CHAPTER 5

Effect of several non-steroidal anti-inflammatory and analgesic drugs on the release of lysosomal enzymes from platelet granules under normal and leukaemic conditions
Introduction

Platelets contain lysosomal granules, the role of which in the platelet functions has also been demonstrated by various workers (1,2). Under leukaemic conditions, platelets show some abnormalities in their functions (3,4). O'Brien (5,6) first suggested that platelet function is directly connected with inflammatory process and almost all the compounds having some effects on platelet function also have anti-inflammatory properties and compounds inactive in the platelet tests (e.g. analgesic and pyretic drugs) have almost no anti-inflammatory activity. Recently the effect of several steroidal and non-steroidal anti-inflammatory drugs on the lysosomal membrane has been firmly established (7). The purpose of the present work is to find out the effect of some pharmacologically active non-steroidal anti-inflammatory and analgesic drugs on the stabilization of the membrane of human platelet granules to release acid phosphatase and \( \beta \)-glucuronidase under either normal or leukaemic conditions.

Materials and Methods

Methods

Conditions of patients, collection of blood, isolation of platelets have been described in Chapter One.
Isolation of platelet granules for drug action

Normal and leukaemic platelets were suspended in 0.34 M sucrose - 0.04 M Tris-acetate buffer, pH 7.4, in the ratio of 1:8 (v/v) of the packed cell. The suspensions were homogenized in Potter-Elvehjem homogenizer fitted with mechanically driven Teflon pestle (radial clearance of 0.003 to 0.005 inch) for 30 seconds and centrifuged at 1,800 g for 15 minutes to sediment cell debris. The sediment was washed and the washings were mixed with the supernatant. The supernatant was then centrifuged at 23,000 g for 20 minutes to obtain platelet granules. Microscopic analysis showed that there is no intact or damaged cells or cell debris in the supernatant.

Determination of latency of the enzymes in platelet granules in hypotonic sucrose solution (experimental medium)

0.5 ml of the granule preparation of both normal and leukaemic platelets were suspended in 9.5 ml of 0.04 M sucrose - 0.04 M Tris-acetate buffer, pH 7.4, at 37°C and after a definite interval of time (30 minutes, 60 minutes, 90 minutes and 120 minutes) 2.0 ml of the suspensions from the incubation mixture were taken and centrifuged at 27,000 g for 20 minutes. The supernatants were used for the assay of released enzymes. Total lysosomal enzyme activities were determined by treatment of 0.2 ml of the granule suspension with 0.1% (v/v) of Triton X-100. After repeated freezing and thawing it was centrifuged at 27,000 g for 20 minutes and the supernatant was used for the enzyme assay.
Preparation of drug solution

Chloroquine phosphate and paracetamol were dissolved in water. Phenylbutazone, oxyphenbutazone, acetylsalicylic acid and phenacetin were dissolved in NaOH solution of equivalent molarity. The pH was adjusted to 7.2.

In vitro effect of the anti-inflammatory and analgesic drugs on the activities of platelet lysosomal enzymes

In experiments to check the *in vitro* effect, if any, of the drugs used on the platelet lysosomal enzyme activities, the granules were preincubated with the drug solutions containing 0.1% (v/v) Triton X-100 and after preincubation, the specific activities of acid phosphatase and β-glucuronidase in the control and the treated groups were estimated. After suspension of the granules in 0.25 M sucrose-0.04 M Tris-acetate buffer pH 7.4, the granules were preincubated at first with 50 mM acetate buffer, pH 5.0, containing 0.1% (v/v) Triton X-100 with or without drugs at 37°C for 10 minutes or for 90 minutes. The enzymes were then assayed and specific activities of the enzymes in each group were estimated. Percent specific activities of the corresponding control groups were calculated for each drug.

