Free Radical Research, May 2010; 44(5): 529–540

informa
healthcare

The manganese superoxide dismutase mimetic, M40403, protects adult mice from lethal total body irradiation

JOHN S. THOMPSON[1,2], YANXIA CHU[2], JUDITH GLASS[2], ANDREA A. TAPP[2] & STEPHEN A. BROWN[1,2]

[1]*The Veterans Affairs Medical Center, and* [2]*The Department of Internal Medicine, University of Kentucky, Lexington, KY, USA*

(Received date: 12 December 2009; In revised form date: 20 January 2010)

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

Abstract
Over-expression of manganese superoxide dismutase (MnSOD) protects tissues from radiation. M40403 is a stable non-peptidyl mimetic of MnSOD that crosses cell membranes and is effective in reducing experimental inflammation. Male BALB/c mice were injected intraperitoneally (i.p.) and subcutaneously (s.c.) with M40403, 30 min before 6.5, 7.5 and 8.5 Gy total body irradiation (TBI). Whereas all control injected mice died after receiving 8.5 Gy TBI by day 17, 30 day survival of mice pre-treated i.p.with 40, 30, 20 or 10 mg/kg was 100%, 90%, 81% and 25%, respectively. The Dose Reduction Factor 50/30 for animals treated with 30 mg M40403 s.c. 30 min prior to TBI was 1.41. Decreased apoptosis of the large and particularly the small bowel and marked recovery of both lymphoid and hematopoietic tissues occurred in the M40403 pre-treated animals. M40403 is effective in reducing TBI-induced tissue destruction and has potential as a new radioprotective agent.

Keywords: *M40403, radiation, bowel, lymphoid, protection*

Copyright © 2010 Informa Healthcare
Not for Sale or Commercial Distribution
Unauthorised use prohibited. Authorised users can download, display, view and print a single copy for personal use

Introduction

Ionizing radiation (IR) and chemotherapy are mainstays of current anti-cancer therapy. Successful treatment of cancer commonly depends on the effects of the therapy on molecules involved in cell cycle regulation, membrane integrity and repair of normal host tissues. IR generates the release of free radicals that not only act directly on the genome of tumour target cells, but also on adjacent normal and indirectly on distant normal cells causing inflammation and apoptosis that may substantially limit therapy. Manganese superoxide dismutase (MnSOD) significantly influences the balance of cellular protection vs cell death [1–4]. It catalyses the dismutation of superoxide in mitochondria, it is essential for life after birth and it scavenges free radicals. Whether by reducing apoptosis or by other mechanisms, MnSOD may also influence ageing, asthma [3] and perhaps transplant outcome [4]. MnSOD also inhibits apoptosis induced by ultraviolet and ionizing radiation [5,6], cardiotoxic drugs, ischemia reperfusion [7] and high cytokine injury [8]. In contrast to copper/zinc superoxide dismutase (Cu/ZnOD), MnSOD exerts its primary anti-apoptotic effect at the mitochondrial membrane [3].

The potential of MnSOD as a therapeutic agent is limited because exogenously administered MnSOD is relatively unstable, unable to easily cross cellular membranes, has a short half-life and is often ineffective at reducing inflammation [4,9]. To overcome these limitations, mimics of MnSOD have been created. M40403 is a novel, highly stable manganese-containing macrocyclic ligand complex with a molecular weight of 483, whose activity mimics that of naturally occurring superoxide dismutase (SOD) enzymes. It is therefore a prototype of a new class of drugs termed SOD mimetics (SODm). Pre-treatment with MnSOD (M40403) has been effective in reducing

Correspondence: John S. Thompson, Department of Internal Medicine, University of Kentucky, 800 Rose Street, Lexington, KY, 40536, USA. Tel: 859-281-4927. Fax: 859-281-4989. Email: jsthom1@uky.edu

ISSN 1071-5762 print/ISSN 1029-2470 online © 2010 Informa UK Ltd. (Informa Healthcare, Taylor & Francis AS)
DOI: 10.3109/10715761003649578

RIGHTSLINK

inflammation and ischemia-reperfusion injury in the rat [9,10]. It also restored vasopressor responses to norepinephrine and reversed the hypotension of septic shock [11] in a rat model. Recently, M40403 has been reported to prevent apoptosis in neuronl/glial forebrain cells induced by N-Methyl-D-aspartate [12] and reduce superoxide-induced hypertension [13]. In conjunction with catalase, it also decreased the accumulation of myeloperoxidase and thiobarbaturic reactive substances (TBARS) in a model of ischemia-reperfusion of rat testis [14]. Based on the fact that all of these models were associated with oxidant stress, we began a series of investigations to determine whether M40403 could reduce the oxidant stress of ionizing radiation and thereby be radioprotective. In this study we report that M40403 is radioprotective to normal lymphoid and hematopoietic tissues *in vivo* and provide haematological, histological and immunochemical evidence as to how it is radioprotective.

Materials and methods

Mice

Male BALB/c mice were purchased from Jackson Laboratories (Bar Harbor ME). The mice were routinely held for 1 week or more before use. All mice in these experiments were 8–12 weeks of age, housed four to a micro-isolator cage and maintained on Teklad-irradiated 29-18 (Harlan, Indianapolis, IN) food and water *ad libitum*. The Institutional Animal Care and Use Committee (IACUC) of the VA Medical Center approved all experimental procedures used in these studies.

M40403

M40403 [$C_{21}H_{35}Cl_2MnN_5$] is one of a pentaazamacrocycle class of manganese (II) complexes. It has four asymmetric centres, but is a single enantiomer in the

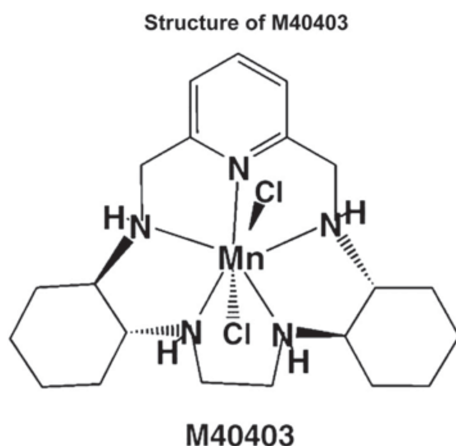

Figure 1. Structure of M40403.

R,R,R,R configuration (Figure 1). Typically, it is a monohydrate and it is a free flowing white-to beige powder. It is soluble in alcohols, chlorinated solvents, nitriles, dimethylformamide and water (ca. 50 g/L). The solubility of M40403 in 26 mM sodium bicarbonate buffered saline (pH 8.3) is ca. 30 mg/mL. It has a catalytic SOD rate $>2 \times 10^7$ M^{-1} s^{-1}, comparable to that of the native manganese SOD enzyme at a pH of ~6. The oxidative state of manganese in M40403 is Mn(II). Mn(II) does not dissociate from M40403 under physiologic conditions *in vitro* or *in vivo*. At pH 7.4, the half-life of M40403 diluted in 26 mM sodium bicarbonate buffer (Sigma-Aldrich, St. Louis , MO) (SBC) is ca. 4 h. Pharmacokinetic studies in plasma (performed by the manufacturer) after a 3.0 mg/kg dose ip were the following: C_{max} = 1374 ng/ml and C_{min} = 4.53 ng/ml; $T_{½}$ = 1.19 h with detectable levels until 4 h.

M40403 was kindly provided in powder form by ActivBiotics, Inc. (Lexington, MA) and prepared as follows: 30 mg dry powder was dissolved in 6.0 ml SBC (26 mM sodium bicarbonate (Sigma-Aldrich, St. Louis, MO) adjusted to pH 8.3 with 1 M NaOH according to the recommendations of ActivBiotics). This stock solution was diluted four times to equal 1.25 mg/ml in SBC.

Treatment

In dose and route finding experiments, normal male BALB/c mice were injected intra-peritoneally (i.p) or subcutaneously (s.c) with doses of 20, 40 and 80 mg/kg and observed for 5 days. All mice injected with 20 mg/kg i.p (5/5) or s.c (5/5) survived; 7/10 injected i.p and 9/10 injected s.c with 40 mg/kg survived; 1/5 injected i.p and 0/5 injected s.c. with 80 mg/kg survived and several of these animals died within 15 min of injection. Next, groups of 10 mice were injected either with a single or with five daily doses of 30 mg/kg M40403 i.p or s.c and all survived.

Two experimental models were tested. In one, the dose of IR was held constant at 8.5 Gy total body irradiation (TBI). The mice were injected i.p. with a single dose of 40 mg/kg, 30 mg/kg, 20 mg/kg or 10 mg/kg M40403. Thirty minutes later the mice received 8.5 Gy total body irradiation (TBI). Control animals received 0.1 ml of SBC buffer prior to TBI. In the other model, groups of 20 mice received either 6.5 or 7.5 Gy TBI. One half of each group was treated with 2.0 mg/kg M40403 i.p. and the other with SBC 30 min before TBI. All were followed for survival.

