Toxicology Letters 190 (2009) 298–302

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

In vivo genotoxicity of EMS: Statistical assessment of the dose response curves

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ARTICLE INFO

Article history:
Available online 21 March 2009

Keywords:
DNA damage
Threshold
Linear dose response
lacZ mutations
Micronucleus
Risk assessment

ABSTRACT

EMS induced micronuclei and lacZ mutations in in vivo studies in mice with a clearly sublinear dose dependency. As reported elsewhere in this issue, NOEL dose values of between 25 mg/kg/day and 80 mg/kg/day were observed for the different endpoints and tissues analysed. Here we show that statistical assessment of the data provides solid support that the induction of mutagenic and clastogenic effects after in vivo treatment with the directly DNA damaging mutagen EMS adheres to a thresholded dose response relation. These data corroborate similar evidence obtained in in vitro studies. We conclude that cells are fully capable of repairing large amounts of DNA ethylations induced by EMS without experiencing elevated mutation frequencies. The stochastic, linear risk assessment model generally employed for DNA damaging genotoxins can therefore be refuted for EMS. While presently this conclusion cannot be generalized to other genotoxins a change of paradigm appears to be indicated at least for alkylating agents inducing a comparable type and spectrum of DNA lesions as EMS.

1. Introduction

The detailed results of our in vivo genotoxicity studies with EMS are described elsewhere in this issue (Gocke et al., 2009a). We observed that EMS did not induce micronuclei in mouse bone marrow at doses ≤80 mg/kg/day and did not increase lacZ mutation frequencies in Muta™mouse at doses ≤25 mg/kg/day (bone marrow, small intestine) and ≤50 mg/kg/day (liver). Above these NOEL doses the frequencies appeared to be linear correlated with dose. In order to define the location of the bend of the dose response curve (i.e. the threshold) and its confidence interval we present here the statistical analysis of the data.

The assessment of the genotoxicity data consisted out of four steps:

(1) Comparison of control groups (to allow cumulation).
(2) Rejection of linear dose response relationship (entire dose range).
(3) Acceptance of linear dose response relationship below the NOEL.
(4) Application of threshold software developed by Lutz and Lutz (in press) to calculate the threshold values including confidence limits.

Viracept patients had been accidentally exposed to tablets contaminated with EMS at a maximal dose of 0.055 mg/kg/day (Mueller and Singer, 2009). In order to set limits to the maximal response at this dose of specific interest we determined additionally the

- confidence limits for the slope of the linear regression line below the NOEL;
- confidence limit for genotoxic effects at 0.055 mg/kg (as % of control).

Finally, we elaborate on the apparent hormetic response relation of micronuclei induction by EMS.

Figs. 1–4 present the individual MNT and lacZ mutant frequencies of the studies in graphical form. The detailed data of the studies are tabulated in Gocke et al. (2009a).

2. Step 1: comparison of control groups

In the MNT study two independent control groups of six animals were included, in the Muta™mouse study four controls groups of seven animals were included for each of the three organs (except for liver, where one control group consisted of six animals, and bone marrow, where two control groups consisted of six animals).

For each organ, the control groups were compared by means of analysis of variance. The resulting p-values are listed in Table 1. No significant differences between the control groups were revealed. For the further analysis the control groups were, therefore, cumulated.

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doi:10.1016/j.toxlet.2009.03.008

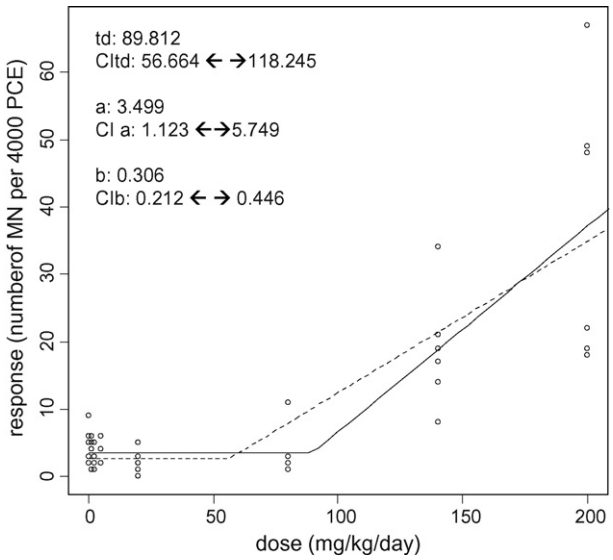

Fig. 1. Hockeystick curve for the MNT data. Each dose group consists out of six animals (data overlap, so the graph does not show six data points at each dose). The dose of 260 mg/kg has been omitted since the toxicity (depression of bone marrow proliferation, see Fig. 1 of Gocke et al., 2009a) at this dose would compromise the dose response.

