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A study of Naphthionin in the management of the bleeding defect in patients with thrombocytopenia

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SYNOPSIS Sodium-α-naphthylamine-4-sulphonate (Naphthionin) appears to shorten the bleeding time in normal subjects and to be of value in the management of the bleeding defect in patients with thrombocytopenia when given by intravenous or intramuscular injection. A good response was achieved in three patients suffering from severe haemorrhage and the prolonged bleeding time in other cases of thrombocytopenia was shortened. There is no evidence that Naphthionin is of value in haemostasis in other patients in whom haemorrhage is not due directly to thrombocytopenia.

The intravenous administration of Congo Red was said to be of value in the management of bleeding in thrombocytopenia (Bruhl, 1933), and sodium-α-naphthylamine-4-sulphonate (Naphthionin) was claimed by Esteve, Oriol, and Regné (1949) to be the active principle of Congo Red. Poller (1955) showed that intravenous injection of Naphthionin produced a shortening of the bleeding time in normal adults. Naphthionin is believed to lower the isoelectric point of fibrinogen to a pH in the region of the blood level of 7-3, and thus fibrinogen may be converted more easily from a sol to a gel state; the haemostatic action of Naphthionin is attributed to this effect on fibrinogen. In the present study Naphthionin was given intravenously to a group of patients with thrombocytopenia and its action assessed by measuring the bleeding time and by the clinical effect.

METHOD OF STUDY

The effect of Naphthionin was first observed in 10 normal adults by estimating the Lee and White clotting time, the bleeding, prothrombin, and heparin clotting times, and the only significant change was observed in the bleeding time, the greatest change being one hour after injection. A group of 10 patients suffering from thrombocytopenia with associated purpura was also studied, six of whom had idiopathic thrombocytopenia and the remaining four secondary thrombocytopenia. Bleeding times were, therefore, estimated before injection and one hour after the intravenous dose (10 ml.) in the patients with thrombocytopenia. Platelets were counted immediately before injection. Initially platelets were also counted one hour after injection but as these results were similar, this practice was discontinued. Some of the patients were suffering from acute haemorrhagic episodes and in these the clinical effects were carefully observed. In several cases after the initial intravenous dose, a course of Naphthionin was given, 5 ml. intramuscularly six-hourly, and the effects observed over several days.

TECHNIQUE

The bleeding time was estimated by the method of Duke (1915). Blotting was performed at 15 sec. intervals. Times were recorded up to 12 minutes but in cases in which bleeding had not stopped the results were recorded simply as exceeding 12 minutes.

LEE AND WHITE (1913) clotting method (modified)

Blood (5 ml.) was withdrawn into a dry syringe from an antecubital vein of a healthy adult in the minimum time after the application of a tourniquet. The whole process was accomplished in under 30 seconds. Three 1 ml. amounts of blood were delivered into three × 3½ in. test tubes which had been allowed to warm to 37°C. in a water-bath. The residual 2 ml. of blood in the syringe was discarded to reduce contamination by tissue juices rich in thromboplastin. As soon as the first blood had entered the syringe timing was begun with a stop-watch. The first tube was tilted gently at 30-second intervals until a firm, totally adherent clot formed, which could not be dislodged by complete inversion of the tube. This was regarded as the end-point in the clotting reaction and the same procedure was repeated on the second and third tubes.

The paper was written a failure has been encountered with the drug in a case of aplastic anaemia and thrombocytopenia following the administration of Thiotepa. This may be due to alteration of the fibrinogen molecule by Thiotepa.

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PROTHROMBIN ESTIMATIONS

Prothrombin times were based on the Quick single-stage principle (Quick, 1935) using a human brain thromboplastin extract. This gave a normal reading of 12 to 14 seconds.

HEPARIN PLASMA CLOTTING TIMES

The method of Poller (1954) was used. A slight deviation from the technique previously described was the performance of the test 30 minutes after collection. This has resulted in longer clotting times owing to the reduction of the effects of storage in glass on oxalated plasma.

PLATELET COUNT

The method of Lempert (1935) was employed.

RESPONSE TO INTRAVENOUS NAPHTHIONIN IN HEALTHY ADULT MALES

The effect of the intravenous administration of 10 ml. (lg.) Naphthionin was observed in 10 healthy young adult males. Before injection the bleeding time, clotting time (by the Lee and White method), prothrombin time, and the heparin retarded clotting time were estimated.

Ten millilitres (lg.) of the drug was injected. Bleeding, clotting, and prothrombin times were repeatedly tested at 30-minute intervals over a three-hour period while heparin-retarded tests were repeated hourly. The effect on bleeding, clotting, and prothrombin times may be observed in Table I.

