Dose dependent reduction of erythroid progenitor cells and inappropriate erythropoietin response in exposure to lead: new aspects of anaemia induced by lead

W Osterode, U Barnas, K Geissler

Abstract

Objective—To determine whether haematopoietic progenitor cells and erythropoietin (EPO), which is an essential humoral stimulus for erythroid progenitor (BFU-E) cell differentiation, are affected by lead intoxication.

Methods—In male subjects chronically exposed to lead with and without anaemia, pluripotent (CFU-GEMM), BFU-E and granulocyte/macrophage (CFU-GM) progenitor cell counts in peripheral blood were measured with a modified clonal assay. Lead concentrations in blood (PbB) and urine (PbU) were measured by the atomic absorption technique, and EPO was measured with a modified radioimmunoassay.

Results—PbB in the subjects exposed to lead ranged from 0.796 to 4.4 µmol/l, and PbU varied between 0.033 and 0.522 µmol/l. In subjects exposed to lead with PbB > 2.896 µmol/l (n=7), BFU-E cells were significantly reduced (p<0.001) whereas the reduction in CFU-GM cells was only of borderline significance (p = 0.037) compared with the age matched controls (n=20). The CFU-GEMM cells remained unchanged. Furthermore, BFU-E and CFU-GM cells were reduced in a dose dependent fashion, with increasing PbB or PbU, respectively. In the subjects exposed to lead EPO was in the normal range and did not increase in the presence of anaemia induced by lead. No correlations existed between EPO and PbB or PbU, respectively. In subjects with no renal failure, serum EPO concentrations were significantly reduced (p<0.001) compared with the age matched controls (n=20). The mean (SD) of EPO in normal controls and anaemic patients was 50.4±10.6 mU/ml and 10.7±2.9 mU/ml, respectively. Reduced packed cell volume in subjects exposed to lead was significantly correlated with EPO in anaemic patients (r=0.61, p<0.05).

Conclusion—The data suggest new aspects of lead induced anaemia besides the currently acknowledged shortened life span of erythrocytes and inhibition of haemoglobin synthesis. Two additional mechanisms should be considered: the reduction of BFU-E cells, and inappropriate renal EPO production in the presence of severe exposure to lead, which would lead to an inadequate maturation of BFU-E cells.

Keywords: blood lead; progenitor cells; erythropoietin

Anæmia in subjects exposed to lead is at present thought to be mainly caused by the inhibition of haemoglobin synthesis (basically by the inhibition of δ-aminolevulinic acid dehydratase activity) and shortened life span of red blood cells. Apart from a haemolytic component, it seems likely that additional mechanisms are involved in anaemia induced by lead. The profound effect of lead on red cell precursors in bone marrow was suspected some years ago. An antimimetic effect of lead on erythroblast nuclei at metaphase has been shown previously. However, at present no in vivo data are available in regard to haematopoietic progenitor cells and exposure to lead. Such progenitor cells form colonies of haematopoietic cells in vitro which represent the differentiation of an individual precursor cell in the presence of an appropriate humoral stimulus (colony stimulating factor (CSF) or erythropoietin (EPO)). In determining the size of the total progenitor cell compartment peripheral blood progenitors have been shown to be better indicators than those in bone marrow where the concentrations depend on the variable blood dilution in bone marrow obtained by puncture. Moreover, it has been shown that there is a stable equilibrium between the pool of progenitor cells in bone marrow and that of peripheral progenitor cells.

We therefore investigated the question whether peripheral progenitor cells or at least...
Table 1 Data of subjects exposed to lead (n=20) and age matched controls (n=20)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Exposed group (n=20)</th>
<th>Control group (n=20)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PbB (µmol/l)</td>
<td>2.195</td>
<td>0.198</td>
<td>0.001</td>
</tr>
<tr>
<td>PbU (µmol/l)</td>
<td>0.225</td>
<td>0.018</td>
<td>0.007</td>
</tr>
<tr>
<td>Erythrocytes (x10^12/l)</td>
<td>4.8</td>
<td>5.1</td>
<td>0.34</td>
</tr>
<tr>
<td>Packed cell volume (x10^12)</td>
<td>43.2</td>
<td>44.2</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.8</td>
<td>14.9</td>
<td>NS</td>
</tr>
<tr>
<td>Reticulocytes (G/l)</td>
<td>56</td>
<td>43</td>
<td>NS</td>
</tr>
<tr>
<td>EPO (mU/ml)</td>
<td>13.5</td>
<td>11.7</td>
<td>NS</td>
</tr>
<tr>
<td>BFU-E (cells/ml)</td>
<td>819</td>
<td>98</td>
<td>NS</td>
</tr>
<tr>
<td>CFU-GM (cells/ml)</td>
<td>269</td>
<td>339</td>
<td>NS</td>
</tr>
<tr>
<td>CFU-GEMM (cells/ml)</td>
<td>39</td>
<td>51</td>
<td>NS</td>
</tr>
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</tr>
</tbody>
</table>

