Methylene Blue-Treated Plasma: Toxicological Profile of Methylene Blue and Its Photoproducts

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参考資料3 マコファルマ社

Purpose: The MacoPharma Theraflex ®System uses Methylene blue (MB) and visible light for virus inactivation of plasma for transfusion (MB plasma). MB is added at a concentration of 1µM. After illumination most of the photosensitizer and its photoproducts are removed by an integrated depletion filter. A considerable number of toxicological data on MB are available in the literature. However, long-term studies are lacking. They are necessary because in certain indications MB plasma is administered for several weeks, for example in the treatment of thrombotic thrombocytopenic (TTP) patients. Recent studies with MB were conducted by the American National Toxicological Program (NTP). They focused on the endpoints: Short-term toxicity (4 and 13 weeks), conventional teratology and long-term carcinogenicity (2 years). However, in these studies MB was administered orally and therefore this application route differs from the mode of application used for MB plasma (intravenous route). It was the aim of the present investigation to elucidate whether toxicological data from the NTP studies can be used to assess the toxicological properties of MB after intravenous administration.

Furthermore, as part of a preclinical testing program, the toxicological profile of MB and its photoproducts was investigated.

Methods

The adsorption, distribution and excretion (ADE) of ^{14}C-labeled MB. The ADE following oral and 24h infusion, respectively, were investigated in rats. A nominal dose level of 20 mg/kg body weight was administered by gavage or by 24h infusion. The observation time was 96 hours.

13 week-gavage toxicity study. The study was conducted by the NTP in male and female F344 rats and B6C3F$_1$ mice. 10 animals/sex/species/group were administered Methylene blue in a suspension with 0.5% aqueous methylcellulose at dose levels of 0 (vehicle only), 25, 50, 100 and 200 mg/kg b.w./day on 5 days/week. In addition, 20 males and 20 females per group were used for interim sacrifices.

Teratology: Teratogenic effects were carried out by intravenous bolus injection of Methylene blue to rats and rabbits. Methylene blue was administered daily to the dams at 4, 12, 36 mg/kg b.w. (rat) and 2, 6, 18 mg/kg b.w. (rabbit).

Tolerance test in Beagles. In a tolerance test 5 ml/kg b.w. of autologous light-treated plasma (1 or 10 µM Methylene blue) was administered to 5 male Beagles per group by intravenous administration. After 3 weeks 3 dogs/group were treated for a second time and sacrified 24h later. Following parameters were examined: haematology, clinical biochemistry and ECG. A complete histopathology was carried out.

Genotoxicity studies. Methylene blue, Azure B, Azure A/C have been tested in a variety of genotoxicity assays, namely: Bacterial reverse mutation test (Ames test); in vitro mammalian cell gene mutation test (HPRT test); in vitro mammalian chromosome aberration test with human lymphocytes); in vivo micronucleus test with rat bone marrow and peripheral blood cells (dose 20 mg/kg b.w., 24h infusion); in vivo UDS test in rats (dose 20 mg/kg b.w., bolus infusion).

All studies were conducted according to GLP and international guidelines.

Results

Pharmacokinetics

Excretion and Recovery of Radioactivity Following Oral and 24h Intravenous Administration of Methylene Blue

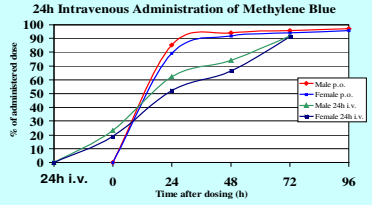

Fig. 1 Recovery of ^{14}C-labelled Methylene blue (MB). The recovery was examined in Sprague-Dawley rats following oral administration (p.o.) and 24h i.v. infusion at a dose level of 20 mg MB/kg body weight. Urine, faeces, organs, expired air, rinse water and infusion site were analysed. The radioactivity recovery rate in organs and at the infusion site was determined 96h

Distribution of Radioactivity 96h After Administration of ^{14}C-Methylene Blue

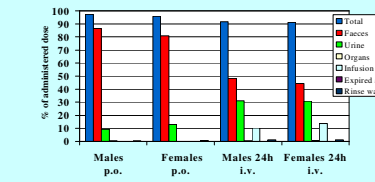

Fig. 2 Mean recovery of radioactivity after oral (gavage) application and 24h i.v. infusion of 20 mg MB/kg body weight in rats.