Total body irradiation (TBI)

TBI was delivered at a dose rate of 2.4 Gy/min by a Mark IV ^{137}Cesium γ-irradiator (J.L. Shepherd,

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

RIGHTSLINK

San Fernando, CA). The mice were radiated on a rotating platform.

Experimental plan

In the initial survival experiments, groups of 8–10 week old male BALB/c mice were injected (i.p.) with control SBC buffer or M40403 30 min prior to 8.5 Gy TBI and observed for 30 days. Moribund animals that met specific criteria established by the IACUC were euthanized and counted as an experimental death. The animals were inspected and weighed daily with the exception that they were inspected and weighed twice daily if they exhibited >20% loss of starting weight coupled with a loss of 2 g or more body weight within 48 h. Following Association for Assessment and Accrediitation of Laboratory Animal Care International (AAALAC) and our local guidelines, animals that met the following criteria were euthanized and counted as an experimental death. Principle criteria included: 'abnormal response to external stimuli, i.e. slow or no movement after gentle prompting, abnormal ambulation, severe diarrhea, uncontrolled bleeding, seizures, skin ulcerations and rapid/panting respirations' and lesser ones included: 'hunched posture, >20% loss of starting weight, a loss of 2 g or more body weight within 48 h, abnormal hair and exudates around the eyes and/or nose. Presence of one of the principal criteria or two or more of the lesser criteria constituted the criteria for euthanasia. Euthanasia was accomplished by CO_2 asphyxiation followed by cervical dislocation. Mice that were found dead were not necropsied.

To investigate the mechanism of M40403 protection from otherwise lethal TBI, other groups of identically aged male BALB/c mice received M40403 or SBC buffer followed by 8.5 Gy TBI. These animals were euthanized at selected times post-TBI for analysis of the effects of TBI and M40403 on tissues most susceptible to TBI. The details of this analysis are presented with the indiviual experiments.

Morphologic criteria

Necropsy was performed at each at the indicated time points and from untreated normal controls. The times were chosen as follows: 4 h to determine the extent and distribution of early radiation-induced apoptosis; day 10 to detect changes that were likely to lead to death but before the majority of the SBC-injected control mice were dying; day 14 because the majority of the SBC control animals had died but the majority of the 20, 30 and 40 mg/kg injected mice were alive; day 21 to detect what histologic and hematologic events had occurred that were associated with survival; and day 53 when it was clear that the surviving mice were gaining weight and apparently completely recovered. The spleen, thymus, liver, small bowel and large bowel were removed *in toto*. The spleen was weighed and ~50% was taken for histology and the other 50% for the isolation of cells for phenotypic analysis. Both aliquots were weighed so that the cell counts could equate to the whole spleen. Samples of the spleen, liver, thymus, skin and bowel were examined by routine haematoxylin-eosin (H&E) light microscopy to determine the preservation or loss of lymphoid and haematopoeitc tissues and the degree of tissue damage (e.g. crypt and villi necrosis in the gut, thinning of the epidermis, bile duct necrosis, etc). In addition, the degree of apoptosis was determined by *in situ* terminal deoxynucleotide transferase-mediated dUPT nick-end-labelling (TUNEL) histochemistry. Modifications of the TUNEL assay were performed on fixed tissue specimens using the Apoptag Plus Peroxidase *in situ* Apoptosis Detection Kit (Chemicon International, Temecula, CA). Positive control sections were included with each group of experimental sections that were treated with DNAse and negative control sections in which the TdT was omitted,

Phenotypic analysis

The viability, phenotype and absolute numbers of T-cells, B-cells, monocytes, NK cells and granulocytes were compared in the radiated M40403 treated and SBC treated and in the non-radiated-untreated control animals. The spleen was obtained from groups of animals killed at 4 h and at 10, 14, 21 and 43 days. After isolating and washing the spleen cells at 10°C, they were resuspended in 5 ml cold Flow Cytometry Medium [FCM] (0.5% Bovine Serum Albumin [BSA] + 0.02% Azide in phosphate buffered saline (PBS) pH 7.4 [FCS]), counted with Crystal Violet [5%] + Acetic Acid [0.0125%] and resuspended in FCM at 20×10^6 cells/ml. To prevent non-specific antibody binding, Fc receptors were blocked with CD16/32 (MFCR00) for 5 min following which they were incubated with antibodies purchased from Caltag (Camrillo CA) unless otherwise noted: T-cell/Cluster of Differentiation (CD) 3 (HM3401); B-cell/CD19 (RM7704); T-cell/CD4 (MCD0401); T-cell/CD8 (RM2204); Macrophage/F4/80 (RM2920); Granulocyte/GR-1 (RM3004); NK/DX5 (BD PharMingen 553858, San Diego, CA). Hamster Immunoglobulin G (IgG) (HM01); rat IgG2a (R2a01, R2a04); rat IgG2b (RGM 04); served as the isotype controls. After incubation for 15 min on ice, the cells were washed twice in cold Wash Buffer (0.01% Azide in PBS), following which 0.2–0.3 ml 1% paraformaldehyde (in PBS pH 7.4) was added and analysed by flow cytometry. Two colour flow cytometry measured the percentage cells of each phenotype and the absolute numbers were calculated from total number of cells determined by crystal violet (see above) × the percentage of phenotype.

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

RIGHTSLINK

Dose reduction factor (DRF)

The LD 50 (lethal dose for 50% of the animals) for control BALB/c mice was determined by injecting groups s.c. with SBC buffer 30 min before doses of 6.0, 6.5, 7.0, 7.5 and 8.5 Gy TBI. The LD 50 of BALB/c mice treated with M40403 was determined by injecting 30 mg/kg 30 min before doses of 8.5, 9.0, 9.5, 10.0, 11.0, 11.5 and 12.0 Gy TBI. The 30 day survival of each set of data was recorded and the LD 50 of the control and experimental set of data was determined by probit analysis. The DRF was calculated by dividing the LD 50 of the M40403 treated animals by the LD 50 of the control SBC treated animals.

Statistics

For the survival data, the data were analysed by log rank statistics. The p-values reflect the difference between the survival curves. For the other data, two tailed T-test statistics assuming unequal samples were performed. The data are expressed as means plus or minus standard deviation (SD). $p<0.05$ was taken as statistically significant.

Results

M40403 protects mice from lethal TBI in a dose-related manner

M40403 was injected i.p. 30 min before 8.5 Gy total body irradiation (TBI) into 8–12 week old BALB/c male mice. Figure 2A represents the composite of three separate experiments in which survival was followed until day 30. Whereas all control injected mice died by day 17, 30 day survival of mice pre-treated with 40, 30, 20 or 10 mg/kg was 100%, 90%, 81% and 25%, respectively: 40 mg/kg, 30 mg/kg, 20 mg/kg and 10 mg/kg-all vs SBC ($p<0.0001$); 40, 30 and 20 mg/kg M40403 were superior to 10 mg/kg ($p<0.001$). Thus, M40403 improved survival after lethal 8.5 Gy TBI in a dose-related manner. We also tested 5 mg/kg M40403, but there was early evidence of extensive injury in the bowel (Figure 3) and survival was less than that of the 10 mg/kg pre-treated animals (data is not shown).

The effect of pre-treatment with 2.0 mg/kg M40403 i.p. on the 30 survival of mice that received either 6.5 or 7.5 Gy TBI was also determined (Figure 2B). Thirty day survival was: 6.5 Gy (57%), 6.5 Gy + M40403 (93%), 7.5 Gy (20%) and 7.5 Gy + M40403 (40%). At both TBI doses, M40403 was radioprotective: 6.5 Gy vs 6.5 Gy plus M40403 ($p<0.001$); 7.5 Gy vs 7.5 Gy plus M40403 ($p<0.05$).

Based on this information, it was clear that M40403 was radioprotective. To determine how effective it was in comparison to other reported radioprotective agents, we determined the LD 50 of mice treated only with SBC buffer alone vs those that received 30 mg/kg of M40403 (Figure 2A). Although survival was nearly the same with pretreatment with 20 mg/kg, 30 mg/kg was superior ($p<0.05$). For this comparison, we chose to treat the SBC control and M40403 experimental animals by the s.c. rather than i.p. or intravenously (i.v.) since the effect on survival was similar (30 day survival: 30 mg/kg i.p. = 90% vs s.c. = 93%). For the control LD 50, there were 10 mice for each dose. Survival was: 6.0 Gy (100%); 6.5 Gy (80%), 7.0 Gy (50%) and 7.5 Gy (30%) (Figure 3A). These data resulted in an LD 50 of 7.09 with fiducial limits of 6.80–7.51. For the LD 50 Gy of the M40403 treated animals, there were 15 mice for the 8.5, 9.0, 9.5 Gy doses of TBI; 20 mice for the 10.0 Gy dose; 10 mice for the 11.0 Gy dose; and five mice received 11.5 or 12.0 Gy TBI (Figure 3B). Thirty day survival for each dose was: 8.5 Gy (93%), 9.0 Gy (73%), 9.5 Gy (73%), 10 Gy (60%), 11 Gy (20%) and both 11.5 and 12 Gy (0%). Probit analysis of the 30 day survival data resulted in an LD 50 of 10.01 with fiducial limits of 9.68–10 (Figure 3C).

