Factors affecting the pharmacokinetics of parenteral chloramphenicol in enteric fever

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Chloramphenicol pharmacokinetics were studied in 29 Nepalese adults diagnosed with uncomplicated enteric fever and randomized to receive succinate ester 30 mg/kg iv or im. Serial plasma concentrations of chloramphenicol, and iothalamate (to estimate glomerular filtration rate), antipyrine (hepatocellular function) and Indocyanine Green (liver blood flow) were measured by HPLC and kinetic parameters estimated by non-compartmental analysis. In culture-positive patients (n = 16), mean residence times (MRTs) and steady-state volumes of distribution (V\textsubscript{d,ss}) for iv chloramphenicol (mean ± s.d.; 4.9 ± 0.9 h and 1.9 ± 0.8 L/kg; n = 7) were less than after im chloramphenicol (12.3 ± 7.3 h and 3.7 ± 2.5 L/kg; n = 9; P < 0.05), with a higher peak plasma concentration after iv (16.2 ± 9.1 versus 7.8 ± 3.6 mg/L; P < 0.05); plasma clearance (Cl\textsubscript{p}) was similar in the two groups (368 ± 172 and 310 ± 224 mL/kg/min after iv and im respectively). In 17 patients examined during convalescence, MRT and V\textsubscript{d,ss} were less than in acute illness regardless of route chloramphenicol administration. There were similar changes in chloramphenicol kinetic parameters in culture-negative patients. Antipyrine Cl\textsubscript{p} and liver blood flow correlated weakly with chloramphenicol Cl\textsubscript{p} in culture-positive patients (P ≤ 0.1) and were higher in convalescence; no such associations were seen for iothalamate Cl\textsubscript{p}. These data indicate that iv chloramphenicol produces peak plasma concentrations which are on average twice those after im injection of the same dose, due principally to a smaller V\textsubscript{d,ss}. Cl\textsubscript{p} is uninfluenced by route of administration and is determined more by hepatic metabolism than renal excretion. Intramuscular treatment may result in sub-therapeutic chloramphenicol concentrations initially, but continued regular iv dosing is more likely to produce levels at which bone marrow toxicity occurs.

Introduction

Chloramphenicol is a widely available and inexpensive antibiotic. Despite the emergence of resistant strains of Salmonella typhi, chloramphenicol is still used as first-line treatment for enteric fever in many parts of the world. It can be given by mouth or parenterally but the bioavailability of oral formulations is better than that achieved after im or iv injection of the succinate ester pro-drug. Nevertheless, parenteral dosing is indicated in patients with nausea, vomiting and/or altered consciousness. Venous access cannot always be obtained where health-care facilities are basic and, even when high quality care is available, patient discomfort and inconvenience, staff time, and risks such as overhydration and thrombophlebitis may make iv injection less attractive than im administration. Early data relating to the relative magnitude of plasma concentrations following im and iv injection were conflicting. Although an inadequate therapeutic response has been reported after im administration, more recent studies in enteric fever

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have suggested that peak plasma chloramphenicol concentrations are not influenced significantly by the route of parenteral administration.\textsuperscript{2,7}

A fter iv or im chloramphenicol succinate injection, approximately one-third of the dose is excreted unchanged in the urine while the remainder is hydrolysed to free drug and either excreted in the urine or conjugated in the liver.\textsuperscript{8} Renal chloramphenicol clearance correlates with indices of renal function including creatinine clearance\textsuperscript{9} and an association between hepatic function and plasma chloramphenicol concentrations has been reported.\textsuperscript{10,11} However, creatinine clearance is an imprecise measure of glomerular filtration rate (GFR) and conventional biochemical tests of liver function reflect more than hepatocellular injury. Iothalamate, a widely used radiological contrast medium, is excreted exclusively by the kidney and its clearance from plasma can provide a valid index of GFR.\textsuperscript{12} A ntipyrrine and Indocyanine Green (ICG) clearance can act as surrogate markers of hepatocellular function and liver blood flow respectively.\textsuperscript{13}

In order to characterize the factors affecting chloramphenicol pharmacokinetics after its parenteral administration as treatment for enteric fever, we studied the disposition of chloramphenicol after iv or im injection of the succinate ester, together with simultaneous estimates GFR (iothalamate clearance), hepatocellular function (antipyrrine clearance) and liver blood flow (ICG clearance).

