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Pharmacokinetics and organ distribution of intravenous and oral methylene blue

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Abstract *Objective*: To determine the pharmacokinetics and organ distribution of i.v. and oral methylene blue, which is used to prevent ifosfamide-induced encephalopathy in oncology.
Methods: The concentration of methylene blue in whole blood was measured using high-performance liquid chromatography in seven volunteers after i.v. and oral administration of 100 mg methylene blue with and without mesna. The distribution of methylene blue in different tissues was measured in rats after intraduodenal and i.v. application.
Results: The time course of methylene blue in whole blood after i.v. administration showed a multiphasic time course with an estimated terminal half-life of 5.25 h. Following oral administration, the area under the concentration–time curve was much lower (9 nmol/min/ml vs 137 nmol/min/ml). Co-administration of mesna, which could influence distribution by ion-pairing, did not alter the pharmacokinetics. The urinary excretion of methylene blue and its leucoform was only moderately higher after i.v. administration (18% vs 28% dose). Intraduodenal administration to rats resulted in higher concentrations in intestinal wall and liver but lower concentrations in whole blood and brain than i.v. methylene blue.
Conclusions: Differences in organ distribution of methylene blue are mainly responsible for the different pharmacokinetics after oral and i.v. administration. If methylene blue acts in the liver, where ifosfamide is primarily activated to reactive and potentially toxic metabolites, oral and i.v. methylene blue are likely to be equally effective. However, if the site of action is the central nervous system, i.v. methylene blue which results in much higher concentrations in brain seems preferable.

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Introduction

Methylene blue is increasingly used in cancer chemotherapy regimens based on the use of ifosfamide. Ifosfamide is associated with dose-limiting central nervous side effects, particularly following oral administration where the incidence of this encephalopathy is approximately 50% [1, 2]. After the systematic study of a patient receiving an accidental overdose of ifosfamide revealed a glutaricaciduria – the hallmark of a type-II defect in mitochondrial electron transfer – methylene blue was introduced in the treatment of ifosfamide encephalopathy [3]. The efficacy of i.v. and oral methylene blue for the treatment and prophylaxis of ifosfamide-associated encephalopathy has since been confirmed by several groups [4–8]. The mechanism and site of action of methylene blue in this condition, however, is not clear. Also, the optimal dose and route of administration has not been defined.

In the present study, the pharmacokinetics and organ distribution of methylene blue following oral and i.v. administration were determined. Since most patients treated with ifosfamide also receive mesna in order to prevent the urotoxicity of metabolites of ifosfamide [9] and since mesna might form an ion pair with methylene blue and thus influence its kinetics, the effect of mesna on the kinetics of methylene blue following oral administration was also investigated.

Patients and methods

Seven healthy volunteers, four males and three females, 19–53 years of age, participated in the study which was approved by the local ethics committee. After an overnight fast, they received methylene blue on three occasions at least 1 week apart in randomised sequence: on one occasion, 100 mg (313 μmol) methylene blue dissolved in 0.9% NaCl at a concentration of 20 mg/ml was

administered i.v. over a 30-s period; on one occasion, two gelatine capsules containing 50 mg methylene blue were administered followed by a glass of water; and, on one occasion, the same dose was administered orally together with 800 mg sodium mercaptoethanesulphonate (mesna, Uromitexan, Asta Medica). Methylene blue was prepared by the local hospital pharmacy. Venous blood samples of 4 ml each were collected in tubes containing ethylene diamine tetraacetic acid (EDTA) before and 5, 10, 15, 20, 30 40, 50, 60, 120 and 240 min after administration of the drug. Urine was collected in fractions for 24 h. Samples of whole blood and urine were stored at −18 °C in test glass tubes with polytetrafluoroethylene (PTFE)-covered screw caps (Schott-Duran) until analysis. The choice of glass tubes for storage and handling of samples is critical since methylene blue is adsorbed on polymer plastic material [10].

