Safety Aspects in Treatment of Cancer by Carbamate Insecticides as Measured by Osmotic Fragility of Erythrocytes

Mohammed Amanullah*, Gaffar Sarwar Zaman¹ and Naseem Begum¹

Abstract
Cancer chemotherapy using carbamate insecticides has been published by the first author. This study was to evaluate their effects on other cell types because; pesticides and insecticides can affect the health of living organisms through different mechanisms such as membrane denaturation. The evaluation of the deleterious effects of chemical agents on biological membranes can be performed through the analysis of the stability of erythrocytes against a concentration gradient of certain chemical agent in physiologic saline solution. In the present study, technical grade carbamate insecticides viz., propoxur, carbaryl and carbofuran were tested for their toxicity on erythrocytes in vitro. Osmotic fragility of erythrocytes in different concentrations of saline, following exposure to technical grade carbamates was studied in order to indirectly assess the degree of anaemia and hypoxia, resulting upon exposure to carbamates. The percent hemolysis of sheep erythrocytes without carbamates (control), initiated at 0.45 percent NaCl solution, which reached 50% at 0.40 percent NaCl concentration. Complete hemolysis was seen at 0.35 percent NaCl. Baygon treated erythrocytes showed initial, 50% and complete hemolysis at 0.55, 0.45 and 0.35% NaCl respectively i.e. a little earlier as compared to the control. Carbaryl treated erythrocytes showed initial 50% and complete hemolysis at 0.65% NaCl, 0.55% and 0.45% concentration of NaCl respectively. Carbofuran exhibited less toxic effects on the fragility of erythrocytes as compared to baygon and carbaryl, where in the initial, 50% and complete haemolysis was seen at 0.50, 0.45 and 0.40 percent NaCl respectively. Though carbamate insecticides affected the integrity of the erythrocytes, the effect is transient and reversible and hence they can safely be used as chemotherapeutic agents for cancer.

Keywords: Carbamate insecticides; Cancer chemotherapy; Carbaryl; Baygon; Carbofuran; Erythrocytes; Osmotic fragility; Anemia; Hypoxia

Introduction
This article is an attempt to further evaluate the toxic effects of carbamate insecticides in support of the earlier papers published by the first author on evaluation of carbamate insecticides as chemotherapeutic agents for cancer [1-3]. The evaluation of the deleterious effects of chemical agents on biological membranes can be performed through the analysis of the stability of erythrocytes against a concentration gradient of certain chemical agent in physiologic saline solution. Keeping this in view, we have tried to assess the consequence in the normal cells while treating cancer with carbamates.

Erythrocytes, or red blood cells, are round disks, concave on two sides, and approximately 7.5 thousandths of a millimeter in diameter. In humans, and most other mammals, the mature red blood cell contains no nucleus; in some vertebrates, it is oval, and nucleated. Hemoglobin, a protein in the red blood cells, gives blood its red color and transports oxygen from the lungs to the body cells, where it picks up carbon dioxide for transport back to the lungs to be expired. Hemoglobin also transports nitric oxide, which regulates blood pressure by expanding or contracting blood vessel walls. Red blood cells are formed in the bone marrow. After an average life of 120 days, red blood cells are broken down and removed by the spleen. However, less work has been done to see the toxic effects of carbamate insecticides on erythrocytes in vitro.

Carbamate insecticides, like baygon, mesurol, carbaryl, carbofuran, aldicarb, primicarb etc., are widely used in the agriculture fields resulting in the contamination of food, feed and fodder, which is being continuously consumed by man and his animals, causing continuous loss of body weight, growth parameters, reproduction and other abnormalities. These insecticides though consumed in minute quantities, have shown to be mutagenic, carcinogenic and teratogenic. However, the degree of their effect on internal tissues like hepatic tissue, lymphatic tissue, immune system and others is not expressed phenotypically within the living organism. Cell and tissue culture techniques provide a novel way to study the effect of these carbamate insecticides on various tissues. Cell and tissue culture has been excessively used in the study of toxic effect of various metabolites on different tissues.

Carbamate insecticides are a group of compounds closely related to the Organophosphate insecticides in chemical structure, mode of action and many other properties. These compounds inhibit the enzyme acetylcholine esterase (AChE) in the neuromuscular junction and cause the death of the organism by neuromuscular paralysis. The carbamates are mainly used in agriculture, as insecticides, fungicides, herbicides, nematocides, or sprout inhibitors. In addition, they are used as biocides for industrial or other applications and in household products. A potential use is in public health vector control. Thus, these chemicals are part of the large group of synthetic pesticides that have been developed, produced, and used on a large scale industrial basis.