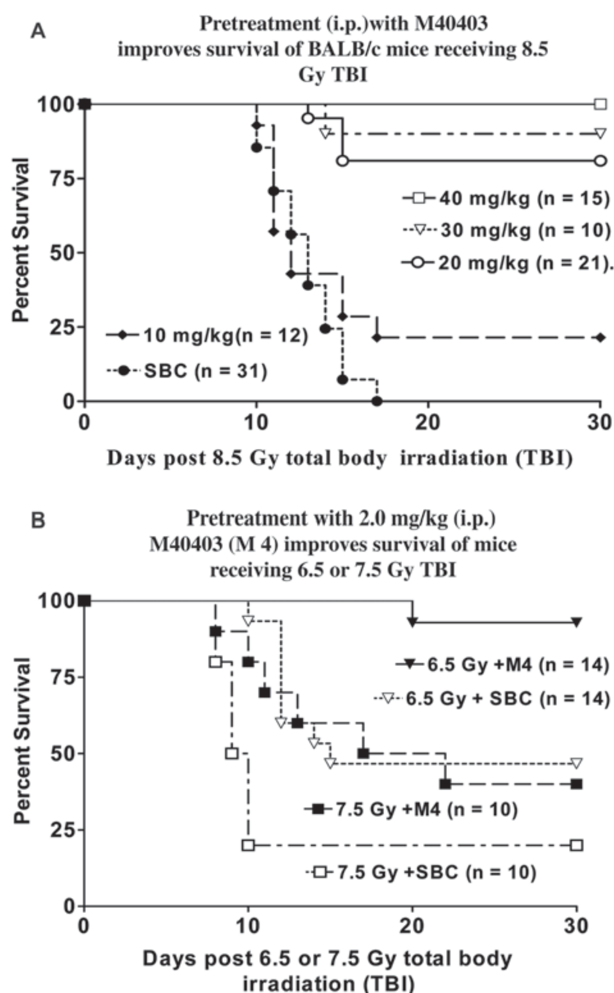

Figure 2. *In vivo* radioprotective effect of i.p. injection of M40403. (A) This figure is a composite of three separate experiments. Ten or more sodium bicarbonate buffer (SBC) treated BALB/c control animals were included with each experiment. (B) Pre-treatment with 2.0 mg/kg M40403 improves 30 day survival of 6.5 and 7.5 Gy total body irradiated BALB/c mice.

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

RIGHTSLINK

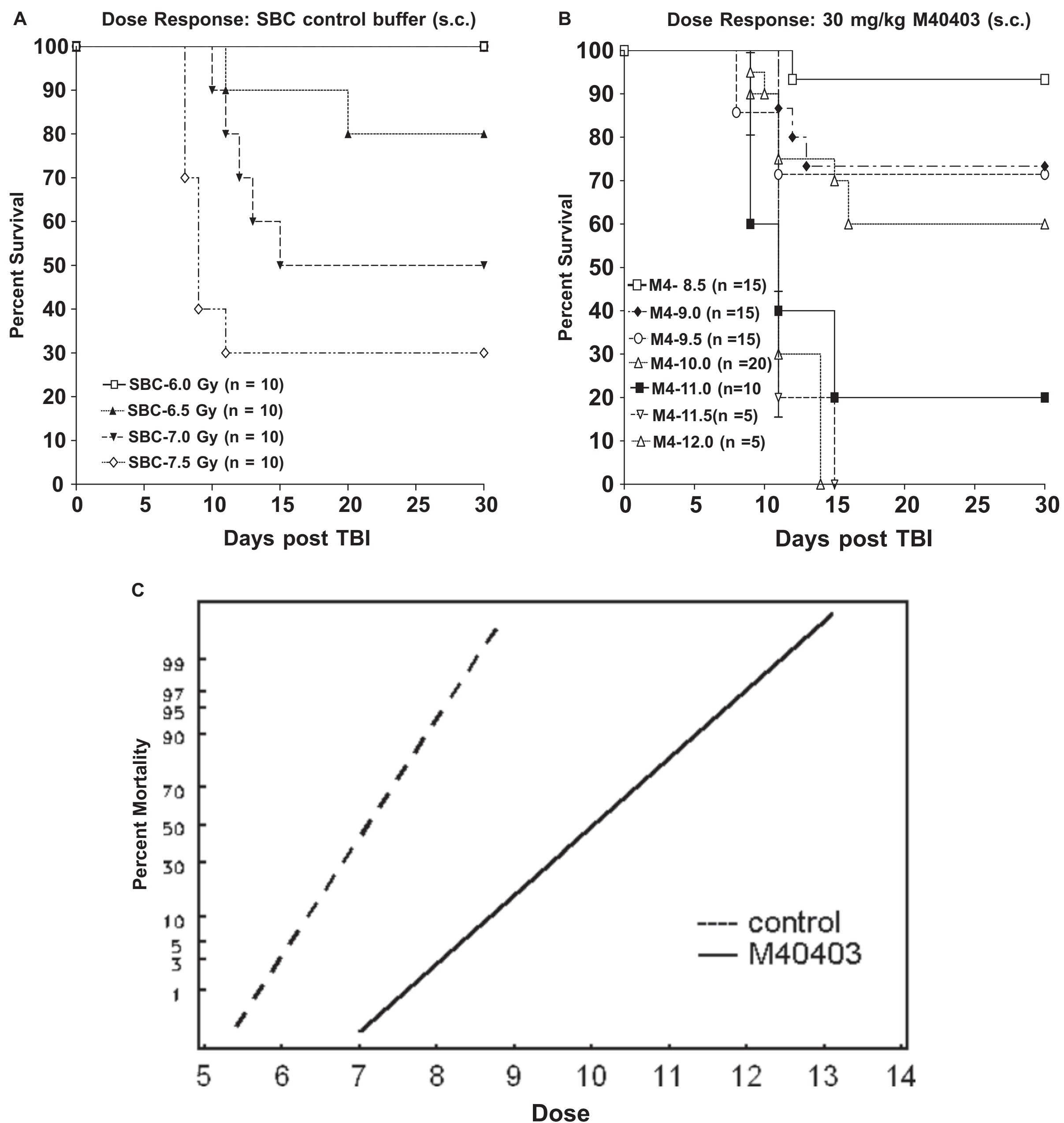

Figure 3. Dose reduction factor induced by subcutaneous pre-treatment with 30 mg/kg M40403. (A) Survival curves of control SBC treated mice receiving 6.0, 6.5, 7.0 and 7.5 Gy TBI. (B) Survival curves of 30.mg/kg treated mice receiving 8.0, 8.5, 9.0, 10.0, 11.0 11.5 and 12 Gy TBI. (C) Probit analysis of Figures 2(A and B) data. Dashed line = no M40403; solid line = pre-treated with M40403.

The difference of the LD 50 Gy for the SBC control treated animals versus the LD 50 for the 30 mg/kg M40403 treated was highly significant (p <0.0001). The Dose Reduction Factor comparing the 30 day 50% survival of experimental as compared to 30 day 50% control survival (DRF 50/30) for animals treated s.c. with 30 mg M40403 30 min prior to TBI was 1.41.