Distribution of methylene blue in tissue of rats after i.v. and intraduodenal administration

Male Wistar rats (285–315 g) bred in the Department of Pathophysiology, University of Bern, were kept in a climatised room with a controlled 12-h/12-h dark/light cycle and had free access to food and water up to the evening prior to the experiment. After an overnight fast they were anaesthetised with 50 mg/kg pentobarbital i.p., and the bile duct and an external jugular vein were cannulated. Methylene blue was administered at a dose of 10 mg/kg in 0.9% saline i.v. to four rats and intraduodenally to four rats by directly injecting the drug just below the pylorus. Bile was collected for 1 h. One hour after the administration of methylene blue, a sample of venous blood was obtained and the animals were killed. The small intestine was rinsed with 12 ml 0.9% NaCl to determine the amount of methylene blue remaining in the intestinal lumen. Samples of liver, small intestinal wall and brain were homogenised in four volumes of water and methylene blue was extracted and measured by means of high-performance liquid chromatography (HPLC) as described below.

Analytical methods

Methylene blue in hemolysate of whole blood was measured by HPLC. (4-Dimethylaminophenylazo)-5-phenylphenanziniumchloride (10 μg), which served as an internal standard, and 0.5 ml 5% sodium hexanesulfonate were added to 0.5–3.0 ml hemolysate in 12 × 100mm glass tubes (Schott-Duran) with tight-fitting PTFE-covered screw caps. After thorough mixing, 2 ml 1,2-dichlorethane was added and the sample was agitated for 15 min [10]. After centrifugation (6 min at 1700 ×*g*), the organic phase was removed and evaporated at 50 °C under a stream of nitrogen.

Chromatographic separation was accomplished with a Nucleosil 100-5 CN column (250 × 4.6 mm, Macherey-Nagel) and a mobile phase of 10 mM ammonium-dihydrogen phosphate:acetonitrile:methanol (60:35:5, v:v) at pH 2.75 and a flow rate of 0.7 ml/min. The efflux was monitored at 660 nm.

Methylene blue in urine was measured spectrophotometrically. Each urine sample was measured before and after oxidation of the leucoform of methylene blue. For oxidation, 38 μl 0.5 M HCl was added to 0.25 ml urine and heated to 70 °C [10]. The urine samples were then extracted with hexanesulfonate and dichloroethane using the same protocol as for whole blood. The concentration of methylene blue before and after oxidation was determined spectrophotometrically at 660 nm.

Using the described extraction procedure, the recovery of methylene blue amounted to 87 ± 2% and 83 ± 3% for concentrations of 3 nmol/ml and 30 nmol/ml, respectively (mean ± SD, $n = 4$). Standard curves were linear in the range of 9–900 nmol/l for the HPLC assay and 0.15–30 nmol/ml for the spectrophotometric assay. Aliquots of blood samples spiked with 10 nmol/l methylene blue were measured on different days with a coefficient of variation of 13.6% ($n = 4$).

Calculations and statistics

The area under the blood concentration–time curve (AUC) was determined using the trapezoidal method. Clearance was calculated as dose/AUC. The data were analysed using analysis of variance (ANOVA) followed by the Student Newman-Keuls test for multiple comparisons. All data are presented as mean ± SEM. The pooled concentrations of methylene blue in blood after i.v. administration were analysed by means of multiple regression analysis.

Results

Following i.v. administration, the concentration of methylene blue in blood exhibited a multiphasic time course (Fig. 1). The pooled data were best fitted using three exponential terms ($Conc_{blood} = 9.4 \times e^{-0.237 \times t} + 3.8 \times e^{-0.065 \times t} + 0.3 \times e^{-0.0022 \times t}$ μmol/l). The AUC

