli et al., 2002). Also Moya-Quiles et al., 1995 showed that the release of haemoglobin was notably facilitated by the incorporation of allethrin into human erythrocytes. This effect may be due to the possible aggregation of the insecticide in the lipid bi-layer with a consequent increase in the membrane instability. So it may be concluded that pyrethroid insecticides induce change(s) in the erythrocytes membrane which may consequently affect the osmotic fragility.

Figure 1 (b) showed changes in the osmotic fragility due to daily oral feeding with diet containing pirimiphos-methyl [4.4 ppm] for 14 days. This curve was shifted to right direction indicating an increase in the osmotic fragility where the % haemolysis was increased in all concentrations of saline solution. This effect might find support in table 1 and 2 where the MCF was increased to 89 % (control value 83 %) and the concentration of saline solution at which the haemolysis was first seen was 100% (control value was 97%). The obtained changes in these results due to daily oral feeding for 14 days by a diet containing pirimiphos-methyl were explained according to Blasiak, 1996. This author found that organophosphorus insecticides might inhibit anion exchange indirectly by changing the fluidity of the erythrocyte membrane or directly by binding to the band 3 protein and evoking conformational changes that lead to the inhibition of anion transport. Also the ability of organophosphorus insecticide for phosphorylation might also disturb some regulation processes in the band 3 protein and affect anion transport in this way (Blasiak, 1996). It is worth noting that, as organophosphorus insecticides are known as acetyl choline-esterase inhibitors ( Rajini et al., 1989 ), and this enzyme is only present in the membrane of erythrocytes where it is localized on the outer side of the membrane so it may be used to examine the status of the erythrocyte membrane ( Iguisu et al., 1994 ).

It may be concluded that organophosphorus insecticides may adversely affect the erythrocyte membrane function and may consequently induce change in the osmotic fragility.
The osmotic fragility curve due to pre-feeding with diet containing bendiocarb (carbamate insecticide) [2.0 ppm] in figure 1(c) showed an increase in the osmotic fragility at concentrations of 5% to 35%, at 40% and at 55% and a decrease in the osmotic fragility (increased osmotic resistance) at 85% to 90% saline solution. These changes were confirmed by the results obtained in tables 1 and 2 where table 1 showed a very small decrease in the MCF and table 2 showed an increase in both concentrations of saline solution at which the initial and complete haemolysis of red blood cells occurred (99.2% and 58% respectively), control values were 97% and 51%. Fortunately, no available literature could be obtained regarding the effect of carbamate insecticide on the membrane of the erythrocytes.

It is worth noting that the shape of osmotic fragility curve due to pirimiphos-methyl pre-feeding (Fig.1b) is different from that of the other 2 curves (due to permethrin and bendiocarb pre-feeding) (Fig.1 a and c) where pirimiphos-methyl induced a consistent change in the % haemolysis of red blood cells versus saline concentration. While permethrin and bendiocarb induced inconsistent changes. This observation was clear in the slope of the ascending part of the curves where these values where 10.8, -8.1, -10.3 and -10.5 for control, permethrin, pirimiphos-methyl and bendiocarb respectively. This change in the shape of the curves, may be due to the difference in the mechanism of action between permethrin and bendiocarb in one side and pirimiphos-methyl on another side, but this expectation needs further and extensive works.

So, it may be concluded that the small doses of the tested insecticide have a possible toxic potential on the membrane of red blood cells. Also these insecticides may possess a potential for interfering with drugs whose effects reside on the surface of red blood cells or inside them.

References
exposure by cypermethrin and the heavy metals, lead and cadmium in rats. In J. Immuno-Pharmacol. 21 (11): 735-43.


تأثير مبيد البرمثرين والبيريمايفوس مثيل والبنديكارب على المقاومة الأسموزية لكرات الدم الحمراء في الفئران

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إن التلوث البيئي بالمبيدات يمثل أحد أهم المشاكل في العالم. وقد وجد أن بعض هذه المبيدات يحدث سرطانات وتشوهات وطفرات وراثية حتى عند الاستعمال العادي في الزراعة، من ناحية أخرى فإن متبقيات المبيدات وقاطلات الأعشاب يعتبران من أهم الملوثات الغذائية خاصة في العالم النامي. إن الاستعمال الواسع للمبيدات في الزراعة قد أثار الانتباه لدراسة تأثير هذه المواد السامة على الإنسان والحيوان، ويفيد البحث إلى دراسة تأثير مبيدات البرمثرين والبيريمايفوس مثيل والبنديكارب على الهشاشة الأسموزية لكرات الدم الحمراء في الفئران. وذلك بتغذيتهم يومياً لمدة قصيرة المدى (لمدة أسبوعين) بذبح يحتوي على المبيدات بجرعة تمثل عشرة أضعاف الجرعة المسموح بها للإنسان يوميا (هي عادة الجرعة التي يمكن أن يتعرض لها الإنسان). استخدمت في هذا البحث أن فئران ذكور فصيلة سبراجودولبي وقسمت إلى 4 مجموعات وتحتوي كل مجموعة على 10 حيوان حيث غذيت المجموعة الأولى (المجموعة الضابطة) بغذاء عادي خالي من المبيدات، وغذيت المجموعة الثانية والثالثة والرابعة بغذاء يحتوي على 21.7 جزء لكل مليون مبيد البرمثرين، 4.4 لكل مليون مبيد البيريمافوس مثيل، 2 جزء لكل مليون مبيد البنديكارب على التوالي لمدة أسبوعين. بعد 24 ساعة أخذت عينات من الدم لتقدير الهشاشة الأسموزية. أوضحت نتائج البحث أن المبيدات المستخدمة تسبب زيادة في الهشاشة الأسموزية لكرات الدم الحمراء.