The significant result was the appreciable reduction of the bleeding time, which appeared to be greatest one hour after injection. The bleeding time is a crude test, yet the differences noted are reasonably marked. The mean deviation is 0.775, which is significantly different from zero (P < 1%).

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age</th>
<th>Type of Thrombocytopenia</th>
<th>Platelet Count</th>
<th>Bleeding Time (min.)</th>
<th>Bleeding Time 1 Hour after Naphthionin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D.V.</td>
<td>18</td>
<td>Idiopathic</td>
<td>3,000</td>
<td>&gt; 12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>D.H.</td>
<td>20</td>
<td>Idiopathic</td>
<td>50,000</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F.B.</td>
<td>32</td>
<td>Idiopathic</td>
<td>70,000</td>
<td>2½</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>G.M.</td>
<td>41</td>
<td>Idiopathic</td>
<td>40,000</td>
<td>8½</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M.O.</td>
<td>36</td>
<td>Idiopathic</td>
<td>35,000</td>
<td>&gt; 12</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>H.G.</td>
<td>75</td>
<td>Idiopathic</td>
<td>8,000</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>W.C.</td>
<td>42</td>
<td>Secondary (aplastic)</td>
<td>11,000</td>
<td>25</td>
<td>15 (after ¼ hour).</td>
</tr>
<tr>
<td>8</td>
<td>C.J.</td>
<td>40</td>
<td>Secondary (carcinoma)</td>
<td>30,000</td>
<td>&gt; 12</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>K.H.</td>
<td>33</td>
<td>Secondary (acute leukaemia)</td>
<td>15,000</td>
<td>10½</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>A.B.</td>
<td>29</td>
<td>Secondary (acute leukaemia)</td>
<td>8,000</td>
<td>&gt; 12</td>
<td>2½</td>
</tr>
</tbody>
</table>

RESULTS IN THROMBOCYTOPENA

The initial results in 10 patients treated with Naphthionin are seen in Table II.

CASE 1 D.V., a woman, had a history of bruising for several years. The gums bled excessively after teeth had been extracted and in the subsequent two months before admission she became increasingly tired. On admission she had multiple ecchymoses and petechiae. The Hess test was positive, Haemoglobin was 38%, platelets 10,000, bleeding time 8½ min. She was treated with blood transfusion, iron, and prednisolone, 60 mg. daily. The platelet count improved to reach a peak of 132,000 two months later (Hb 96%) on 30 mg. prednisolone daily. Within a further four weeks the platelets fell to 9,000 (Hb 90%) and a marked Cushing's appearance developed. A narrow puncture confirmed the diagnosis of idiopathic thrombocytopenic purpura. The bleeding time before marrow puncture was over 12 minutes. Naphthionin, 10 ml. was given intravenously one hour before the puncture and the bleeding time was reduced to 2½ minutes. There was no excessive bleeding as a result of the puncture. Splenectomy was carried out two weeks later (platelets 6,000) under steroid and Naphthionin cover (lg. ¼ hr. pre-operatively) and there was no excessive blood loss.

CASE 2 In a woman 15 months after splenectomy for idiopathic thrombocytopenic purpura, a peak platelet count of 310,000 had been reached post-operatively and a slow fall occurred thereafter, despite continued treatment with steroids (prednisolone 15 mg. daily), to 35,000 one year later. The patient was admitted for dental
extraction necessitated by pregnancy. She still experienced occasional bruising and had a large haematoma of the leg after a slight injury. The Hess test was positive (platelets 50,000, bleeding time 4 min.). Naphthionin, 10 ml, was given intravenously and the bleeding time after one hour was $\frac{3}{4}$ minutes. An intramuscular course of Naphthionin, 5 ml, 6-hourly, was commenced and extraction accomplished with only slight blood loss. The bleeding time while on intramuscular therapy fell to 2 minutes. A second extraction was performed 10 days later, again under Naphthionin cover, and haemostasis was easily secured.

CASE 3 This woman had idiopathic thrombocytopenia with spontaneous recovery. She was pregnant for the third time four months before coming to hospital because of recent spontaneous bruising. Platelets numbered 42,000; the Hess test was positive. Twelve days later the platelets numbered 70,000, and the bleeding time was $2\frac{1}{2}$ minutes. One hour after 10 ml. Naphthionin injected intravenously it was 3 minutes.