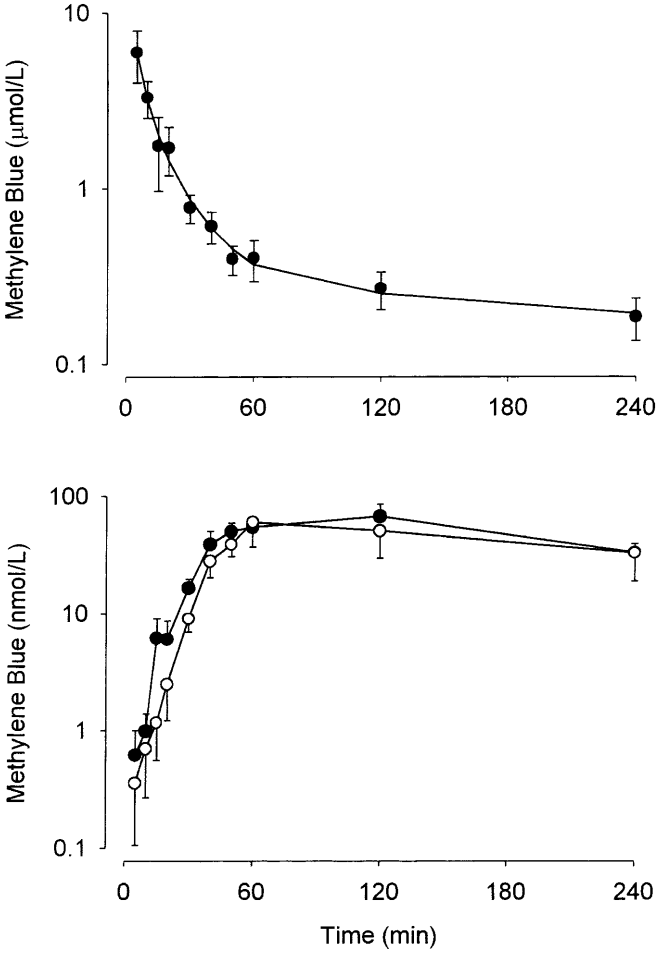

Fig. 1 Concentration of methylene blue in whole blood after the i.v. administration of 100 mg methylene blue (*top panel*) and oral administration of 100 mg methylene blue with (*open circles*, *bottom panel*) and without (*closed circles*, *bottom panel*) 800 mg mesna to seven healthy volunteers. Mean ± SEM, $n = 7$. The *line* connecting the *triangles* represents the equation describing the time course of the blood concentration terms ($Conc_{blood} = 9.4 \times e^{-0.237 \times t} + 3.8 \times e^{-0.065 \times t} + 0.3 \times e^{-0.0022 \times t}$ μmol/l, $R^2 = 0.99$)

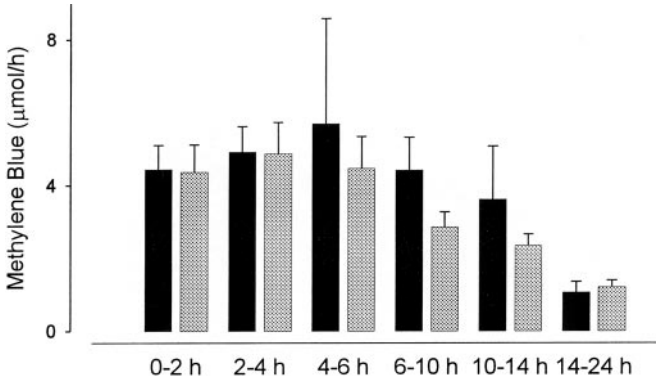

Fig. 2 Rate of urinary excretion of total methylene blue (µmol/h) during the indicated time intervals after i.v. (*filled bars*) and oral (*stippled bars*) administration of methylene blue. Mean ± SEM, $n = 7$

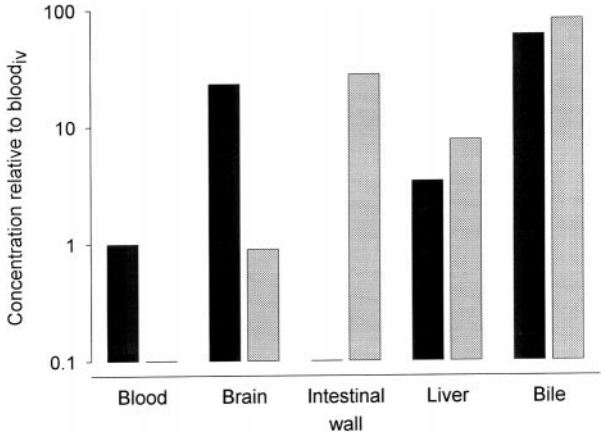

Fig. 3 Distribution of methylene blue in rats after i.v. (*black bars*) and intraduodenal (*shaded bars*) administration. The *bars* represent the mean concentration measured in tissue of four rats in each group relative to the concentration found in blood 1 h after i.v. administration. Except for the concentration in bile, the concentrations were significantly ($P < 0.05$) different in all tissues for the two modes of application

amounted to 0.134 ± 0.025 µmol/min/ml and the systemic clearance 3.0 ± 0.7 l/min.

The time course of methylene blue in blood after oral administration is also shown in Fig. 1. Maximal concentrations were reached after 1–2 h. There was no significant difference in the presence of mesna. The AUC up to 240 min was significantly ($P < 0.01$) lower than after i.v. administration with 0.011 ± 0.004 µmol/min/ml with mesna and 0.010 ± 0.004 µmol/min/ml without mesna.