M40403 protects the bowel and enhances multilineage hematopoietic recovery

To investigate the mechanisms of how M40403 protected mice from lethal TBI, groups of four animals each were euthanized to compare haematopoietic and lymphoid depletion and recovery by routine Haematoxylin and Eosin (H&E) histology and TUNEL immunohistochemistry. The 4 h, days 10, 14 and 21 day time points were chosen to demonstrate the differences in early apoptosis in the spleen and bowel at 4 h; the differences in blood values and lymphoid tissues on day 10 when some of the radiated but otherwise untreated mice were beginning to die; on day 14 when more of the radiated-untreated animals were dying; and on day 21 for characterization of the surviving animals. The remaining animals were necropsied on day 43 when

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

RIGHTSLINK

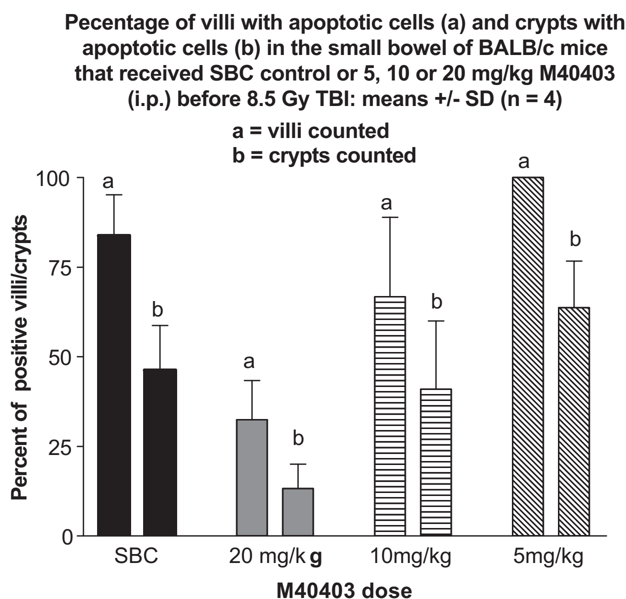

Figure 4. Pre-treatment with 20 mg/kg M40403 i.p. significantly reduced apoptosis in the small bowel 4 h after 8.5 TBI. The percentage of apoptotic cells in the villi are represented by the first bar of each pair of bars. The second bar of each pair represents that percentage of villi with apoptosis in their crypts. The percentages are compared for each pair of bars treated with control SBC, 20 mg/kg, 10 mg/kg and 5 mg/kg M40403 injected i.p before TBI. Error bars indicate means ± SD (n = 4). Apoptosis was determined by the TUNEL assay.

the experiment was terminated. The changes at each time point were compared to each other and to a group of completely normal untreated BALB/c mice of similar age and sex.

Four hours post-TBI: M40403 reduced apoptosis in the bowel

At 4 h after TBI, animals from each treatment group were euthanized. H&E microscopy revealed an early mild loss of cells in the lymph follicles of the spleen and Peyer's patches, but no changes were detectable in the skin or bowel. However, marked apoptosis had already occurred in the large and particularly in the small bowel in the control SBC and 5 mg/kg treated animals. Figure 4 illustrates the percentage of villi exhibiting apoptotic cells in the small bowel and is based on counting more than 100 villi for each measurement. Apoptosis was clearly reduced in the animals pre-treated with 20 mg/kg M40403. The majority of the apoptotic cells were in the bases of the villi, but in more severe cases, crypt injury was also marked and in some of the most severely damaged the terminal tufts of the villi were apoptotic. Apoptosis was also prominent in the lymphoid follicles of the spleen and Peyer's patches (Figures 5 A and B), but, in this case, there was no apparent reduction with any dose of M40403 tested as compared to that occurring in the SBC controls. Flow cytometry analysis of spleen cells from the 8.5 Gy TBI animals pre-treated with SBC or 10, 20 or 30 mg/kg M40403 demonstrated no significant differences between the absolute numbers of CD3, CD4, CD8 lymphocytes, Gr-1 granulocytes, F4/80 macrophages and NK/DX-5 NK cells. However, there was a significant increase in the absolute number of Gr-1 granulocytes (13 ± 5.0) in the radiated animals as compared to untreated controls (5.9 ± 0.9) ($p \leq 0.01$) at this early period post-8.5 Gy TBI. Table I summarizes the haematological and lymphoid changes in the spleen as they progressed from 4 h to day 43 as compared to that of completely normal untreated BALB/c animals of the same age.

Ten days post-TBI

All of the SBC control and M40403 experimental radiated mice initially lost weight following TBI. There was a trend for the 20 or 30 mg/kg treated mice to have a little less loss in weight and in the total cell count as compared to that in the SBC treated animals. Spleen weight had decreased by 2/3 and the total spleen cell count by 97% in both SBC control and experimental animals as compared to control untreated mice of the same sex and age. Light microscopy of the small bowel of SBC and 10 mg/kg M40403 treated mice exhibited severe loss of villi, but much less injury in the mice pre-treated with 20 (Figure 6) or 30 mg/kg M40403. Sections of the spleen demonstrated marked loss of lymphoid tissue (Figure 7). There was little apoptosis in the residual lymphocytes, but prominent apoptosis was now evident throughout the red pulp and with all doses of M40403. Thus, the day 10 analysis primarily reflected radiation damage with little evidence of recovery. With the exception of the bowel histology, no clear differentiation based upon M40403 pre-treatment could be made.

Fourteen days post-TBI: 10 and 20 mg/kg M40403 enhance recovery from TBI damage

At day 14 post-8.5 Gy TBI, only two of the SBC and three of the10 mg/kg treated mice were still alive (Figure 2A). Body weight and spleen weight had not increased significantly from the day 10 weights (Table I). Although there was a trend toward slightly higher total cell counts, leukocyte counts and CD19 B cells in the M40403 treated animals as compared to the SBC controls, these differences did not reach statistical significance. The one exception was an increase in total leukocytes in the 10 mg/kg treated animals on day 14 as compared to day 10 ($p<0.05$). However, this may not be biologically significant since the majority of the 10 mg/kg treated animals did not survive for 30 days. Histologically, spleens from animals killed for analysis on this day still showed marked hypocellularity (Figure 7), but there were now foci of lymphoid cells along trabeculae (data not shown). Apoptosis was even more marked in the red pulp of the spleen (Figure 5C).

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

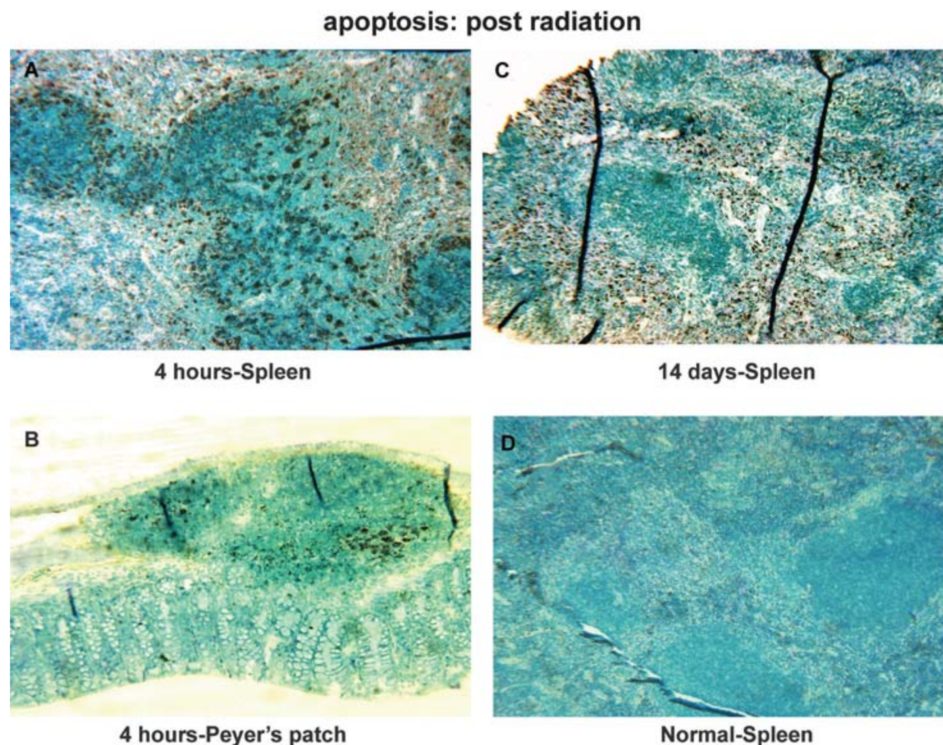

Figure 5. Pre-treatment with M40403 i.p. had no early effect on apoptosis of the spleen and a Peyers patch. Magnification 600×, TUNEL assay. (A) Marked apoptosis in splenic lymphoid tissue at 4 h post-TBI. (B) Marked apoptosis in a Peyer's patch at 4 h post-TBI. (C) Marked apoptosis in splenic red pulp and its absence in residual white pulp 14 days post-TBI. (D) The absence of significant apoptosis in the spleen of a completely normal untreated animal.

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

New erythropoiesis was still scant, but nucleated red blood cells (rbcs) were now seen in the red pulp of some of the 20 mg/kg M40403-treated animals. The haematocrit of the remaining SBC-treated animals had dropped to 17.0 ± 4.08 ($n = 4$) as compared to that of the M40403 treated animals: 20 mg/kg (26.08 ± 0.83; $n = 4$) and 10 mg/kg (23.45 ± 1.27; $n = 4$). The haematocrits of both the 20 mg/kg ($p<0.01$) and 10 mg/kg ($p<0.05$) treated animals were statistically superior to that of the SBC control.