Organ Distribution of ^{14}C-Labelled Methylene Blue Following Oral and 24h i.v. Administration

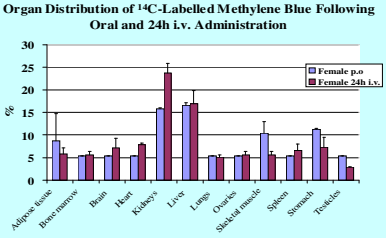

Fig. 3 Distribution of radioactivity in different organs of rats 96h after administration of 20 mg MB/kg body weight. Less than 1% of the dose was found in the different organs.

References:

Hejtmancik MR, Ryan MJ, Toft JD, Persin RL, Kurtz PJ, Chabra RS. Hematological effects in F344 rats and B6C3F1 mice during the 13-weeks gavage toxicity study of methylene blue trihydrate. Toxicol. Sciences 2002; 65: 126-134

National Toxicology Program (NTP): Methylene blue trihydrate. Http://ntp-server.niehs.nih.gov/

Pharmacokinetics

Pharmacokinetics of Radio-Labelled Methylene Blue

Sex/Route of application	C_{max} (µg-eq/ml)	T_{max} (min.)	$T_{1/2}$ α (min.)	$T_{1/2}$ β (h)	AUC $_{0-\infty}$ (µg eq·h/ml)	Systemic bioavail-ability
Males p.o.	1.58	10.9	2.8	13.7	13.1	44%
Females p.o.	1.55	8.5	2.8	18.4	12.2	56%
Males 24h i.v.	0.93	12	3	12.6	30.0	-
Females 24h i.v.	0.77	12	2.8	16.0	22.0	-

Tab. 1 Pharmacokinetic parameters of plasma radioactivity in rats. Pharmacokinetics were determined in male and female rats after oral (gavage) application and 24h i.v. infusion of 20 mg MB/kg body weight.

AUC: Area under the curve

Pharmacokinetic Parameters in Rats Following 24h i.v. Infusion of ^{14}C-Labelled MB and Azure B (AzB)

Compart-ment	C_{max}(µg-eq/g tissue) T_{max} 30 min.		AUC $_{0-\infty}$ (µg eq·h/g)		Terminal $T_{1/2}$ (h)	
	MB	AzB	MB	AzB	MB	AzB
Plasma	0.72 *SD: 0.25*	1.27 *SD: 0.61*	18.7	33.3	17.7	16.1
Bone marrow	0.48 *SD: 0.21*	0.81 *SD: 0.45*	17.04	31.44	19.1	23.3
Heart	1.39 *SD: 0.90*	0.97 *SD: 0.22*	57.85	50.48	29,8	29.1
Kidneys	2.22 *SD: 0.80*	2.74 *SD: 0.99*	88.04	129.83	15.2	37.1
Liver	2.30 *SD: 0.64*	2.94 *SD: 1.28*	75.43	106.35	15.5	20.2
Lungs	1.05 *SD: 0.28*	1.30 *SD: 0.48*	31.90	47.73	15.0	18.2

Tab. 2 Pharmacokinetic parameters following 24h i.v. infusion of 20 mg/kg b.w. ^{14}C-labelled MB or Azure B (AzB). MB and Azure B have an initial half-life of only several minutes and a longer terminal elimination half-life at a very low MB concentration level (< 1% of the dose).

Summary of Pharmacokinetics

- **Pharmacokinetics of ^{14}C-labelled MB after 24h infusion and oral application were comparable in T_{max}, $T_{1/2}$ α and $T_{1/2}$ β. It indicated:**
  - **a biphasic elimination of MB with an initial half-life of only several minutes and a longer terminal half-life of several hours but at a very low MB concentration level**
  - **less than 1% radioactivity in plasma and the examined organs**
  - **that radioactivity was almost completely excreted after 96h**
  - **that the oral dose of Methylene blue was well absorbed. The systemic bioavailability of MB was approx. 50%**
  - **no accumulation or storage of Methylene blue**
- **Administration of degradation product Azure B revealed a similar pharmacokinetic profile as MB**

Results of the National Toxicological Program (NTP) can be used to asses the toxicological profile of Methylene blue following intravenous application

Toxicological Profile

Study Type	BSD	NTP
Route of application	24h or bolus infusion	Oral (by gavage)
Toxicokinetics	MB, Azure B (rat)	MB (rat, mouse)
ADE	MB (rat)	
Acute toxicity	MB, Azure B, Azure A/C (rat)	
4 and 12 weeks toxicity		MB (rat, mouse)
Teratology	MB (rat, rabbit)	MB (rat, rabbit)
Tolerance test	MB light-treated plasma (beagle)	
Genotoxicity in vitro	MB, Azure B, Azure A/C	MB
Genotoxicity in vivo	MB, Azure B (rat)	MB
Carcinogenicity		[1]MB (mouse, rat)