Table 2 Correlation coefficients R (Spearman rank order) between progenitor cells in peripheral blood and blood lead (PbB) and urinary lead (PbU)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>PbB (µmol/l)</th>
<th>PbU (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>p Value</td>
<td>R</td>
</tr>
<tr>
<td>CFU-GEMM</td>
<td>−0.06</td>
<td>0.81</td>
</tr>
<tr>
<td>BFU-E</td>
<td>−0.16</td>
<td>0.49</td>
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</table>

Figure 2 Progenitor cells in peripheral blood in non-exposed control subjects (n=20), in subjects moderately exposed to lead (PbB<2.896 µmol/l, n=13), and in subjects highly exposed to lead (PbB≥2.896 µmol/l n=7).

Erythroid progenitor (BFU-E) cells are reduced in lead intoxication, and as lead has a tubulotoxic effect at a site where EPO is synthesised, whether EPO excretion is also affected. It was hoped that the results would help to elucidate the pathology of anaemia induced by lead.

Patients and methods

SUBJECTS

Twenty male subjects chronically exposed to lead for a mean (SD) of 46 (7) years, working in various manufacturing processes—such as smelting, casting, etc—gave their informed consent to having an additional 7 ml blood sample taken at their regular check up for blood chemistry. Subjects with known haematological, cardiovascular, hepatic, or renal disease were excluded.

Control data were obtained from 20 age matched healthy, non-exposed men (mean (SD) age 42 (9) years).

PROGENITOR CELL ASSAY

Pluripotent (CFU-GEMM), BFU-E, and granulocyte/macrophage (CFU-GM) progenitor cells in peripheral blood were measured with a modified clonal assay. Cultures were stimulated with 100U/ml rhGM-CSF (Sandoz AG), 10U/ml rhIL-3 (Sandoz AG) and 1mU/ml EPO (Toyobo, Osaka, Japan). Peripheral mononuclear cells were plated in triplicate at 2x10^6/ml. After 14 days (at 37°C, 5% CO2, full humidity) cultures were examined under an inverted microscope. Aggregates with more than 50 translucent, dispersed cells were counted as CFU-GM cells. Bursts containing >100 red coloured cells were scored as BFU-E cells. CFU-GEMM cells were identified by their heterogeneous composition of translucent and haemoglobinised cells. Results are expressed as progenitor cells/ml blood.

EPO ASSAY

Serum samples were stored at −20°C until simultaneous measurement of EPO concentrations with a modified specific radioimmunoassay previously described. Concentrations of EPO in a control group (n=25), ranged between 5 and 25 mU/l with a variance between assays <20% (n=7) and a variance within assays <10% (n=7).

In various anaemic patients (n=40) with no renal impairment, the relation between EPO and packed cell volume was determined from a standard curve for packed cell volume between 25% and 50% (fig 1, line) for normal and anaemic patients (each n=8).

LEAD CONCENTRATIONS IN BLOOD AND URINE

The PbB and PbU were measured by atomic absorption with a Perkin Elmer 2380 spectrophotometer with a graphite furnace.

STATISTICS

The Spearman rank order method was used to calculate the correlations. A t test was used for comparisons. If the normality test used (Kolmogorov-Smirnov) failed, a non-parametric alternative (Mann-Whitney) was used. Data are represented as means (SD).

Results

Table 1 summarises haematological data, PbB, and PbU for workers exposed to lead and the data from 20 aged matched subjects not exposed to lead.

No differences existed between median values of haematological variables for exposed subjects and the controls. The same applied to progenitor cells. However, in subjects exposed to lead with PbB≥2.896 µmol/l (n=7), the range in which haematological alterations are mainly expected, BFU-E cell counts were significantly reduced compared with controls (n=20) (484 (317) v 1027 (322), p < 0.001), whereas CFU-GM cells only showed a border-
line reduction (163 (75) vs 396 (268), p=0.034). The CFU-GEMM cells were not altered (fig 2). Moreover, a significant negative correlation existed between PbB and BFU-E or CFU-GM cells in the subjects exposed to lead. This correlation was more pronounced between PbU and BFU-E or CFU-GM, respectively (table 2 and fig 3 A and B).

Median serum EPO concentrations in the subjects exposed to lead did not differ from those in the control group (table 1). However, although EPO increased exponentially in the presence of anaemia and normal renal function (fig 1, open circles), EPO in the subjects exposed to lead (fig 1, filled circles) failed to increase when packed cell volume or haemoglobin was reduced (filled squares). Because EPO consistently remained in the normal range, no correlations were found between EPO and PbB, PbU, or progenitor cells.