The acute toxicity of the different carbamates ranges from highly toxic to only slightly toxic or practically non-toxic. The LD50 for the rat ranges from less than 1 mg/kg to over 5000 mg/kg body weight. The acute dermal toxicity of carbamates is generally low to moderate. Carbamates produce slight to moderate skin and eye irritation, depending on the vehicle used, duration of contact, and on whether...
the substance is applied to the abraded or intact skin. Apart from the anticholinesterase activity, the following changes can be found: an influence on the haemopoietic system, an influence on the functioning of and at higher dosages degeneration of the liver and kidneys and degeneration of testes. These abnormalities in the different organ systems depend on the animal strain and on the chemical structure of the carbamate. A considerable number of reproduction and teratogenicity studies have been carried out with different car bamates and various animal species. Different types of abnormalities were found, i.e., increase in mortality, disturbance of the endocrine system, and effects on the hypophysis and its gonadotropic function. These effects were mainly seen at high dose levels. Generally, the fatal effects included an increase in mortality, decreased weight gain in the first few weeks after birth, and induction of early embryonic death. All these effects can be summarized as embryotoxic effects. Certain car bamates also induce teratogenic effects, mainly at high dose levels applied by stomach tube. When the same dose level was administered with the diet, no effects were seen.

The carbamate insecticides are a group of chemical inhibitors that inhibit the enzyme acetylcholine esterase, especially in the nervous tissue. This inhibition can be selectively reversed by an appropriate dose of atropine. The hydroxyl (OH) groups of the amino acid serine in the enzyme acetylcholine esterase are selectively inhibited by formation of a covalent bond. The toxic effects observed in other tissues where the enzyme acetylcholine esterase is not prominent, is due to the inhibition of other enzymes containing serine moieties at the vital sites of the enzymes. This enzyme inhibition results in inactivation of the enzyme or attenuation of the activity of the enzymes. Most of the enzymes of the energy metabolism (glycolysis), protein synthesis, and nucleic acid metabolism contain serine moieties at their active site or other sites which are responsible for the enzyme action, resulting in energy deprivation to the cell, depleted protein and nucleic acid concentration thereby causing cell stasis or death. Similarly, these car bamates inhibit the enzymes of cancer cells causing suspension in the growth of the tumor or tumor regression due to cell death.

Different researchers reported that osmotic fragility of red blood cells may be caused by physiological [4-7] or pathological [8,9] reasons. Low osmotic resistance may lead to intravascular hemolysis, which may cause a reduction of the erythrocyte life span and hence lead to anemia [8] if not treated.

Carbaryl produced, in vitro, a dose-dependent increase in met haemoglobin (Met Hb) formation at 10 and 100 mg/litre, as well as decreases in reduced glutathione levels in the erythrocytes of Dorset sheep with low erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD), which is similar to humans who have G-6-PD deficiency.

Some of the observations made by Ferguson in 1999 [10], about the effects of primaquine on erythrocytes in vitro/in vivo are (a) extraerythrocytic factors, including hepatic drug metabolism, are important for hemolysis in vivo. (b) Although high concentrations of primaquine may cause lysis of G6PD-deficient erythrocytes in vitro, normal cells are equally susceptible. (c) Sequestration of deformed cells by the reticuloendothelial system (the spleen) in vivo is probably essential for the observed hemolytic effect. However, absence of metabolic potentiation of primaquine in the in vitro system may also account for this discrepancy. (d) Metabolites of several hemolyzing agents (including primaquine) are more effective than the parent compounds in causing increased mechanical fragility of erythrocytes in vitro. (e) Active metabolites produced principally in the liver must be sufficiently stable to reach the erythrocyte before the red cell can be injured. (f) Additional intraerythrocyte catalytic activities probably play a role in the pathogenesis of hemolysis. Several hemolytic drugs can interact with hemoglobin to generate low levels of hydrogen peroxide, which, like organic hydroperoxides, may result in oxidation of GSH via erythrocyte glutathione peroxidase. (g) The glutathione transferases are a potentially important group of enzymes, which has hitherto not been suspected of playing a role in G6PD-deficient hemolysis. Quinones may lead to both oxidation and depletion of GSH, independently of possible epoxide formation.