CASE 4 A woman presented with swelling and painful blue discoloration of two toes and a history of purpura over the arms and chest and spontaneous bruising for three or four months. The Hess test was positive. Platelets numbered 40,000, and the bleeding time was $8\frac{2}{3}$ minutes, which became $3\frac{1}{4}$ minutes one hour after intravenous injection of 10 ml. Naphthionin. Marrow puncture performed under Naphthionin cover produced no excessive bleeding. The diagnosis of idiopathic thrombocytopenic purpura was confirmed. A maximum platelet response of 180,000 was reached on prednisolone, 40 mg. daily, and then the response fell off so splenectomy was performed (platelets 87,000) under Naphthionin cover (10 ml. intravenously half an hour pre-operatively, then 5 ml. intramuscularly six-hourly for 48 hours). Excellent haemostasis was secured. A post-operative platelet peak of 300,000 was reached and the count then stabilized at 250,000, its present level.

CASE 5 A woman presented with spontaneous bruising and continuous heavy vaginal bleeding since the onset of her last menstrual period three weeks previously. The Hess test was positive. Platelets numbered 35,000. Bleeding continued despite vaginal packing, and the bleeding time exceeded 12 minutes. Naphthionin, 10 ml., was given intravenously and within one hour the bleeding time was 4 minutes. Within a few hours of the injection haemorrhage ceased and did not recur. An intramuscular course of Naphthionin was not required. Subsequently, administration of prednisolone produced a satisfactory and maintained response.

CASE 6 A woman presented with a history of melena and severe epistaxis for two months, worst in the two weeks before admission. Spontaneous bruising had been observed for about a year previously. On admission she was very pale with multiple bruises and petechiae. The Hess test was positive. Haemoglobin was 23% and platelets 10,000. Transfusions and cortisone were commenced and produced a rise in haemoglobin to 38%, but melena continued and in eight hours the Hb dropped to 32%. The bleeding time at this point was 7 min.; one hour after 10 ml. intravenous Naphthionin it was $1\frac{1}{2}$ min. Within a few hours melaena ceased and the next day the Hb was 29%. Naphthionin was continued intramuscularly for the next three days and 2 more pints of blood were given and iron administered. On the fourth day (Hb 40%, platelets 17,000), after Naphthionin had been discontinued, further melaena occurred which rapidly responded to another three-day course of Naphthionin. Uninterrupted progress was made for the next eight days (Hb 51%, platelets 48,000) when a further slight melaena occurred which again responded to intramuscular Naphthionin.

CASE 7 A woman with rheumatoid arthritis, who had been treated with steroids for the past three years and gold injections before that, had been well until her last period two weeks before when she began losing blood heavily and had several epistaxes and one small haemoptysis. On admission she was pale with numerous bruises on the arms and legs, and still bleeding from the vagina. The Hess test was positive, Hb 36%, W.B.C.s 2,500 with an absolute neutropenia (polymorphs 34%, lymphocytes 63%, monocytes 3%), and platelets 20,000. The bleeding time was over 18 minutes. Despite blood transfusion and vaginal packing, haemorrhage continued. The Hb was raised to 58% by transfusion and a marrow puncture performed which showed an aplastic picture thought to be due to the gold injections. Within three days the Hb had fallen again to 36% (platelets 11,000). Bleeding time was estimated until bleeding ceased (25 min.), and then 10 ml. intravenous Naphthionin was given which reduced the bleeding time to 15 min. in half an hour. Within a few hours bleeding had ceased entirely. Treatment was continued intramuscularly for the next three days (5 ml. six-hourly) and improvement was thereafter maintained, despite the failure of the platelet count to rise, apart from a small loss three weeks later, which was immediately controlled by a further short course of Naphthionin.

CASE 8 A woman was well until two weeks before admission when her normal menstrual period started and she began to feel tired and listless. Within a week she developed breathlessness, pyrexia, and nasal bleeding. Blood then oozed from the gums and lips, and bruises appeared spontaneously on the arms and legs. There was a history of pain and swelling of the left breast one month before which settled in two or three days. A small, soft, tender mass in the left breast and multiple bruises were the only positive physical findings. Haemoglobin was 70%, platelets 30,000, prothrombin activity 24%, and the plasma clotting time 3 min. (normal 1½-3 min.). Poor clots in these tests suggested a fibrinogen deficiency and on estimation the level was 40 mg%. In view of the uncertainty of diagnosis, marrow puncture (which showed a reactive picture with thrombocytopenia) was performed and a haematoma occurred at the site of aspiration. The bleeding time was then found to be over 12 min., so 10 ml. Naphthionin was given intravenously with reduction of the bleeding time to 8 min. in one hour. In view of the fibrinogen deficiency, transfusions of
quadruple-strength plasma and fibrinogen were given. As well as a deficiency in fibrinogen, coagulation tests showed a combined defect involving both 'extrinsic' and 'intrinsic' systems which was treated by transfusion of fresh blood. Naphthionin was continued intramuscularly (5 ml. six-hourly) and as the fibrinogen level rose in response to therapy so the bleeding time shortened (Fig. 1) reducing to 4 min. after 48 hours. Improvement, however, was not maintained and the fibrinogen dropped to 40 mg. % while the bleeding time again exceeded 12 minutes. Despite further fibrinogen and fresh blood transfusions her condition continued to deteriorate and she died the next day.