Tab. 3 Toxicology program for Methylene blue and its photoproducts. Test items are mainly administered clinically as 24h infusion. ADE: Absorption, Distribution, Excretion. NTP: American National Toxicology Program

[1] Laboratory reports completed but not yet published by NTP

Mutagenicity of Methylene Blue and Methylene Blue-Treated Plasma *in Vitro*

	MB	AzB	AzA/C	[1]MB-plasma (10µM)
In vitro				
Ames-test (TA98)	Pos. (>1µg/plate)	Pos. (> 0.3 µg/ml)	Neg.	Neg.
Chromosome aberration test (human lymphocytes)	Pos. (> 1.25µg/ml)	Pos. (> 2.5 µg/ml)	Neg.	Neg.
HPRT test V79 cells	Neg.	Neg.	Neg.	Neg.
In vivo				
[1]Micronucleus test (rat bone marrow and peripheral blood cells)	Neg.	Neg.		
[1]UDS test	Neg.	Neg.		

Tab. 4 Treatment conditions *in vivo*: 24h i.v. infusion of 20 mg/kg body weight Methylene blue (MB) or Azure B (AzB). [1]Light treated MB plasma before removal of MB and its photoproducts.

Study Type	Clinical signs	Thresholds
4 and 12 weeks toxicity rat, mouse 0, 25, 50, 100, 200 mg/kg b.w.(oral)	Dose related increase in haematopoises, methaemoglobinemia, Heinz bodies,	Threshold for haematological effects (slight methaemo-globinemia) < 25 mg/kg b.w.
Teratology rat 4, 12, 36 mg/kg b.w.(i.v.)	Increase in resorption rate No teratogenic properties	NOEL (foetal organism) 4 mg/kg b.w.
Rabbit i.v. 2, 6, 18 mg/kg b.w.	Highest dose leads to death of the foetus and increase resorption rate. No significant teratogenic properties	NOEL (foetal organism) 6 mg/kg b.w.
Tolerance test Beagle light treated MB plasma (1.6 and 16 µg MB/kg b.w.)	No signs of intolerance	

Tab. 5 Toxicological findings detected during 12 week-toxicity (NTP), developmental toxicity (teratogenicity) and tolerance test after application of methylene blue and light-treated MB plasma (tolerance test only)

NOEL: No observed effect level

Safety Margins for Toxicity from *in vivo* Studies with Methylene blue and Methylene Blue Treated Plasma

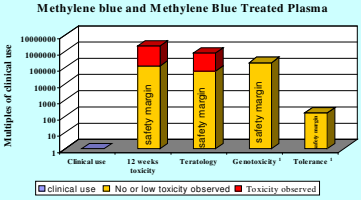

Fig.4 Safety margins for toxicity from in vivo studies with methylene blue. Calculations are based on the thresholds NOEL: no observed effect level (teratology) and LOAEL: lowest observe effect level (slight methaemoglobinemia) (12 weeks toxicity) and a normal clinical exposure of 0,1µg MB/kg body weight (= 6 units MB plasma). [1] No toxicity occured; therfore the safety margins are based on the highest dose tested.

Summary of toxicological findings:

- **The threshold for a haematological effect was below 25 mg MB/kg body weight. Subchronic administration (13 weeks) of Methylene blue in rats and mice resulted in gross and microscopic findings which are consistent with the development of haemolytic anaemia.**
- **The no observed effect level (NOEL) for the foetal organism was 4 mg and 6 mg/kg b.w./day in rats and rabbits, respectively. High dose of Methylene blue (≥12 mg/kg b.w.) intravenously administered to pregnant rats and rabbits resulted mainly in loss of implants and increased number of early resorptions. No teratogenic properties were detected.**
- **Genotoxicological (i.e. clastogenic) effects of MB and Azure B were only found *in vitro*.**
- **No indication of genotoxic effects on bone marrow, peripheral blood cells and hepatocytes after application of 20 mg/kg b.w. MB and Azure B.**
- **No signs of intolerance (haematology, clinical biochemistry and ECG) or sensitization after infusion of 1µM or 10µM MB light-treated plasma before removal of MB and photoproducts were observed.**

Thresholds for no or low toxic properties which occurred after administration of MB in preclinical studies are > 160 to 200000 fold higher than the estimated clinical exposure of MB after infusion of 6 units MB-light treated plasma.