**Patients and methods**

**Patients**

Twenty-nine Nepalese adults (20 males and nine non-pregnant females) aged 14–56 years were recruited. Most were farmers or rural labourers living in the Kathmandu valley. A ll had been admitted to Tribhuvan University Teaching Hospital, Kathmandu, with symptoms of enteric fever including fever for more than 3 days, headache, cough, abdominal pain and distension, and constipation or diarrhoea. Those who had received recent chloramphenicol treatment or had a history of chloramphenicol allergy were excluded. A ll patients gave informed consent to study procedures which were approved by the Nepalese Ministry of Health, Kathmandu.

**Methods**

Clinical procedures. A fter clinical assessment, a suitable forearm vein was cannulated and blood drawn for full blood count, plasma electrolyte, urea and creatinine concentrations and liver function tests. Three separate 5 mL volumes of blood were drawn at 15 min intervals for culture. A bone marrow aspirate was taken for preparation of a smear and culture, and rectal swab and urine collection for culture were also performed. Patients were randomized to 30 mg/kg body weight chloramphenicol succinate (Chloromycetin, Parke Davis Medical, Eastleigh, UK), equivalent to 21.8 mg/kg chloramphenicol base, to be given either iv or im. Intramuscular injection was into the anterior thigh. I mmediately afterwards, antipyrrine (10 mg/kg), iothalamate (10 mg/kg) and ICG (0.3 mg/kg) were given by rapid injection into the side-arm of a rapid iv infusion of sterile normal saline solution. Heparinized blood samples were taken at time 0 and 4, 5, 6, 8, 10, 12, 14, 16, 20, 25, 30, 45, 60, 90 and 120 min, and at 3, 4, 6 and 8 h. A ll samples were kept on ice and centrifuged promptly, and separated plasma was either assayed within 24 h for ICG concentration or stored and transported at below \(-20^\circ\text{C}\) before assay for chloramphenicol, antipyrrine and iothalamate. A fter the 8 h sample, patients able to tolerate oral medication were given chloramphenicol 600 mg qds by mouth. F urther blood samples were taken each morning immediately before and 30 min after the first chloramphenicol dose of the day. A full blood count was also performed on day 3 of treatment and at discharge (between days 5 and 7). A ll patients were asked to complete a 14 day course of chloramphenicol and to attend for re-examination the day after their last dose. A t this 15 day visit, a second pharmacokinetic study was performed using an identical protocol to that up to the 8 h sample in the acute study. A final follow-up visit 28 days post-admission was scheduled for a repeat full blood count and biochemical profile, in addition to clinical reassessment and repeat stool culture.

**Microbiological techniques.** Blood and bone marrow cultures were performed in 50 mL Columbia broth incubated at 37°C and routinely subcultured at 12–24 h, 36–48 h and after 7 days of incubation, on to chocolate and MacConkey agars. U rine and rectal swabs were cultured routinely, including selective culture on desoxycholate citrate agar (DCA) and in selenite broth. Isolates were identified as S. typhi or Salmonella paratyphi using conventional biochemical tests\textsuperscript{14} and serological testing for O, H and V\textsubscript{1} antigens. Biochemical results were subsequently confirmed using the API20E system (bio-Mérieux, Basingstoke, UK). A ntiibiotic susceptibility to chloramphenicol, ampicillin, trimethoprim, sulphafurazole, cefotaxime and ciprofloxacin was tested initially by disc diffusion\textsuperscript{15} and MICs were determined subsequently. Serological tests were not included as these have been shown to be unreliable for the diagnosis of enteric fever in adults.\textsuperscript{16}