The rate of urinary excretion of total methylene blue, i.e. methylene blue and leucomethylene blue, after i.v. and oral administration of the compound is shown in Fig. 2. Between 4 h and 14 h, the rate tended to be higher after i.v. administration, such that the total urinary excretion of methylene blue amounted to 28.6 ± 3.0% in 24 h, which was slightly but significantly ($P < 0.01$) higher than the 18.5 ± 1.9% and 18.4 ± 2.4% of the administered dose after oral methylene blue with and without mesna, respectively. After 4 h, the urinary excretion of total methylene blue decreased with an average half-life of 6.6 h. This corresponds well with the half-life of 5.25 h calculated from the third exponential term of the equation describing the disappearance of methylene blue from blood after i.v. administration. The fraction excreted in the leucoform, approximately one-third of the excreted methylene blue, was not different with the different modes of administration.

The distribution of methylene blue in various compartments in rats is shown in Fig. 3. One hour after administration of the drug, the concentrations in blood and brain were significantly ($P < 0.05$) higher after i.v. than after intraduodenal application. In contrast, the concentrations in the intestinal wall and in the liver were significantly ($P < 0.05$) higher after intraduodenal administration. The concentration in bile and the biliary excretion were similar with the two modes of application. Less than 3% of the administered dose were found in the intestinal lumen 1 h after intraduodenal administration.

Discussion

Methylene blue exhibits complex pharmacokinetics. After i.v. administration, the disappearance is multiphasic with extensive distribution into deeper compartments and a slow terminal rate of disappearance. The decline in the rate of urinary excretion between 4 h and 24 h and the estimated terminal phase after i.v. administration ($-0.0022\ \text{min}^{-1}$) indicate that the half-life of methylene blue is around 5–6.5 h. Following the administration of radio-iodinated methylene blue, a half-life of 4.5 h for the tracer has been reported [11].

After oral administration, the concentrations of methylene blue in blood and the AUC were one order of magnitude lower than after i.v. administration. This could be due to poor gastrointestinal absorption. The almost identical urinary excretion after oral and i.v. administration and the experiments in rats, however, argue against this possibility. Alternatively, an extensive first-pass metabolism could account for the low bioavailability. Again, the urinary excretion argues against this possibility. Moreover, no major metabolites of methylene blue other than the two redox forms have been described. Finally, differences in the distribution in various compartments, such as intestinal wall and liver, prior to reaching the blood stream with subsequent redistribution could account for the low circulating concentrations in whole blood after oral administration. This hypothesis is supported by the animal experiments where no methylene blue was detectable in blood after intraduodenal administration but significantly higher concentrations were found in intestinal wall and liver. The animal experiments also demonstrate that, depending on the mode of administration, substantially higher concentrations of methylene blue are reached in various organs than in blood. Tenfold higher concen-

trations of radio-iodinated methylene blue than in blood have also been found in tumours following administration of radio-iodinated methylene blue to patients [11].

Approximately one-third of the methylene blue excreted in urine was in the leucoform. The leucoform of the compound is generally rapidly oxidised in air [12]. However, in urine – but not in blood – it forms stable complexes [13]. The observed urinary excretion agrees with the excretion of radioactivity after administration of labelled methylene blue [11].

Mesna, which is used as part of most ifosfamide regimens, could potentially alter the distribution of methylene blue by ion pairing. The identical time course of the concentration of methylene blue in blood after oral administration with and without mesna indicates that mesna does not influence the pharmacokinetics of methylene blue. Thus, co-administration of the two compounds should pose no problems in clinical practice.

The present data indicate that oral methylene blue is well absorbed from the gastrointestinal tract but differs markedly with regard to organ distribution compared with i.v. methylene blue. Rather than first-pass metabolism, methylene blue is thus subjected to extensive 'first-pass distribution' and, depending on the mode of administration, markedly different tissue concentrations are achieved. The site of action of methylene blue in the prevention of ifosfamide-associated encephalopathy is not known. If it is the liver, where ifosfamide is primarily activated to reactive and potentially toxic metabolites, oral and i.v. methylene blue are likely to be equally effective. However, if the site of action is the central nervous system, i.v. administration, which results in much higher concentrations of methylene blue in brain, seems preferable. Similar distribution phenomena with other pharmacologically active agents might markedly influence the effects of such compounds with various mode of administration.

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