Safety Of Methylene Blue Treated Plasma

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OBJECTIVES

The procedure using methylene blue (MB) to inactivate viruses in therapeutic plasma is well established worldwide. It includes membrane filtration (Plasmaflex, 0.65 µm pore size), addition of MB (dry pill, 85 µg, resulting in 1 µmole/L at 266 ml), illumination (approx. 20 min, 590 nm), and filtration of MB and photoproducts (Blueflex). More than 4 million units of plasma were transfused without any unusual adverse event reported.

Aim of this study was to prove the toxicological safety of MB, its photoproducts azure A, B and C, and that of MB-treated plasma.

Figure 1: Global status of Theraflex MB-Plasma use

METHODS

Adsorption, distribution and excretion of 14C-labeled MB following 24 h infusion were investigated at a dose level of 20 mg/kg body weight (b.w.) in rats. Observation time was 96 hours.

Studies on teratogenic effects were done by intravenous bolus injection of MB into rats and Beagle dogs. MB was administered daily to the dams at 4, 12, 36 mg/kg b.w. (rat) and 2, 6, 18 mg/kg b.w. (rabbit).

In a tolerance test 5 ml/kg b.w. of autologous light-treated plasma (1 or 10 µM MB) was administered to 5 male Beagles per group by intravenous administration. After 21 d 3 dogs/group were treated again and sacrificed 24 h later. Hematology, clinical biochemistry, and electrocardiogram were examined. A complete histopathology was done.

MB and Azure A/B/C were tested in: bacterial reverse mutation test (Ames test), in vitro mammalian cell gene mutation test, in vitro mammalian chromosome aberration test with human lymphocytes, in vivo micronucleus test with rat bone marrow and peripheral blood cells (20 mg/kg b.w., 24 h infusion), in vivo unscheduled DNA synthesis test in rats (20 mg/kg b.w., bolus infusion).

RESULTS

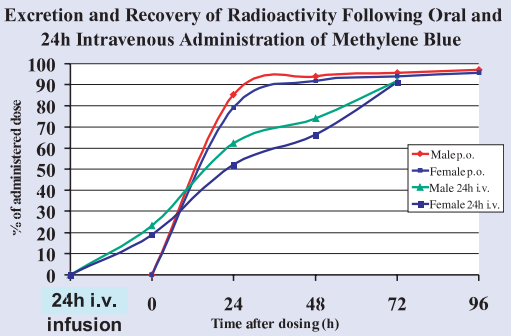

Fig. 2 **Recovery of ^{14}C-labelled Methylene blue (MB).** The recovery was examined in Sprague-Dawley rats following oral administration (p.o.) and 24 h i.v. infusion at a dose level of 20 mg MB/kg body weight. Urine, faeces, organs, expired air, rinse water and infusion site were analysed. The radioactivity recovery rate in organs and at the infusion.

1. Pharmacokinetics of ^{14}C-labeled MB after 24 h infusion were determined in T_{max}, $T_{1/2}$ α and $T_{1/2}$ β It indicated:

- biphasic elimination of MB with an initial half-life of 3 min and a longer terminal half-life of 12.6 h (male) and 16.0 h (female)

- less than 1 % radioactivity in plasma and examined organs

- Excretion of radioactivity was almost complete after 96 h

- no accumulation or storage of MB

2. The no observed effect level (NOEL) for the fetal organism was 4 mg and 6 mg/kg b.w./day in rats and rabbits.

3. Clastogenic effects of MB and Azure B were found in vitro.

4. No genotoxic effects on bone marrow, peripheral blood cells and hepatocytes after application of 20 mg/kg b.w. MB and Azure B.

5. No signs of intolerance or sensitization after infusion of 1 µM or 10 µM MB light-treated plasma before removal of MB and photoproducts were observed.

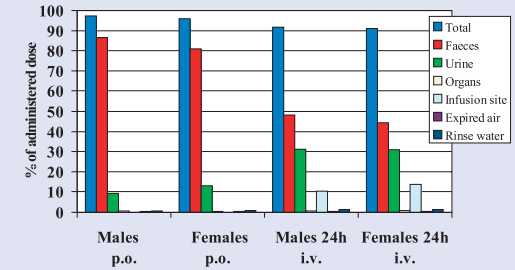

Fig. 3 Mean recovery of radioactivity after oral (gavage) application and 24 h i.v. infusion of 20 mg MB/kg body weight in rats.

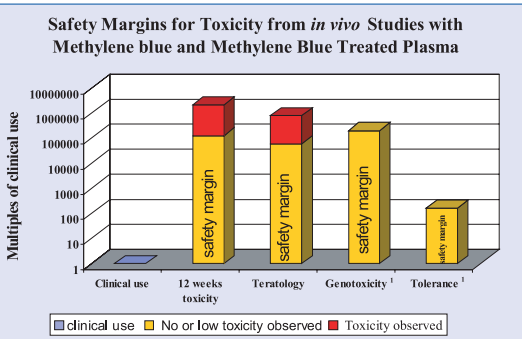

Fig. 4 Safety margins for toxicity from in vivo studies with emthylene blue. Calculations are based on the treshold NOEL: no observed effect level (slight methaemoglobinemia) (12 weeks toxicity) and a normal clinical exposure of 0,1 µg MB/kg body weight (=6 units MB plasma). [1] no toxicity occured;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

CONCLUSIONS

Thresholds for no or low toxic properties which occurred after administration of MB in preclinical studies varied depending on the amount of MB applicable in the specific test system. They are > 160 to 200,000 fold higher than the estimated clinical exposure of MB after infusion of 6 units MB-light treated plasma.

REFERENCES

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2. Pohler et al. 2004 Transfus Med Hemother 31 (suppl 3):1-84, PS305