**Assay techniques.** Plasma chloramphenicol assay was performed on samples at 0, 14, 30, 60 and 120 min, at 3, 4, 6 and 8 h, and on all pre- and post-dose daily samples, using HPLC as described previously.\textsuperscript{17} I ndocyanine Green was assayed within 24 h in plasma from all samples taken between 0 and 120 min using the method of Grainger et al.\textsuperscript{18} Plasma antipyrrine and iothalamate concentrations were also determined on these samples by HPLC techniques which ensure no interference between analytes.\textsuperscript{19}
Parenteral chloramphenicol in enteric fever

Pharmacokinetic analysis. Pharmacokinetic analysis of plasma chloramphenicol, iothalamate and antipyrine concentrations was by model-independent methods. The Lagrange algorithm was used to calculate area under the concentration-time curve (AUC) and the product-moment curve (AUMC). Mean residence time (MRT) is given by the formula \( \frac{dose \times AUMC}{AUC} \), the volume of distribution at steady state \((V_{dss})\) is given by the formula \( \frac{dose \times AUMC}{AUC^2} \), and the plasma clearance \((Cl_p)\) by \( \frac{dose}{AUC} \). For both iv and im administration, \( V_{dss} \) and \( Cl_p \) are given as divided by the fraction of drug absorbed or available (f). Liver blood flow was estimated using rate constants derived from a two-compartment pharmacokinetic model with elimination from the peripheral (liver) compartment.\(^\text{18}\)

Statistical analysis. Data were analysed using parametric tests (SPSS for Windows, SPSS Inc., Chicago, IL, USA) and are, unless otherwise stated, reported as mean ± standard deviation (s.d.). Two-sample comparisons were performed by Student's t-test, multiple comparisons by analysis of variance, and associations between variables by Pearson's product-moment correlation coefficient.

Results

Clinical course

In 16 patients (55%), Salmonella spp. were isolated from blood and/or bone marrow cultures. Details of culture-positive patients are summarized in Table I. Of these patients, 13 were positive for S. typhi and three for S. paratyphi A. All cultured organisms were fully antibiotic-sensitive. None of the patients was obtunded or shocked or had evidence of gastrointestinal bleeding or perforation or other manifestations of severe infection. All 16 responded to treatment with a median fever clearance time of 5 days (range 2–9 days). None of the 14 culture-positive patients who attended the 28 day review had new symptoms of enteric fever.

In the other 13 patients (see Table I), no pathogen was isolated from cultures, but all were given a standard course of chloramphenicol with a satisfactory clinical response. Five of these culture-negative patients had received antibiotics active against local strains of S. typhi (ampicillin, amoxycillin or co-trimoxazole before presentation. Eleven of the 16 culture-positive and six of the 13 culture-negative patients were restudied on day 15.

Haematological indices

None of the 29 patients was anaemic at presentation (haemoglobin 13.1 ± 3.0 g/dL) and there was only a minor fall in the mean haemoglobin concentration, with the lowest point being 12.1 ± 1.4 g/dL immediately after the 14 day chloramphenicol course. There was a similar small fall in the mean total white cell count from 6.1 ± 2.6 to 5.3 ± 1.5 \( \times 10^9 \) /L on day 15, but no patient developed significant leucopaenia or neutropaenia during treatment. The platelet count remained stable throughout (172,100 ± 76,000 and 176,000 ± 86,500 \( \times 10^9 \) /L on days 1 and 15 respectively). In the 14 patients who attended for review on day 28, the mean haemoglobin concentration (13.3 ± 1.5 g/dL), and white cell (7.6 ± 1.2 \( \times 10^9 \) /L) and platelet (261,400 ± 85,000 \( \times 10^9 \) /L) counts were all within normal limits.

Chloramphenicol pharmacokinetics

Seven patients randomized to iv treatment proved culture-positive and seven were culture-negative. The equivalent patient numbers for im treatment were nine and six, respectively. Mean ± s.d. plasma chloramphenicol concentrations for culture-positive patients are shown in Figure 1; the plasma concentration profiles after iv and im administration for culture-negative patients were similar (data not shown). Derived pharmacokinetic parameters by culture result and route of administration are summarized in Table II. There was a significantly greater peak concentration after iv compared with im administration \((P < 0.012)\), and this peak was attained earlier \((P < 0.015)\) regardless of the culture result. The MRT was significantly shorter after iv