Injury was also evident in the small bowel of a SBC-treated mouse euthanized on day 14 (Figure 6B) as compared to the nearly normal bowel from a 20 mg/kg-M40403-treated animal (Figure 6E). In contrast, the small bowel of the animals that had been euthanized because they were moribund, e.g. on day 15, exhibited markedly short villi, necrosis of their crypts and debris in the lumen (Figure C). Thus, by day 14, there was further evidence of destruction of the small bowel in the animals that had received control SBC as contrasted to much less injury in the mice pre-treated with 20 and 30 mg/kg M40403.

Twenty-one days post-TBI: M40403 treated mice exhibit resurgent erythroid and granulopoiesis in the spleen

By day 21 there were dramatic increases in the weights of the majority of spleens from the mice pre-treated with 20 or 30 mg/kg (Table I). The data from the 20 and 30 mg/kg (listed as 20 mg/kg in Table I) treated animals has been pooled since there was essentially no difference between the two dosages and the changes reflecting their recovery post-TBI were so dramatic. To a less quantitative degree, there were similar changes in the mice that received 10 mg/kg M40403 and survived to day 21. The mean weight of the spleens from animals pre-treated with 20 or 30 mg/kg M40403 was much greater at day 21 (333.0 mg) as compared to day 14 (31.5 mg) ($p <0.0001$), but also greater than that of untreated control weights: untreated control vs 20 or 30 mg/kg M40403 ($p<0.001$). This was due to a >15-fold increase in total leukocytes compared to that on day 14 and included increases in lymphocytes, monocytes and NK cells, but was predominately related to a profound increase in immature and mature granulocytes (50.7 ± 10^6 vs 0.6 ± 10^6) ($p<0.0001$). Megakaryocytes were definitely increased, indicating impending recovery of platelets. However, the predominant change was a massive increase in the number of nucleated rbcs by day 21 (405.7 ± 10^6) as compared to day 14 (0.31 ± 10^6). There were no surviving SBC animals to compare, but there was a statistically significant difference ($p<0.05$) in the haematocrit of the 20 mg/kg mice (24.34 ± 2.14; $n = 7$) vs that of the 10 mg/kg mice (19.10 ± 3.97; $n = 4$). Histologically, it was difficult to confirm increased granulopoiesis because

RIGHTSLINK

Table I. Effect of M40403 on lymphoid and haematological recovery in mice receiving 8.5 Gy TBI.

Time p-TBI	M40403 dose		BW (g)	Spl W (mg)	Total cells	Total × 106**		Absolute cell counts						
						rbcs[a]	leuks[b]	CD19	CD3	CD4	CD8	Gr-1	F4/80	DX5
4 h	30 (4)[c]	*M*	25.6	76.0	132.0	2.3	127.7	56.6	29.9	28.5	12.2	12.4	8.0	8.7
		± SD	3.0	9.3	36.8	0.9	33.0	0.6	1.3	6.2	2.8	5.3	1.8	3.3
	20 (4)[c]	*M*	24.9	78.1	136.4	2.4	147.2	75.8	40.7	32.9	3.7	4.7	9.5	10.0
		± SD	1.8	1.9	20.3	0.2	24.9	11.1	8.8	4.5	2.2	1.1	2.7	2.1
	10 (4)[c]	*M*	26.1	77.8	155.5	2.5	153.0	88.7	33.1	29.7	14.7	13.9	11.3	9.8
		± SD	2.0	4.7	41.0	2.5	42.0	20.0	10.3	5.3	2.2	8.3	0.5	2.5
	SBC (4)[c]	*M*	21.0	78.9	154.0	1.6	152.4	84.0	42.1	31.0	15.0	13.0	0.3	8.1
		± SD	3.7	4.9	39.1	0.1	39.6	20.2	6.4	5.4	1.0	5.0	1.6	2.5
10 days	30 (3)[c]	*M*	27.2	27.7	1.6	0.5	1.4	0.1	0.8	0.7	0.1	0.2	0.2	0.4
		± SD	0.3	0.6	0.7	0.2	0.6	0.0	0.4	0.3	0.1	0.1	0.1	0.3
	20 (2)[c]	*M*	24.8	35.5	2.1	0.4	1.7	0.1	0.7	0.6	0.1	0.1	9.3	0.7
		± SD	0.8	2.6	1.0	0.2	0.8	0.0	0.4	0.3	0.0	0.0	0.2	0.5
	10 (4)[c]	*M*	26.1	28.5	1.5	0.6	0.8	0.0	0.5	0.4	0.1	0.1	0.3	0.7
		± SD	0.1	3.0	0.2	0.4	0.3	0.0	0.1	0.0	0.1	0.0	0.1'	0.3
	SBC (4)[c]	*M*	24.9	26.8	0.9	0.4	0.6	0.0	0.5	0.3	0.0	0.0	0.1	0.2
		± SD	0.2	3.7	0.5	0.4	0.6	0.0	0.3	0.3	0.0	0.0	0.1	0.2
14 days	20 (4)[c]	*M*	24.8	31.5	2.7	0.3	4.7	0.8	1.1	1.1	0.4	0.2	0.2	0.4
		± SD	0.78	5.0	1.1	0.1	4.0	0.7	0.5	0.4	0.5	0.1	0.1	0.2
	10 (4)[c]	*M*	23.6	28.4	5.6	0.6	4.9	1.4	1.9	1.5	0.3	0.1	0.2	0.4
		± SD	3.8	4.6	3.3	0.4	2.6	1.4	0.7	1.0	0.2	0.1	0.1	0.3
	SBC (3)[c]	*M*	21.7	20.4	2.1	0.7	2.0	0.3	0.9	0.7	0.1	0.0	0.1	0.3
		± SD	1.5	5.4	0.2	0.7	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.1
21 days	20 (6)[c]	*M*	23.5	333.9	464.1	405.7	79.2	7.3	11.8	7.8	4.5	50.7	4.1	6.4
		± SD	1.2	32.6	186.6	137.0	18.7	4.1	3.9	2.4	1.1	15.3	1.3	2.6
	10 (4)[c]	*M*	19.1	237.5	186.0	155.8	30.2	3.1	8.7	6.2	3.4	9.4	2.4	4.2
		± SD	3.4	104.3	76.3	71.1	6.6	1.4	2.2	2.3	1.7	5.9	1.0	2.2
54 days	30 (8)[c]	*M*	26.3	68.0	116.3	2.6	115.9	60.8	36.7	25.5	9.1	6.9	8.6	9.7
		± SD	0.8	4.3	14.1	0.8	13.6	6.7	5.6	3.4	1.4	1.0	1.5	1.7
	Con. (4)[c]	*M*	25.4	81.2	112.1	4.8	107.7	60.2	34.4	23.7	11.7	5.9	7.3	5.1
		± SD	0.5	6.8	35.0	3.7	31.4	16.9	12.9	7.6	2.6	0.0	3.3	1.5

BW = body weight; Spl W = spleen weight; rbcs = nucleated red blood cells; leuks = leukocytes; M4 = M40403; SBC = sodium bicarbonate; Control = completely untreated BALB/c mice.
[a]Total nucleated rbcs = total cell count – total number of leukocytes × 10^6.
[b]Absolute leukocyte and phenotypic leukocyte counts: determined by multiplying the total number of cells × 10^6 counted by haemocytometer by the % CD45 = total leukocytes; and *** by the % CD19 = B-cells; % CD3, CD4 and CD8 = T-cells; % Gr-1 = granulocytes; % Dx-5 = NK cells; and % F4/80 = monocytes/macrophages.
$(n)^c$ = number of animals examined.

the red pulp was overshadowed by the marked erythropoiesis (Figures 8B1 and B2).

By 43 days post-TBI, animals receiving 30 mg/kg M40403 appear clinically, histologically and hematologically recovered

A group of eight radiated mice treated with 30 mg/kg M40403 were observed until day 43 at which time they had regained or exceeded their initial body weight and they appeared to be entirely healthy. The striking increases in total leukocytes and nucleated rbcs noted on day 21 had reversed to that of untreated control values, as had all of the sub-sets of leukocytes that were measured (Table I, Figures 8C1, 2 and D1, 2). Thus, these data demonstrate that 20 or 30 mg/kg M40403 generally promoted a strong recovery in erythroid, myeloid, lymphoid and megakaryocytic cells and help to explain why M40403 treatment promoted survival after lethal TBI.