Materials and Methods

The erythrocyte osmotic fragility test is used to measure the osmotic resistance of the erythrocyte membrane [11]. Fresh sheep blood sample with heparin (50 IU/ml) was collected from jugular vein of slaughtered healthy and young sheep (one year old). A volume of 2.5 ml of whole blood was diluted with 7.5 ml normal saline (0.9% NaCl) solution to wash the erythrocytes. After centrifugation at 1,000 RPM for 15 min, 3 ml of the sediment was collected. Then the washing procedure was repeated thrice. Technical grade carbamate insecticides at a final concentration of 400 ppm were added and incubated for 6 hours. The fragility of RBC was tested by a routine method in which the cells were placed in different concentrations of NaCl and hemolysis was determined by colorimetric method. The hemolysis percentage of the samples in each solution was calculated on the basis of the 100% hemolysis in distilled water.

16 test tubes were taken and labeled 1-16, then different concentrations of saline in each of the test tubes were prepared as given in the Table 1.

Then, 0.05 ml (50 μl) of each of the carbamate treated blood (as described above) was added to each of the test tube. Mixed well and kept aside for 30 min., mixed again and centrifuged at 2500 RPM for 10 minutes. Then, 3.5 ml of the supernatant was taken without disturbing the sediment, in a cuvette and the optical density (OD) of each tube was read in a colorimeter at 545 nm using the saline as blank. The hemolysis in distilled water (tube 16) was taken to be 100%. Depending upon this, the % hemolysis in other tubes was calculated as:

\[
\%\text{Hemolysis} = \frac{\text{O.D. of Unknown}}{\text{O.D. of tube with water}} \times 100
\]

Table 1: Preparation of different concentrations of saline.

<table>
<thead>
<tr>
<th>Test tube No.</th>
<th>1%NaCl (ml)</th>
<th>H₂O (ml)</th>
<th>Resolvent solution (%NaCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.25</td>
<td>0.75</td>
<td>0.85</td>
</tr>
<tr>
<td>2</td>
<td>4.00</td>
<td>1.00</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>3.75</td>
<td>1.25</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>3.50</td>
<td>1.50</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>3.25</td>
<td>1.75</td>
<td>0.65</td>
</tr>
<tr>
<td>6</td>
<td>3.00</td>
<td>2.00</td>
<td>0.60</td>
</tr>
<tr>
<td>7</td>
<td>2.75</td>
<td>2.25</td>
<td>0.55</td>
</tr>
<tr>
<td>8</td>
<td>2.50</td>
<td>2.50</td>
<td>0.50</td>
</tr>
<tr>
<td>9</td>
<td>2.25</td>
<td>2.75</td>
<td>0.45</td>
</tr>
<tr>
<td>10</td>
<td>2.00</td>
<td>3.00</td>
<td>0.40</td>
</tr>
<tr>
<td>11</td>
<td>1.75</td>
<td>3.25</td>
<td>0.35</td>
</tr>
<tr>
<td>12</td>
<td>1.50</td>
<td>3.50</td>
<td>0.30</td>
</tr>
<tr>
<td>13</td>
<td>1.25</td>
<td>3.75</td>
<td>0.25</td>
</tr>
<tr>
<td>14</td>
<td>1.00</td>
<td>4.00</td>
<td>0.20</td>
</tr>
<tr>
<td>15</td>
<td>0.50</td>
<td>4.50</td>
<td>0.10</td>
</tr>
<tr>
<td>16</td>
<td>0.00</td>
<td>5.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Results

The detailed results are presented in Table 2 and the same has been summarized in Table 3 and Figure 1. The percent hemolysis of erythrocytes in control (untreated blood) was negligible at 0.8 percent of NaCl concentration and the initial hemolysis began at 0.45% NaCl which reached 50% at 0.40 percent NaCl concentration. Complete hemolysis was seen at 0.35 percent NaCl. Baygon treated erythrocytes showed initial hemolysis at 0.55 percent NaCl concentration (29.76%). 50% hemolysis was seen at 0.45% NaCl (51.01 ± 0.65%). However, the hemolysis completed at 0.35% NaCl i.e. a little earlier as compared to the control. Carbaryl treated erythrocytes showed more toxic effects with the initial hemolysis beginning much earlier i.e. at 0.65% NaCl (20.95%). 50% hemolysis was seen with 0.55% NaCl concentration of NaCl. However, the maximum hemolysis reached at 0.45% concentration of NaCl i.e. carbaryl treated erythrocytes were more fragile as compared to the control, carbaryl (0.40%) or even carbofuran (0.40% NaCl) treated erythrocytes. Carbofuran treated erythrocytes exhibited less toxic effects on the fragility of erythrocytes as compared to baygon and carbaryl, where in the initial, 50% and complete haemolysis was seen at 0.50, 0.45 and 0.40 percent NaCl (Table 3 and Figure 1).