Incubation of platelet granules with drug solution

The granule fractions were resuspended in cold 0.4 M sucrose - 0.04 M Tris-acetate buffer, pH 7.4. An aliquot of 0.2 ml was added to each incubation mixture containing 1.6 ml.
of 0.04 M Tris-acetate buffer, pH 7.4, with or without drugs. The final volume of the incubation medium was maintained to 2.0 ml. The mixtures were incubated in incubator shaker (80 strokes per minute) for 60 minutes at 37°C. After incubation the mixtures were centrifuged at 27,000 g for 20 minutes at 0°C and the supernatants were used for the assay of the released enzymes.

**Enzyme assays**

The details of the assay system for acid phosphatase and β-glucuronidase were the same as described in Chapter One.

**Estimation of protein**

Protein content of all the experiments were determined according to the method described by Lowry et al. (8).

**Materials**

All the materials used in this study have been described in Chapter Three.
Results

In vitro lysosomal enzyme release in hypotonic sucrose solution (experimental medium)

The in vitro release of acid phosphatase and $\beta$-glucuronidase from platelet granule fractions under normal and leukaemic conditions in hypotonic sucrose (0.04 M sucrose - 0.04 M Tris-acetate buffer, pH 7.4) at a temperature of 37°C was plotted in Figure 1. Total activities of the enzymes were assayed after complete disruption of the granule fractions by repeated freezing and thawing in 0.1% (v/v) Triton X-100. The above figure illustrates that the latency of the enzymes in the granule fractions in the hypotonic sucrose decreases steadily in respect of time initially but at 90 minutes onward the activities of released enzymes reach a stationary state. It is also noted from Figure 1 that $\beta$-glucuronidase is released more rapidly than acid phosphatase in both the cases. The extent of release of the enzymes from normal platelet granules is more than that of leukaemic platelets.

In vitro effect of several anti-inflammatory and analgesic drugs on the activities of platelet lysosomal enzymes under normal and leukaemic conditions

Table 1 shows that the activities of acid phosphatase and $\beta$-glucuronidase of platelet granules under either normal or leukaemic conditions are not severely affected by the addition of anti-inflammatory and analgesic drugs at a concentration
Effect of anti-inflammatory and analgesic drugs on platelet lysosomal enzyme release under normal and leukaemic conditions

Table 2 illustrates that the specific activities of acid phosphatase and $\beta$-glucuronidase in the supernatants after preincubation of the platelet granules with the anti-inflammatory drugs are very low in comparison to that of the control under either normal or leukaemic conditions. Table 2 further shows that the extent of release of acid phosphatase in the supernatant is less inhibited by the above drugs than that of $\beta$-glucuronidase from both normal and leukaemic platelet granules. The response of these drugs on the release of these enzymes from normal and leukaemic platelet granules appears to be the same. It is also noted from Table 2 that the two analgesics of the chemical nature viz. paracetamol and phenacetin do not alter markedly the specific activities of the two enzymes in the supernatants under both normal and leukaemic conditions.

Discussion

Human normal and leukaemic platelet lysosomal enzymes are found to be released in hypotonic sucrose solution at 37°C and the extent of release increases with time. $\beta$-glucuronidase is released more rapidly than acid phosphatase with respect to time of incubation and the
extent of release of the lysosomal enzymes from normal platelet granules is higher than that of leukemic platelet granules in the hypotonic sucrose solution (Fig. 1). Previous observation on the distribution study of platelet lysosomal enzymes under either normal or leukemic conditions showed that the leukemic platelet lysosomes are less fragile in isotonic sucrose medium (Chapter Four) and it is noted here that leukemic platelet lysosomes behave in a similar fashion also in hypotonic medium. It can be concluded from the present investigation that the leukemic platelet lysosomes are less permeable to sucrose than normal making the leukemic platelet lysosomes less fragile.

Several steroidal and non-steroidal anti-inflammatory drugs are known to stabilize lysosomes isolated from different sources (9). It was also found that some acidic non-steroidal anti-inflammatory drugs have some biphasic action on the release of lysosomal enzymes depending on the concentration of the drugs used (10).