Table I. Details of the 16 patients who were culture-positive for S. typhi \((n = 13)\) or S. paratyphi and 13 who were culture-negative. Data are expressed as mean ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>Culture-positive</th>
<th>Culture-negative</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>21 ± 4</td>
<td>28 ± 13</td>
</tr>
<tr>
<td>Number of males</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Route (iv/im)</td>
<td>7/9</td>
<td>7/6</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>14.3 ± 1.6</td>
<td>11.8 ± 3.9</td>
</tr>
<tr>
<td>White cell count ( \times 10^9 ) /L</td>
<td>5.6 ± 1.3</td>
<td>6.8 ± 3.5</td>
</tr>
<tr>
<td>Serum creatinine (( \mu )mol/L)</td>
<td>98 ± 14</td>
<td>91 ± 14</td>
</tr>
<tr>
<td>Serum bilirubin (( \mu )mol/L)</td>
<td>7 ± 3</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>Serum aspartate transaminase (iU /L)</td>
<td>32 ± 9</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>37 ± 6</td>
<td>37 ± 4</td>
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</tbody>
</table>
Table II. Model-independent pharmacokinetic parameters on admission and at follow-up in culture-positive and culture-negative patients randomized to either iv or im chloramphenicol

<table>
<thead>
<tr>
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<th>Acute illness</th>
<th>Convalescence</th>
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<tbody>
<tr>
<td></td>
<td>culture-positive</td>
<td>culture-negative</td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>im</td>
</tr>
<tr>
<td>Number of patients</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Peak concentration (mg/L)</td>
<td>16.2 ± 9.1</td>
<td>7.8 ± 3.6[^a]</td>
</tr>
<tr>
<td>Time to peak (h)</td>
<td>0.7 ± 0.3</td>
<td>1.9 ± 1.0[^a]</td>
</tr>
<tr>
<td>AUC_{0-∞} (mg.h/L)</td>
<td>72 ± 32</td>
<td>100 ± 66</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>4.9 ± 0.9</td>
<td>12.3 ± 7.3[^a]</td>
</tr>
<tr>
<td>V_{dss} (L/kg)</td>
<td>1.9 ± 0.8</td>
<td>3.7 ± 2.5[^a]</td>
</tr>
<tr>
<td>Cl_{p} (mL/kg/h)</td>
<td>368 ± 172</td>
<td>310 ± 224</td>
</tr>
</tbody>
</table>

[^a] P < 0.05 versus iv administration.
[^b] P < 0.05 versus acute illness.
Liver blood flow

Estimates of liver blood flow were available for 18 patients in acute illness (14 of whom were culture-positive) and for seven in convalescence (see Table III). There was a positive association between estimates of liver blood flow and chloramphenicol clearance at presentation which was of borderline statistical significance ($r = 0.39$, $n = 14$, $P = 0.08$). Hepatic blood flow also tended to parallel antipyrine clearance in the acute study ($r = 0.37$, $n = 13$, $P = 0.10$). Although liver blood flow estimates in convalescence were generally higher than in acute illness (see Table III), no
significant differences were observed in the series as a whole or in culture-positive patients (P > 0.22).

Discussion

Plasma chloramphenicol profiles in both salmonella culture-positive and culture-negative patients in our series indicated that peak concentrations were significantly lower and occurred significantly later after im than iv injection. This pattern was also found in convalescent studies performed in a sub-group of the patients. There has been debate in the literature over several decades as to whether the route of parenteral administration of chloramphenicol succinate is an important determinant of plasma chloramphenicol per se.8 Our data suggest that im administration could lead to subtherapeutic concentrations of chloramphenicol, at least during initial therapy, in a significant proportion of patients with enteric fever.