Discussion
We describe for the first time the effect of exposure to lead on peripheral blood progenitor cells. Our results show that BFU-E and CFU-GM cells in patients with high PbB are significantly reduced, and that the reduction is dose dependent.

Blood traversing the bone first passes through a surface region of rapid exchange before entering the metabolically active region of bone. After leaving the larger vessels, plasma superfusate enters the canalicules that supply bulk bone. Lead can thus diffuse into the total bone compartment and establish contact with bone marrow. As shown in table 2, a much higher level of significance was achieved between BFU-E and PbU than between these cells and PbB. Lead in the blood pool and that in bone are in equilibrium. The loss of lead by renal excretion is mainly restored by a lead fraction from bone, where lead gets into contact with bone marrow. We assume that this may be the reason why the correlation between BFU-E and PbU is more significant than that with PbB. On the other hand, the higher correlation in table 2 for PbU than for PbB could be simply due to the larger range of PbU values.

Recent investigations showed that progenitor cells are affected by lead. Inhibition of haem synthesis induced apoptosis in human BFU-E cells. In these experiments succinyl-lactetone, a specific inhibitor of δ-aminolevulinic acid dehydratase or isonicotinic acid hydracide, an inhibitor of δ-aminolevulinic acid synthetase was used. Experiments were done on highly purified human erythroid colony forming cells (CFU-E) which differentiate from BFU-E cells. Addition of haemin to the culture medium could reverse this effect. Lead is known to be an effective inhibitor of haem synthesis in red blood cells as well. Although the mechanism by which the inhibition of haem synthesis produces apoptosis in progenitor cells is not clear, we assume that our results are due to apoptosis, and could thus explain a dose dependent reduction of BFU-E cells during exposure to lead. Haematological effects of lead are the only ones for which dose-response relations have been established. Moreover, it was shown that zinc porphyrins are potent inhibitors of haematopoesis in animal and human bone marrow. Colony growth of BFU-E and CFU-GM cells was inhibited by zinc protoporphyrin. This is endogenously formed and found in high concentrations in red blood cells, in the presence of exposure to lead. It was suspected that zinc protoporphyrin may itself participate in bone marrow toxicity by lead.

Erythropoietin is a humoral factor that stimulates the differentiation of BFU-E cells. Reduced EPO and increased PbB were found in pregnant women. In a group of 1502 women, analysis of variance showed that women with higher PbB had lower serum EPO concentrations. In our smaller group, the serum EPO values showed no difference between subjects exposed to lead and controls. However, as shown in figure 3, no increase in EPO was registered in patients with anaemia who were exposed to lead. Because the main fraction of EPO (>90%) is produced in peritubular capillary lining cells of the kidney, we suspect that the tubulotoxicity of lead is the reason why EPO is not adequately generated at higher PbB.

The mechanism by which lead intoxication reduces EPO is not clear, but it is suspected that lead interferes with calcium. An increased entry of calcium into renal cells capable of producing EPO is necessary if tissue hypoxia is found. In general, it has been shown...
that lead and calcium interfere in many tissues. Also, electrophysiological studies have shown that lead (active from inside the erythrocytes) can mimic Ca$^{2+}$ ions and activate calcium dependent potassium channels even at low concentrations. An impaired calcium entry may cause EPO concentrations to be reduced.

Surprisingly, we registered a decrease in BFU-E cells as well as CFU-GM cells in the presence of severe exposure to lead. Two reasons may account for this finding. Administration of EPO to patients with renal failure increased BFU-E and CFU-GM cell concentrations suggesting that in vivo EPO increased BFU-E and CFU-GM cell counts. This may be a stimulator of the CFU-GM cell compartment as well. In fact, endogenous EPO concentrations in subjects exposed to lead were not increased in the presence of anaemia as expected. The second reason may be the endogenously formed zinc protoporphyrin in exposure to lead, which could also have reduced CFU-GM cells.

A delayed blood regeneration was registered in subjects exposed to lead who had donated 0.45 l blood, as shown by lower haemoglobin concentrations 15 days after blood donation. This phenomenon could be explained by our results. Reduced BFU-E cell concentration and inappropriate EPO production in these subjects may account for their suspected reserve capacity for blood formation.

In conclusion, our data suggest a new aspect of lead induced anaemia. It seems that two mechanisms besides the shortened life span of red blood cells are additionally involved. They are: (a) the reduction of BFU-E cells and (b) an inappropriate secretion of EPO, which may impair the differentiation of BFU-E cells.

The skilful and excellent assistance of Ms DIJ Jäger in measuring PbB and PbU is gratefully acknowledged.

References:

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