The results presented in Table 2 revealed that several non-steroidal anti-inflammatory drugs have some effect on the stabilization of the human normal and leukemic platelet lysosomes to release their enzymes in hypotonic solutions. The results presented in Table 1 also point out that the non-steroidal anti-inflammatory and analgesic drugs have no direct adverse effect on the enzymes of platelet granules even on 90 minutes of preincubation at 37°C. The decreased activities of
acid phosphatase and β-glucuronidase in the supernatant medium after preincubation of the platelet granules in presence of non-steroidal acidic and basic anti-inflammatory drugs are entirely due to the stabilizing effect of these drugs on the membrane of platelet granules under both normal and leukemic conditions.

The nature of binding of different enzymes on lysosomes was noted earlier (10,11). Figure 1 and Table 2 indicate the nature of binding of acid phosphatase and β-glucuronidase on human platelet lysosomes both under normal and leukemic conditions. It is observed that β-glucuronidase is more readily released and is also more sensitive to the drug action than that of the firmly bound acid phosphatase in hypotonic medium. Similar observations were noted earlier using the leucocyte system (Chapter Three).

It is indicated that the responsiveness of these drugs on the stabilizing action of normal platelet granules is not different from that of leukemic platelet granules. The two analgesics, paracetamol and phenacetin, which have either none or very mild effect on human platelet functions (5) have very poor stabilizing effect on human normal and leukemic platelet granules.
References

Table 1

*In vitro* effect of some non-steroidal anti-inflammatory and analgesic drugs on the activities of platelet lysosomal enzymes under normal and leukaemic conditions

(Each result is expressed as mean ± S.D. of four individual experiments)

<table>
<thead>
<tr>
<th>Drugs (10^-5 M)</th>
<th>Normal platelet lysosomal enzyme activity as % activity of corresponding control</th>
<th>Leukaemic platelet lysosomal enzyme activity as % activity of corresponding control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid phosphatase</td>
<td>10 minutes</td>
</tr>
<tr>
<td></td>
<td>10 minutes</td>
<td>90 minutes</td>
</tr>
<tr>
<td>Chloroquine phosphate</td>
<td>100 ± 7.9</td>
<td>98 ± 6.0</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>95 ± 6.6</td>
<td>92 ± 8.2</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>105 ± 9.3</td>
<td>95 ± 7.4</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>98 ± 7.9</td>
<td>96 ± 6.7</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100 ± 9.2</td>
<td>94 ± 7.8</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>97 ± 8.9</td>
<td>95 ± 10.2</td>
</tr>
</tbody>
</table>
### Effect of some anti-inflammatory and anesthetic granules under normal and leukaemic conditions

(Each result is expressed as mean ± S.D. of five

<table>
<thead>
<tr>
<th>Conditions of experiments</th>
<th>Acid phosphatase</th>
<th>Sp. activity in the supernatant of release of platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.92 ± 5.6</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>Control + Chloroquine phosphate</td>
<td>29.88 ± 5.1</td>
<td>49 ± 7.1</td>
</tr>
<tr>
<td>Control + Phenylbutazone</td>
<td>27.10 ± 4.6</td>
<td>55 ± 6.1</td>
</tr>
<tr>
<td>Control + Oxyphenbutazone</td>
<td>29.95 ± 5.9</td>
<td>49 ± 6.7</td>
</tr>
<tr>
<td>Control + Acetylsalicylic acid</td>
<td>32.20 ± 3.1</td>
<td>46 ± 7.1</td>
</tr>
<tr>
<td>Control + Paracetamol</td>
<td>52.80 ± 6.2</td>
<td>15 ± 3.7</td>
</tr>
<tr>
<td>Control + Phenacetin</td>
<td>53.90 ± 8.5</td>
<td>11 ± 3.0</td>
</tr>
</tbody>
</table>

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**Table 2**
In vitro release of lysosomal enzymes in hypotonic sucrose solution from platelet granules under normal and leukemic conditions.

Fig. 1

In vitro release of lysosomal enzymes in hypotonic sucrose solution from platelet granules under normal and leukemic conditions.

- Leukaemic
- Normal
- (-) α-glucuronidase
- (---) Acid phosphatase

% Relative specific activity

30 60 90 120
Time in min