After im injection in our culture-positive patients, mean plasma chloramphenicol concentrations were 6.7 and 5.4 mg/L at 2 and 5 h, respectively. These values are, allowing for dose and age, generally low compared with those in previous studies. McCrumb et al.3 and Ciocatto & Marchiaro reported mean concentrations of 4–6 mg/L at these times after 15 mg/kg body weight chloramphenicol succinate (half the dose used in the present study) given by im injection to adult patients with acute infections. Ross et al.21 and Shann et al.7 have reported higher concentrations (14–17 mg/L) in children at equivalent times after doses of 50 mg/kg and 25 mg/kg respectively. Consistent with concentration profiles after iv and im injection which did not differ significantly in the latter study,2 Glazko et al.2 reported plasma chloramphenicol levels at least as high after im as after iv injection of the succinate ester in small numbers of healthy adults. These results indicate that factors such as age and severity of illness make it difficult to come to firm conclusions regarding the adequacy of im administration.

Changes in assay methodology over time and the anatomical site of im injection are also important considerations when reviewing available data. HPLC assays have been used since the 1970s, replacing microbiological, colorimetric and other methods.8 Our patients had im injections into the anterior thigh which would reduce the risk of inadvertent intralipomatous administration as compared with when given into the buttock,22 but mean concentrations were still lower than those after iv administration. Although all our patients recovered, the spread of resistant S. typhi may mean that the chance of therapeutic failure after repeated im administration increases, as reported previously by other authors.5,6

Therapeutic plasma concentrations of chloramphenicol are considered to range from a trough of 5–10 mg/L to a peak of 10–20 mg/L.23,24 The majority of our patients treated initially by im injection were well below these values during the first 8 h of the study. Institution of oral therapy after this time and its greater bioavailability resulted in satisfactory plasma chloramphenicol concentrations the next day regardless of initial route of parenteral administration. However, none of our patients had infections severe enough to warrant prolonged parenteral treatment.

Pharmacokinetic analyses of chloramphenicol concentrations after injection of the succinate ester are complicated by the rate of hydrolysis and the associated renal losses of the succinate pro-drug. Nevertheless, Clp estimates in our patients were consistent with those reported previously for patients with essentially normal hepatic and renal function.8 The significant differences in MRT are likely to reflect differences in rates of absorption from the two administration sites.25 Although no statistically significant differences were found in AUC, estimates were generally greater after im compared with following iv injection, suggesting greater bioavailability after im administration. This could result, at least in part, from greater renal excretion and/or hepatic metabolism of chloramphenicol at the higher concentrations achieved after iv injection.

The majority of our patients had normal hepatorenal function as judged by conventional biochemical tests. Nevertheless, sequential determination of antipyrine and iothalamate clearance revealed that hepatocellular but not renal function was significantly altered during typhoid
fever. Weak associations between chloramphenicol clearance, antipyrine clearance and liver blood flow are consistent with previously published preliminary data relating serum transaminase and chloramphenicol concentrations in children with enteric fever. A similar relationship in cirrhosis has been debated but serum chloramphenicol levels in patients with a variety of liver diseases appear to be highest in those with the most severe hepatocellular dysfunction.

Our patients exhibited no evidence of bone marrow toxicity during a standard course of chloramphenicol and no other significant side-effects were observed. A lthough adequate patient follow-up in developing countries is often difficult to achieve, we are not aware of recurrent infections in any of the patients studied, regardless of their culture status. A proportion of the culture-negative patients, especially those who received antimicrobial treatment before admission, may have had typhoid or paratyphoid fever. The results from culture-positive and -negative groups were generally similar, especially in the comparisons between plasma chloramphenicol concentrations after iv and im injection. We found chloramphenicol to be safe and effective for the treatment of clinically suspected enteric fever in adult Nepalese patients. However, im administration resulted in relatively low plasma chloramphenicol concentrations which may have remained subtherapeutic for several doses had this route of administration been maintained. Although our cases were not severe, there was some evidence of an association between chloramphenicol metabolism and both hepatocellular function and liver blood flow, which could have clinical consequences in patients with more severe and complicated infections. A susceptibility of S. typhi to chloramphenicol declines, this will become of increasing relevance. Bone marrow toxicity is most marked when the plasma chloramphenicol concentration is >25 mg/L. A lthough such concentrations were not achieved in most of our patients in the first few days of treatment, jaundiced patients and those with significantly raised transaminase concentrations should be assessed haematologically at regular intervals in order to minimize the risk of toxicity occurring.

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G. P. A charya et al.


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