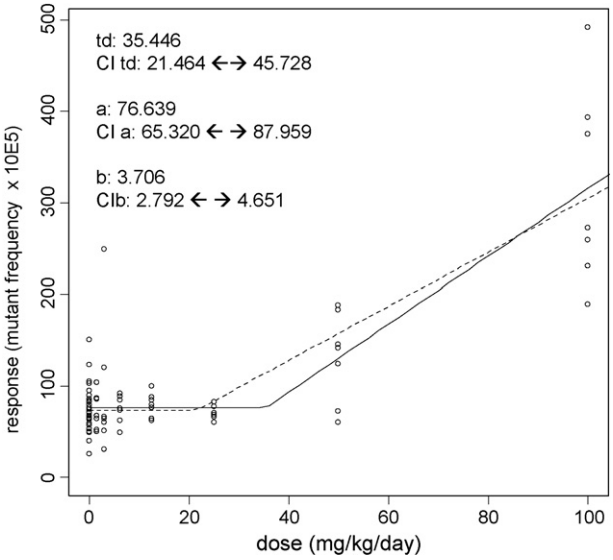

Fig. 2. Hockeystick curve for the bone marrow Muta™mouse data.

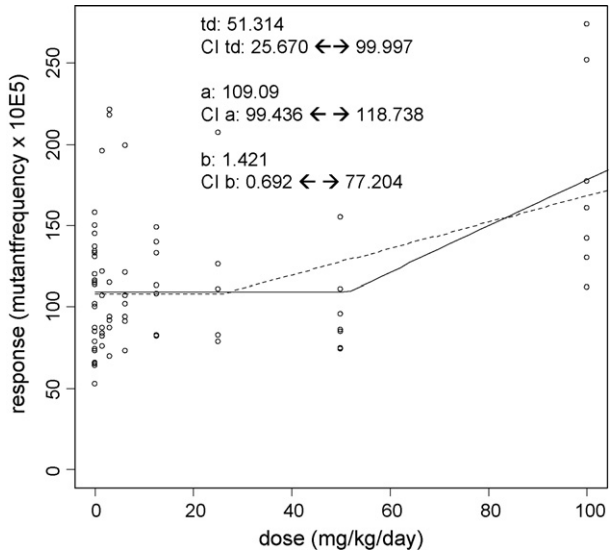

Fig. 3. Hockeystick curve for the liver Muta™mouse data.

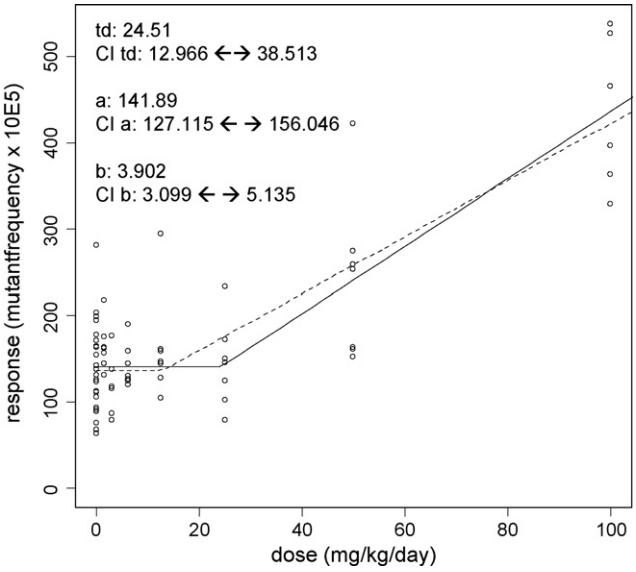

Fig. 4. Hockeystick curve for the GI tract Muta™mouse data.

3. Step 2: rejection of linear dose response relationship (entire dose range)

The dose response relationship of each organ was fitted by a second degree polynomial regression. The coefficient of the quadratic term was tested against zero. For the Muta™mouse study all dose groups (controls, 1.56 mg/kg, 3.13 mg/kg, 6.25 mg/kg, 12.5 mg/kg, 25 mg/kg, 50 mg/kg, and 100 mg/kg) and for the MNT study all dose groups ≤200 mg/kg (controls, 1.25 mg/kg, 2.5 mg/kg, 5 mg/kg, 20 mg/kg, 80 mg/kg, 140 mg/kg, and 200 mg/kg) were included. The top dose of 260 mg/kg was excluded since the apparent saturation of effects at this dose could lead to an inappropriate rejection.

Linear dose response relations can be rejected for all curves as shown in Table 2.

Table 1
Comparison of control groups.

Study	Organ	*p*-Value
MNT	Bone marrow	0.6848
Muta™mouse	Bone marrow	0.2021
Muta™mouse	Liver	0.0776
Muta™mouse	GI-tract	0.1086

Table 2
Assessment of linearity of entire dose range.

Study	Organ	*p*-Value
MNT	Bone marrow	≤0.001
Muta™mouse	Bone marrow	≤0.001
Muta™mouse	Liver	0.047
Muta™mouse	GI-tract	0.004