Post-mortem examination showed a carcinoma of the left breast with multiple metastases, particularly affecting the liver, where there were tumour masses and malignant cells lying free in the sinusoids. The thrombocytopenia appeared to be part of the deficiency syndrome.

CASE 9 A woman presented with a history of malaise and cough. Her last two periods had been heavier than usual lasting eight days, with only a three- instead of a four-week interval. Examination showed multiple bruises over the arms, legs and body, anaemia, and ulcerated, oozing gums. The Hess test was positive. Haemoglobin was 36%, platelets 15,000, and W.B.C.'s 9,500, (10% blasts). Acute myeloid leukaemia was confirmed by marrow puncture performed under Naphthionin cover. The bleeding time was 10½ min. and one hour after 10 ml. intravenous Naphthionin it was 6½ minutes. Intramuscular Naphthionin, 5 ml. six-hourly, was continued for the next three days. The formation of a poor clot suggested that the fibrinogen level was low (75 mg.%). Administration of 5g. fibrinogen after packed cells produced a small rise to 90 mg.% and a corresponding reduction in the bleeding time to 6 minutes. Transfusions were continued and next day the bleeding time had shortened to 5 min. and the fibrinogen level risen to 170 mg.% At this stage the patient died from broncho-pneumonia.

CASE 10 A man presented with a history of weakness and malaise. Platelets numbered 8,000. Acute leukaemia was confirmed by marrow puncture, again carried out under Naphthionin cover. The bleeding time before an intravenous injection of 10 ml. Naphthionin was in excess of 12 min. and one hour later it was 2½ minutes. Haemostasis after marrow puncture was easily secured.

**COMMENTS**

The present study suggests that sodium-α-naphthylamine-4-sulphonate (Naphthionin) is of considerable value in the management of the bleeding defect resulting from thrombocytopenia. The effect of the drug can be rapidly achieved by intravenous injection. It may also be given intramuscularly on a prolonged basis where necessary. In the present study in all patients in whom the bleeding time was prolonged, it was appreciably reduced by the intravenous administration of Naphthionin. In the two subjects with platelet counts of 50,000 and 70,000 and a normal bleeding time (cases 2 and 3) there was little or no effect. The observations in case 8 were of extreme interest owing to the fluctuations in the patient's fibrinogen level. The haemostatic effect of Naphthionin is believed to be due to a direct action on fibrinogen. In this case there appeared to be a reciprocal relationship between the fibrinogen level and the bleeding time (Fig. 1). This, together with the observations in case 9, tends to confirm the view that Naphthionin acts directly upon fibrinogen.

**TABLE III**

**EFFECT OF 1G. NAPHTHIONIN INTRAVENOUSLY IN PATIENTS WITH BOTH THROMBOCYTOPENIA AND PROLONGED BLEEDING TIMES**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Platelet Count</th>
<th>Bleeding Time (min.)</th>
<th>Bleeding Time after Naphthionin (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F.</td>
<td>10,000</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>F.</td>
<td>40,000</td>
<td>8½</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>F.</td>
<td>35,000</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>F.</td>
<td>10,000</td>
<td>7</td>
<td>1½</td>
</tr>
<tr>
<td>5</td>
<td>F.</td>
<td>20,000</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>F.</td>
<td>30,000</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>F.</td>
<td>15,000</td>
<td>10½</td>
<td>6½</td>
</tr>
<tr>
<td>8</td>
<td>M.</td>
<td>8,000</td>
<td>12</td>
<td>2½</td>
</tr>
</tbody>
</table>
The dramatic clinical improvement produced by the drug when administered to the three patients suffering from severe haemorrhage (cases 5, 6, and 7) is encouraging, and suggests that Naphthoin should be given to all cases of thrombocytopenia during the bleeding phase. It may be of interest to add that no effect was produced by intravenous injection (1g.) in a further patient with diffuse purpura associated with myelosclerosis and gross splenomegaly. There was a prolonged bleeding time (9½ min.) and the Hess test in this case was positive but the platelet count was 120,000. This suggests that the drug is not useful in the management of non-thrombocytopenic purpura. Indeed Naphthoin appears to have no place in haemostasis after operation in non-thrombocytopenic patients (Kirkman, unpublished data).

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