Discussion

The pro-drug amifostine is the only drug approved by the Federal Drug Administration (FDA) as a cytoprotective agent [15–17]. There are at least five mechanisms whereby amifostine may exert this effect: (1) free radical scavenging; (2) facilitation of DNA repair; (3) activation of NF-κB [6]; (4) induction of MnSOD [18,19]; and (5) induction of transitory intracellular hypoxia, making the cell less susceptible to free radical generation. Supershift analysis has confirmed that amifostine specifically activates the p50/p65 heterodimer of nuclear factor-kappaB (NF-κB) and that inhibition of either dimer diminishes the capacity of amifostine to induce MnSOD [20]. Furthermore, there appears to be an important interaction between MnSOD and p53. Pani et al. [21] observed higher levels of the anti-oxidant MnSOD and resistance to oxidant injury in $p53^{-/-}$ cells. This resistance could be temporarily reduced by the transfection of p53, suggesting that MnSOD was negatively regulated by p53. Recently, Dhar et al. [22] demonstrated that over-expression of

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

RIGHTSLINK

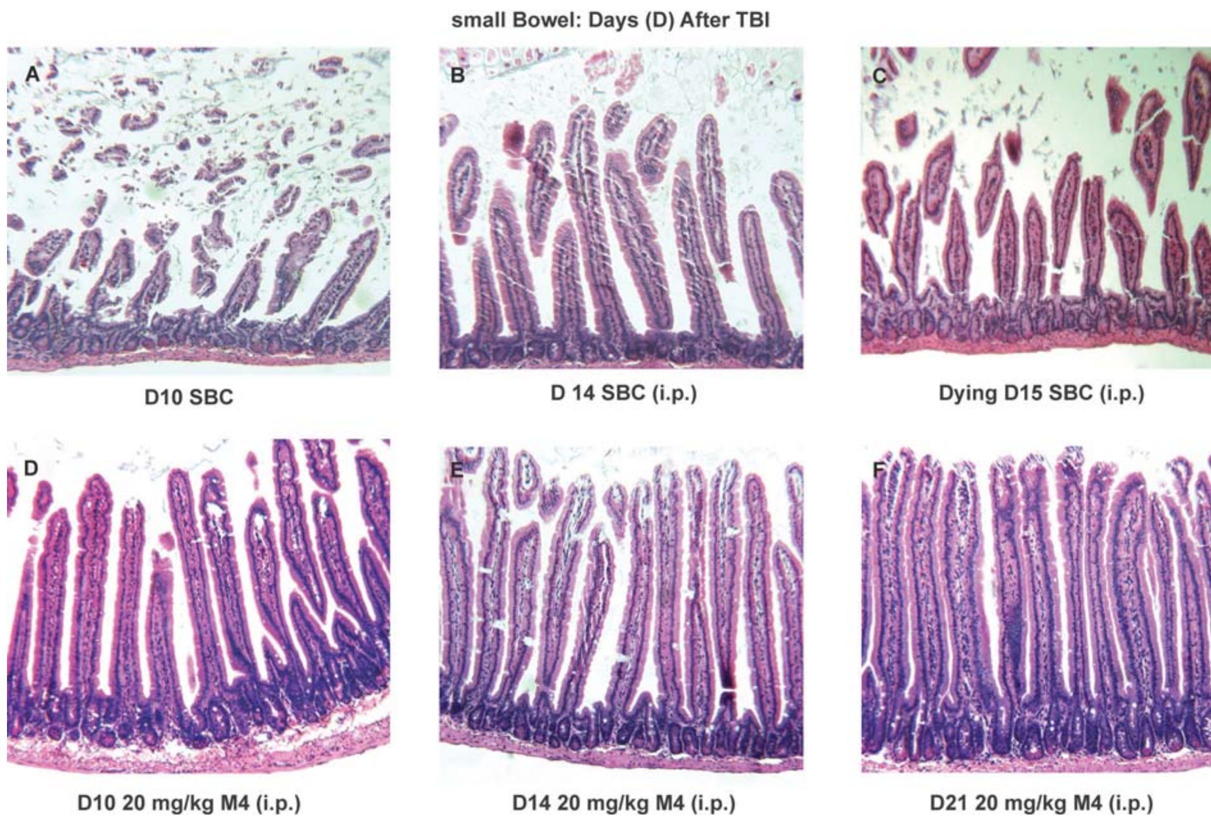

Figure 6. Tissue injury and recovery of the small bowel and spleen in lethally irradiated mice pre-treated i.p. with sodium bicarbonate buffer [SBC] or M40403. Haematoxylin and Eosin (H&E) staining. Magnification: 600×. (A–C) The small bowel at days 10, 14, and prior to death on day 15 in control mice treated with SBC. (D–F) The comparison of small bowel histology in mice treated with 20 mg/kg M40403 i.p. on days 10, 14 and 21.

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

p53 inhibits MnSOD gene expression by its binding to specificity protein 1 (Sp-1), i.e. one of the factors critical for induction of MnSOD. The calculated DRF of 1.41 for M40403 indicates that it is a radioprotective drug. A key question was how does this compare to the DRF for amifostine and other similar radioprotective agents? It is difficult to directly make this comparison because the strain of mice, the route of administration and the doses tested vary in the reported studies. The route of administration is important since extrapolation of animal results should take into consideration what routes could be possible for human self-administration quickly after radiation exposure. Some of the older investigations cite amifostine's DRF 50/30 >2.0 [23,24]. Brown et al. [24] studied the radioprotective effect of a large group of compounds in male BALB/c mice receiving TBI and reported DRFs as high as 2.3 for those pre-treated with WR-2721 (amifostine). It is difficult to compare this result to that we observed with M40403 because their reported LD 50/30 of 9.60 ± Gy is unusually high for BALB/c mice that are known to be one of the more radiosensitive strains [25–27]. For comparison, the LD-50/30 we observed for 10–12 week old male BALB/c mice housed in micro-isolater cages, fed laboratory chow and provided with acidified water, was 7.09 Gy.

Interest in the possibility that mimics of superoxide dismutase could be radioprotective dates back at least to the report of Sorenson [28], who observed that pre-treatment with a copper containing mimetic improved the LD50/30 dose survival of B6VDF1 mice from 6.3 to 7.0 Gy TBI. In 1993 this same group also reported that 40 μmol/kg of a manganese-salicylate containing compound administered s.c. 1–3 h after TBI improved survival from an ~LD 50 dose of 8.0 Gy to C57BL/ mice. They suspected that the benefit was due in part to an unknown effect in addition to acting as superoxide dismutase. Two recent reports are of particular interest because they both may involve protection via initiation of MnSOD (SOD2). EUK-189 is a superoxide dismutase/catalase mimetic. Administration of 70 mg/kg s.c. 1 h before TBI to CD2F1 mice resulted in a DRF = 1.15 [29]. Burdelya et al. [30] injected 0.2 mg/kg s.c. of a derivative of flagellin (CBLB502) 30 min before TBI to Swiss mice that was less immunogenic and toxic than the parent flagellin and determined a dose modifying factor of 1.6 if administered 30 min before TBI. In addition, a mitigating effect was also observed if it was injected up to 1 h after radiation. CBLB502 activated NF-κB and increased superoxide dismutase 2 (SOD2 = MnSOD) in the lamina propria of the small bowel. NF-κB induction of SOD2 may be the key, since it has also been observed with amifostine [4,6,17].

RIGHTSLINK

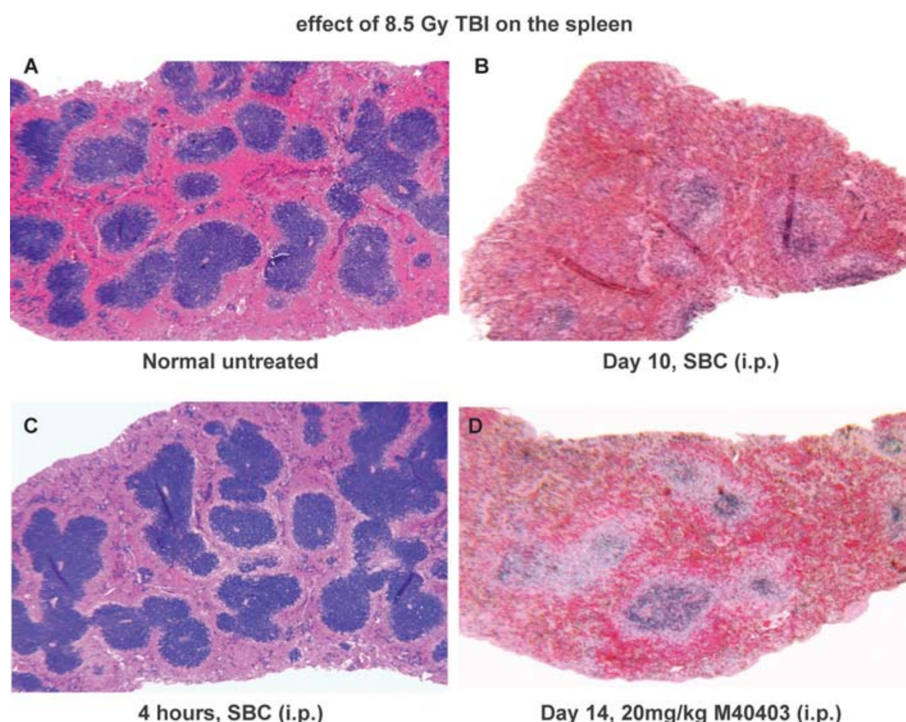

Figure 7. Loss of splenic lymphoid tissue at 10 and 14 days in lethally irradiated mice pre-treated i.p. with either control SBC or 20 mg/kg M40403. Haematoxylin and Eosin (H&E) staining. Magnification: 350×. (A) Normal untreated spleen. Note numerous well developed lymphoid follicles. (B) Spleen 4 h after 8.5 Gy TBI in an SBC treated animal. Note, no major change seen. (C) Spleen 14 days after 8.5 Gy TBI in an SBC treated animals showing loss of lymphoid tissue. (D) Spleen 14 days after 8.5 Gy TBI in an animal treated with 20 mg/kg M40403. Note the marked loss of lymphoid tissue in both (C) and (D).