Discussion

The purpose of undertaking the study of osmotic fragility of erythrocytes due to the effect of carbamate insecticides in the present research work was to assess the degree of anemia that could result by the use of these insecticides as cancer chemotherapeutic agents. Further, erythrocyte fragility is an indirect measure of the integrity of cell membranes of other tissues, thereby gives an idea of the degree of damage to normal tissues when treatment with drugs is undertaken.

The osmotic fragility (OF) test measures the stability of erythrocytes by testing the cells’ ability to withstand hemolysis in decreasing concentrations of saline solution. The OF test is a rough index of red cell surface-to-volume ratio. When erythrocytes are placed in a hypotonic solution, water osmotically enters the cells and causes them to swell. The cell reaches a point where the membrane starts to leak, and, finally, the cell bursts and releases hemoglobin. Damage to the red cell membrane also allows hemoglobin to escape from the cells but does not cause osmotic swelling. A positive correlation exists between abnormal OF test results and decreased survival time for red cells. The mean OF expresses the concentration of NaCl in which 50 percent hemolysis occurs. Increased as well as decreased osmotic fragility of erythrocytes have been described in different types of anemia in human medicine, dog and cat.

There are many factors that influence the osmotic fragility of erythrocytes viz., mechanical damage of erythrocyte membrane, red blood cell age [7], changes in red blood cell shape, hemocoagulation and acidosis, elevation of body temperature, exercise stress and catecholamine [5], lysolceithin (hemolytic agent released by the contracting spleen into circulation), peroxidation of the erythrocyte membrane, any factor inhibiting or attenuating glycolysis can cause erythrolysis as glycolysis is the most important source of energy in red cells. The carbamate insecticides tested in the present study were all potent inhibitors of not only glycolysis but many other enzymes of cell metabolism. Therefore, all the drugs rendered erythrocytes more fragile to the changes in the osmolality of the solution in which they were taken into. Carbaryl affected the erythrocytes most followed by baygon and carbofuran. Similar results were reported by Foul et al. [13] wherein the carbamates methomyl and trichlorofon significantly produced a reduction in the number of erythrocytes (due to increased fragility), haemoglobin level and haematocrit value in in vivo experiments on birds. Willi et al. [14] reported an increase in osmotic fragility of the erythrocytes due to infection by hemotrophic Mycoplasma species in Swiss cats. Humberto et al. [15], published a similar trend of 100% erythrocyte hemolysis with the pesticide Roundup® at concentration limit recommended for agricultural purposes. Chiediere et al. [16], reported that lipid peroxidation can be measured indirectly by erythrocyte osmotic fragility as a molecular mechanism implicated in chlorpyrifos poisoning. Milea et al. [17], verified the effects of vibrations on the osmotic fragility (OF) of red blood cells (RBC) isolated from whole blood submitted from Whole body vibration (WBV) exercises in oscillating platforms (OP), wherein they found considerable level of decreased osmotic fragility.

Cancer anemia is classified as an anemia of chronic diseases, whose pathogenic mechanism has been difficult to establish. Bone marrow production of RBC is usually slightly increased but insufficient to

Table 2: Percent in vitro hemolysis due to the effect of technical grade carbamates.

