Abnormal haemoglobin electrophoresis caused by BW 12C

F van Rhee, I Ware, T Littlewood

Abstract
BW 12C, a substituted benzaldehyde, stabilises haemoglobin in the oxy-conformation and has attracted interest as an anti-sickling agent. The drug causes a left shift in the oxygen saturation curve and may induce tumour anoxia and enhance the effect of cytotoxic drugs. During clinical trials in patients with cancer abnormal bands on haemoglobin electrophoresis strips were observed. This made correct diagnosis of abnormal haemoglobins impossible. Solubility tests for sickling disorders (Itano) also proved unreliable.

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BW 12C is a substituted benzaldehyde which causes a dose dependent left shift in the oxygen saturation curve by binding to oxyhaemoglobin. The drug inhibits sickling by increasing red cell oxyhaemoglobin and reducing the polymerisation of deoxyhaemoglobin. It was initially designed to treat the crises of sickle cell anaemia. BW 12C induces tumour anoxia and necrosis by reducing peripheral oxygen delivery, and is currently being evaluated in clinical trials for its anti-neoplastic activity. BW 12C may synergise with and enhance the cytotoxic effects of chemotherapeutic agents such as mitomycin C which are selectively toxic towards hypoxic or anoxic cells.

![Haemoglobin electrophoresis pH 8.3 (TRIS/EDTA/borate buffer)]

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<tbody>
<tr>
<td>1</td>
<td>Normal blood no drug</td>
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<tr>
<td>2</td>
<td>Normal blood + 350 μg/ml</td>
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<td>3</td>
<td>Normal blood + 700 μg/ml</td>
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<td>4</td>
<td>Sickle cell trait no drug</td>
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<td>Sickle cell trait + 350 μg/ml</td>
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Haemoglobin electrophoresis of normal and sickle cell trait blood after incubation with BW 12C.

Case report
An abnormal band was observed during haemoglobin electrophoresis in a 62 year old Chinese man who was receiving treatment with BW 12C and mitomycin C for metastatic adenocarcinoma. His red cell indices suggested thalassaemia trait, and haemoglobin electrophoresis on cellulose acetate at pH 8.9 showed a fast moving band relative to the HbA position. Haemoglobin electrophoresis on citrate agar at pH 6.2 was unaffected. When haemoglobin electrophoresis was repeated one day later the abnormal band had disappeared. In published studies with healthy volunteers no clinically relevant effect of BW 12C on the oxygen saturation curve could be shown after 24 hours and the elimination half life was estimated to be 8-4 hours.

The abnormal band could be reproduced in vitro by incubating lysates of red cells with therapeutic doses of BW 12C, indicating that BW 12C rather than an active metabolite was responsible for the observed effect (figure). With red cells of patients with structural variants such as sickle cell trait, HbC trait, and Hb S C disease were studied, additional bands were noted after incubation with BW 12C, making correct interpretation of the results impossible. In homozygous sickle cell anaemia an additional band close to the HbA position was observed.

Routine screening tests (sickle cell preparations and Itano solubility tests) for sickling disorders gave false negative results after BW 12C in heterozygous conditions such as sickle cell trait; in homozygous sickle cell anaemia only low grade positive reactions were recor-ded. By stabilising sickle cell haemoglobin in the oxy-conformation BW 12C protected against the formation of insoluble deoxyhaemoglobin induced by reducing agents (metabisulphite and dithionite) used in screening tests.

Accurate diagnosis of thalassaemia disorders was difficult because the mobility of the haemoglobin A2 band was also altered significantly. Haemoglobin F as measured by the Betke alkali denaturation method was not affected.

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