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

Within the dosage range of 10–30 mg/kg, our studies demonstrate that the MnSOD mimetic M40403 reproduces some of the cytoprotective effects attributed to MnSOD and to amifostine. For example, 20–30 mg/kg M40403 dramatically reduced the apoptosis in the bowel that developed in the control animals within 4 h after TBI. Subsequently, the bowel in animals receiving this dose of M40403 was spared much of the destruction of villi seen in the control treated mice. This is similar to the observations reported by Guo et al. [8] using gene transfer of MnSOD. This benefit may be accounted for by the early ability of M40403 to reduce reactive oxidant species induced by TBI. The fact that the mice receiving 20–30 mg/kg (and to a lesser extent 10 mg/kg) M40403 did not suffer marked destruction of intestinal villi is clearly one critical factor in the survival advantage related to M40403 pre-treatment.

On the other hand, apoptosis of the splenic lymphoid tissue was prominent at 4 h and marked hypocellularity subsequently occurred in all of the irradiated animals irrespective of M40403 treatment. However, on day 10 there was less apoptosis in the red pulp of the 20 mg/kg treated mice. There was an increase in nucleated rbcs in both 10 and 20 mg/kg at day 14 as compared to the one SBC animal necropsied on day 14. This preceded a very marked increase in nucleated rbcs and the increase in granulocytes that occurred by day 21. Although we did not directly determine recovery of platelets, megakaryocytes were present by day 14 and numerous in the red pulp by day 21. The capacity of amifostine to stimulate bone marrow colony forming units-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM) and burst forming units-erythroid (BFU-E) cell growth has been observed in murine cells after radiation [31] and in normal non-irradiated human marrow [32]. To our knowledge M40403 has not been reported as to whether or not it might share this feature with amifostine, but our histological data is suggestive of an important haematopoietic effect.

Could M40403 mitigate radiation injury if administered following TBI? This has not been tested, but there are reports of its effectiveness after the initiation of other experimental injuries [33–35].

The doses of 20 and 30 mg/kg may appear to be high but were tolerated in these experimental conditions. Extrapolation to man of these doses in the mouse considers three very relevant factors. First, Voison et al. [36], based on weight to body surface area (M^2), recommended a mouse/man conversion factor of 12. Second, this conversion based on body surface area does not take into account the relative clearance of M40403 in the mouse as compared to man. The compound M40403 has been extensively studied in animals (mice, rat and dog) and man (personal communication; Dr Dennis P. Riley, Galera Therapeutics St. Louis, MO). The pharmacokinetics (PK) and

RIGHTSLINK

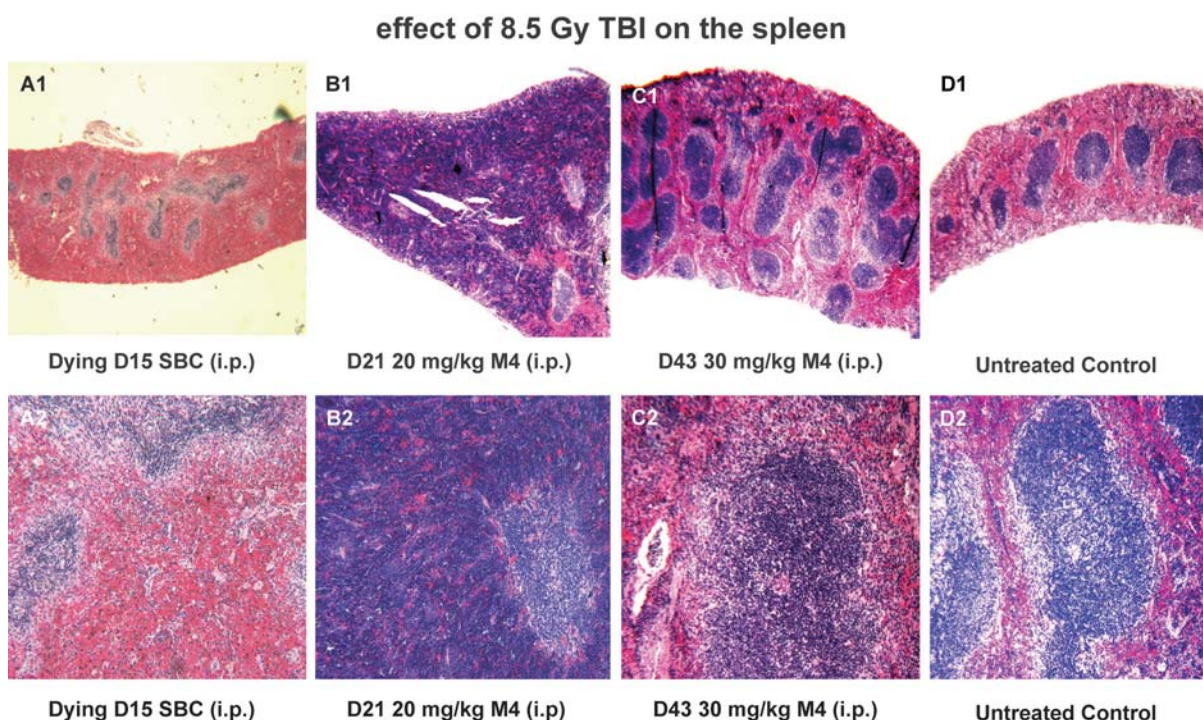

Figure 8. Tissue injury and recovery of the spleen in lethally irradiated mice pre-treated with M40403 or SBC i.p. Haematoxylin and Eosin (H&E) staining. (A1–D1) Magnification ×350. H&E comparison of the histology of the spleen of a mouse dying on day 15 (A1), a mouse treated with 20 mg/kg M40403 and alive on day 21 (B1), a mouse treated with 30 mg/kg M40403 and alive on day 43 (C1) and a completely untreated control mouse (D1). (A2–D2) Magnification ×600. The same spleens at higher power. Note the marked hypocellularity of the SBC-treated animal dying on day 15, the profound increase in nucleated red blood cells in the 20 mg/kg M40403-treated animal at day 21 as compared to the normal white and red pulp architecture of the animal living on day 43 (C1–C2) and the normal untreated control animal (D1–D2).

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

species relationships for M40403 exhibit very standard allometric scaling of dose following administration i.v. of the drug in the rat, dog and man. For example, an i.v. dose in the rat scales to a human equivalent dose of 1/6th of the rat dose in mg/kg and ~50% of the corresponding dog dose in mg/kg. In the mouse, however, the compound exhibits a very different clearance and PK behaviour such that the normal PK scaling to a human equivalent dose based on allometric scaling is altered. The compound clears very rapidly from the blood of mice, resulting in a scaling factor that is less than the surface area scaling of 1/12th. This results in a striking difference in the clearance (CL (L/h/kg) of M40403 in the mouse of 7.08 as compared to that in the human (0.15), rat (0.13) and dog (0.01). Thus, the area under the curve (AUC)/dose is ~40–60-fold greater in humans as compared to the mouse. Therefore, the pharmacology of M40403 in the mouse is characterized by necessarily administering doses much higher than needed in the rat, human or dog to elicit a pharmacological efficacy. However, a bolus injection, as administered to our mice, may manifest a toxic effect related to the potentiation of nitric oxide (NO), resulting in an early reduction in blood pressure. This translates into a maximum concentration (C_{max}) toxicity that can be moderated by slow infusion rates. Consequently, there is a risk of generating an acute NO-induced C_{max} driven toxicity in the mouse but, if this does not occur, there are no longer term negative effects and the benefit of the higher dose can be achieved. Taken together, this accounts for the observation, that when 40 mg/kg was injected, a few animals died within minutes but if they survived this early death, this dose completely protected the 8.5 Gy TBI-mice (Figure 2). Since M40403 has been dosed in over 600 subjects via an i.v. infusion at doses higher than 0.2 mg/kg, one can reasonably conclude that the protective effects observed, when M40403 is administered to mice exposed to radiation, can be achieved in man with clinically relevant doses.