<table>
<thead>
<tr>
<th>%NaCl</th>
<th>0.80</th>
<th>0.75</th>
<th>0.63</th>
<th>0.65</th>
<th>0.50</th>
<th>0.45</th>
<th>0.40</th>
<th>0.35</th>
<th>0.30</th>
<th>0.25</th>
<th>0.20</th>
<th>0.15</th>
<th>0.10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.66 ± 0.00</td>
<td>7.20 ± 0.00</td>
<td>8.14 ± 0.00</td>
<td>8.58 ± 0.00</td>
<td>9.79 ± 0.00</td>
<td>10.51 ± 0.00</td>
<td>28.54 ± 0.00</td>
<td>51.86 ± 0.00</td>
<td>95.82 ± 0.00</td>
<td>99.41 ± 0.00</td>
<td>99.41 ± 0.00</td>
<td>99.41 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>Baygon</td>
<td>0.62 ± 0.00</td>
<td>9.72 ± 0.00</td>
<td>12.87 ± 0.00</td>
<td>15.95 ± 0.00</td>
<td>29.78 ± 0.00</td>
<td>43.02 ± 0.00</td>
<td>51.01 ± 0.00</td>
<td>98.70 ± 0.00</td>
<td>97.99 ± 0.00</td>
<td>99.54 ± 0.00</td>
<td>99.26 ± 0.00</td>
<td>99.51 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>6.21 ± 0.00</td>
<td>8.47 ± 0.00</td>
<td>12.82 ± 0.00</td>
<td>15.95 ± 0.00</td>
<td>29.78 ± 0.00</td>
<td>43.02 ± 0.00</td>
<td>51.01 ± 0.00</td>
<td>98.70 ± 0.00</td>
<td>97.99 ± 0.00</td>
<td>99.54 ± 0.00</td>
<td>99.26 ± 0.00</td>
<td>99.51 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>5.75 ± 0.00</td>
<td>7.48 ± 0.00</td>
<td>8.97 ± 0.00</td>
<td>10.85 ± 0.00</td>
<td>12.67 ± 0.00</td>
<td>16.70 ± 0.00</td>
<td>34.96 ± 0.00</td>
<td>60.26 ± 0.00</td>
<td>88.51 ± 0.00</td>
<td>95.99 ± 0.00</td>
<td>99.26 ± 0.00</td>
<td>99.51 ± 0.00</td>
<td>99.38 ± 0.00</td>
</tr>
</tbody>
</table>

Note:
1. ± = Standard Error
2. Values in the same column bearing the same superscript do not differ significantly (P ≤ 0.01)
compensate for a moderately increased RBC destruction [18-21]. At present, there are no clear explanations for either phenomenon, i.e., bone marrow relative insufficiency and increased RBC destruction. It has been suggested that the activation of the immune and inflammatory systems through the increasing levels of pro-inflammatory cytokines such as interleukins 1 and 6, TNF-α and INF-δ would induce iron retention by the reticuloendothelial system, gastrointestinal tract and liver, therefore exerting an inhibitory effect on erythroid precursors [22-24]. The erythropoietin production and bone marrow response to it are decreased in cancer anemia [25] and treatment with recombinant erythropoietin has been reported to be only of temporary help [18]. Development of hemolytic anemia associated with increased RBC osmotic fragility is not frequent in cancer and has been associated with a) autoimmune antibodies, b) microangiopathic alterations, and c) chemotherapy [26]. The present study emphasizes the complexity of the mechanisms involved in anemia and apoptosis of other cells induced by cancer chemotherapy.

Conclusion

Osmotic fragility of erythrocytes was tested in-order to assess the effect of carbamate insecticides on normal cells while attempting to treat cancer. Nevertheless it’s a fact that cancer chemotherapy using any drug is more deleterious to normal tissues than to the tumor tissue itself and carbamates will be no exceptional. This study shows that the maximum effect of the carbamate, carbaryl on erythrocytes is bearable and reversible by the internal physiology of the human body and hence carbamates can form one among the usable chemicals for cancer chemotherapy. However, we recommend that the use of carbamates in the treatment of cancer cannot be undertaken systematically. These chemicals may be used to target specific tissues through the use of site specific target agents like the ‘smart-bombs’ developed by the team of scientists [27] at the School of Chemistry, Tel Aviv University (TAU), Israel, for the delivery of drugs specifically to the tumor tissue. The systemic toxic effects of these chemicals, especially on the neuro-muscular junctions may be prevented by the concomitant use of specific antidotes as shown by Kimmarle [28] in his experiments, wherein he used 50 mg/kg atropine sulfate prior to the intraperitoneal dose of 100 mg/kg propoxur, without any neurotoxic signs.

Authors’ Contributions

Dr. Mohammed Amanullah has conceived the concept and organized the design of the sequential experiments in the study of toxicity of carbamate insecticides and their use as chemotherapeutic agents for cancer, carried out the osmotic fragility test and drafted the complete manuscript. Dr. Gaffar Sarwar Zaman collected the blood samples from the slaughter house, brought them to the laboratory and processed them for use in the present experiment and helped in the centrifugation and taking the spectrophotometric readings. He was also involved in acquisition of data and revising the manuscript critically for important intellectual content. Dr. Naseem Begum participated in the design of the study, analysis and interpretation of data and performed the statistical analysis, conceived the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgement

The authors are grateful to the authorities of Karnataka VAFS University, Bidar, India for providing necessary facilities to carry out the present work.

References


Author Affiliation

*Department of Clinical Biochemistry, College of Medicine, King Khalid University, Kingdom of Saudi Arabia*