Conclusion

We conclude that M40403 is effective in reducing TBI-induced tissue destruction and has potential as a new radioprotective agent.

Declaration of interest: Supported by a VA Merit Review Grant, a VA Merit Entry Award and a University of Kentucky Research Graduate Studies Award. The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

RIGHTSLINK

References

[1] Wong GHW, Kaspar RL, Vehar G. Tumor necrosis factor and lymphotoxin: protection against oxidative stress through induction of MnSOD. EXS 1996;77:321–333.

[2] Lebovitz RM, Zhang H, Vogel J, Cartwright L, Dionne N, Lu N, Huang S, Matzuk NM. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide-deficient mice. Proc Natl Acad Sci USA1999;93:9782–9787.

[3] Macmillan-Crow LA, Cruthirds DL. Invited review: manganese superoxide dismutase in disease. Free Radic Res 2001; 34:325–336.

[4] Cuzzocrea S , Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev 2001;53:135–159.

[5] Epperly MW, Epstein CJ, Travis E, Greenberger JS. Decreased pulmonary radiation resistance of manganese superoxide dismutase (MnSOD)-deficient mice is corrected by human manganese superoxide dismutase-plasmid/liposome (SOD2-PL) in tracheal gene therapy. Radiat Res 2000;154:365–374.

[6] Epperly MW, Tyurina YY, Nie S, Niu YY, Zhang X, Kagan K, Greenberger JS. MnSOD-plasmid liposome gene therapy decreases ionizing irradiation-induced lipid peroxidation of the esophagus. In-Vivo 2005;19:997–1004.

[7] Masini E, Cuzzocrea S, Mazzon E, Marzocca C, Mannaioni PF, Salvelmini D. Protective effects of M40403, a selective superoxide dismutase mimetic, in myocardial ischemia and reperfusioin injury. Br J Pharmacol 2002;136:905–917.

[8] Guo HL, Wolfe D, Epperly MW, Huang S, Liu K, Glorioso JC, Greenberger J, Blumberg D. Gene transfer of human manganese superoxide dismutase protects small intestinal villi from radiation injury. J Gastrointest Surg 2003;7:229–235.

[9] Salvemini D, Wang Z-Q, Sweier JL, Samouilov A, Macarthur H, Misko TP, Currie MG, Cuzzocrea S, Sikorski JA, Riley DP. A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. Science 1999;286:304–306.

[10] Salvemini D, Muscoli C, Riley DP, Cuzzocrea S. 2002. Superoxide dismutase mimetics. Pulmonary Pharmocol Ther 2002;15:439–447.

[11] Macarthur H, Westfall TC, Riley DP, Misko TP, Salvemini D. Inactivation of catacholamines by superoxide gives new insights on the pathogenesis of septic shock. Proc Natl Acad Sci USA 2000;9:9753–9758.

[12] Mcinnis J, Wang C, Anastasio N, Huffman M, Ye YP, Salvemini D, Johnson KM. The role of superoxide and nuclear factor kappa B signaling in N-methyl-D-asparta-induced necrosis and apoptosis. J Pharmacol Exp Ther 2002;301: 478–487.

[13] Currizoni S, Masson E, Dugo L, Paola R, Caputi AP, Salvemini D. Superoxide: a key player in hypertension. FASEB J 2004;18:94–101.

[14] Lysiak JJ, Nguyen QAT, Turner TT. Peptide and nonpeptide reactive oxygen scavengers provide partial rescue of the testis after torsion. J Andrology 2002;2:400–409.

[15] Cassatt DR, Fazenbaker CA, Kifle G, Bachy CM. Preclinical studies of the radioprotective efficacy and pharmacokinetics of subcutaneously administered amifostine. Semin Oncol 2002;29:2–8.

[16] Grdina DJ, Murley JS, Kataoka Y. Radioprotectants: current status and new directions. Oncology 2002;63(suppl 2):2–10.

[17] Romano MR, Lamberti AN, Bisogni R, Garbi C, Pagnano AM, Auletta P, Tassone P, Turco MC, Venuta S. Amifostine inhibits hematopoietic progenitor cell apoptosis by activating NF-κB/ Rel transcription factors. Blood 1999;94:4060–4066.

[18] Murley JS, Kataoka Y, Hallahan DE, Roberts JC, Grdina DJ. Activation of NF-κB and MnSOD gene expression by free radical scavengers in human microvascular endothelial cells. Free Radic Biol Med 2001;12:1426–1439.

[19] Murley JS, KataokaY, Cao D, Li JJ, Oberley LW, Grdina DJ. Delayed radioprotection by NF-κB-mediated induction of Sod2 (MnSOD) in SA-NH tumor cells after exposure to clinically used thiol-containing drugs. Radiat Res 2004; 162:536–546.

[20] Kahl R, Kampkotter A, Watjen W, Chovolou Y. Antioxidant enzymes and apoptosis. Drug Metab Rev 2004;36: 747–762.

[21] Pani G, Bedogni B, Anzevino R, Colavitti R, Palazzotti B, Borrello S, Galeotti T. Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p53-deficient cells. Cancer Res 2000;60:4654–4660.

[22] Dhar SK, Xu Y, Chen Y, St-Clair DK. Specificity protein 1-dependent p53-mediated suppression of human manganese superoxide dismutase gene expression. J Biol Chem 2006; 281:21698–21709.

[23] Weiss JF, Hoover RL, Kumar KS. Selenium pretreatment enhances the radioprotective effect and reduces the lethal toxicity of WR-2721. Free Radic Res Comm 1987;3:33–36.

[24] Brown DQ, Pittock JW, Rubiinstein JS. Early results of the screening program for radioprotectors. Int J Radiat Oncol Biol Phys 1982;8:565–570.

[25] Roderick TH. The response of twenty-seven inbred strains of mice to daily doses of whole-body x-irradiation. Radiat Res 1963;20:631–639.

[26] Okayasu R, Suctomi K, Yu y, Silver A, Bedford JS, Cox R, Ullrich RI. A deficiency in DNA repair and DNA-PKcs expression in the radiosensitive BALB/c mouse. Cancer Res 2000;60:4342–4345.

[27] Yu Y, Okayasu R, Weil MM, Silver A, McCarthy M, Zabriskie R, Long S, Cox R, Ulrich RL. Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the *Prkdc* (DNA-dependent protein kinase catalytic subunit) gene. Cancer Res 2001;61:1820–1824.

[28] Sorenson JR. Bis(3,5-diisopropylsalicylato)copper(II), a potent radioprotectant with superoxide dismutase mimetic activity. J Med Chem 1984;27:1747–1749.

[29] Srinivasan N, Doctrow S, Singh VJ, Whitnall MH. Evaluation of EUK-189, a synthetic superoxide dismutase/catalase mimetic as a radiation countermeasure. Immunopharmacol Immunotoxicol 2008;30:271–290.

[30] Burdelya L, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, Kurnasov OV, Fort, FL, Osterman AL, DiDonato JA, Feinstein E, Gudkov AV. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science 2008;320:226–230.

[31] Taylor CW, Wang LM, List AF, Fernandes D, Paine-Murrieta GD, Johnson CS, Capizzi RI. Amifostine protects normal tissues from paclitaxel toxicity while cytotoxicity against tumour cells is maintained. Eur J Cancer 1997;33:1693–1698.

[32] List AF, Heaton R, Glinsmann-Gibson B, Capizzi RL. Amifostine stimulates formation of multipotent and erythroid bone marrow progenitors. Leukemia 1998;12:1596–1602.

[33] Coppey LJ, Geollett JS, .Davidson EP, Dunlap JA, Lund LL, Salvemini D, Yorek MA. Effect of M40403 treatment of diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular function of epineurial arterioles of the sciatic nerve. Br J Pharmacol 2001;134:21–29.

[34] Wang Z-Q, Porreca F, Cuzzocrea S, Galen K, Lightfoot R, Masini E, Muscoli C, Mollace V, Ndengele, M, Salvemini D. A newly identified role for superoxide in inflammatory pain. J Pharmacol Exp Ther 2004;309:869–878.

[35] Cuzzocrea S, Mazzon E, Dugo L, Di-Paloa R, Caputi AP, Salvemini D. Superoxide: a key player in hypertension. FASEB J 2004;18:94–101.

[36] Voison EM, Ruthsatz M, Collins JM, Hoyle PC. Extrapolation of animal toxicity in humans: interspecies comparisons in drug development. Regulat Toxicol Pharmacol 1990;12:107–116.

This paper was first published online on Early Online on 17 March 2010.

Free Radic Res Downloaded from informahealthcare.com by University of Saskatchewan on 12/05/11
For personal use only.

RIGHTSLINK