Cancer Chemother Pharmacol (2011) 68:815–821
DOI 10.1007/s00280-011-1649-1

SHORT COMMUNICATION

# Methylseleninic acid inhibits HDAC activity in diffuse large B-cell lymphoma cell lines

**Shireen Kassam · Heidi Goenaga-Infante · Lenushka Maharaj · Crispin T. Hiley · Simone Juliger · Simon P. Joel**

Received: 10 February 2011 / Accepted: 3 April 2011 / Published online: 26 April 2011


**Abstract**
*Purpose* Selenium is a trace element that is fundamental to human health. Research has mainly focussed on its role in cancer prevention, but recent evidence supports its role in established cancer, with high concentrations inducing tumour cell death and non-toxic concentrations sensitising cells to chemotherapy. However, the precise mechanism of selenium action is not clear. The effect of methylseleninic acid (MSA), an organic selenium compound, on histone deacetylase (HDAC) activity in diffuse large B-cell lymphoma cell lines is reported here.
*Methods* Lymphoma cell lines were exposed to MSA under normoxic and hypoxic conditions. Protein expression was determined by western blotting, HDAC activity and VEGF concentration by fluorimetric and electrochemiluminescence assays, respectively, and intracellular selenium metabolites quantified by mass spectrometry.
*Results* MSA inhibited HDAC activity, which resulted in the acetylation of histone H3 and α-tubulin. However, cellular metabolism of MSA to methylselenol was required for this effect. Dimethylselenide, the methylation product of methylselenol, was found to be the major intracellular metabolite. MSA also inhibited HIF-1α expression and VEGF secretion, a possible consequence of HDAC inhibition.
*Conclusion* The ability of methylselenol to inhibit HDAC activity has not been previously reported, thus providing a novel mechanism of selenium action.

**Keywords** Selenium · Methylseleninic acid · Histone deacetylase · HIF1alpha · VEGF

**Electronic supplementary material** The online version of this article (doi:10.1007/s00280-011-1649-1) contains supplementary material, which is available to authorized users.

S. Kassam · L. Maharaj · S. Juliger · S. P. Joel (✉)
Centre for Haemato-Oncology, Barts Cancer Institute,
Queen Mary University of London,
London EC1B 6BQ, UK
e-mail: s.p.joel@qmul.ac.uk

S. Kassam
e-mail: s.a.kassam@qmul.ac.uk

H. Goenaga-Infante
LGC Limited, London, UK

C. T. Hiley
Centre for Molecular Oncology and Imaging,
Barts Cancer Institute, London, UK

## Introduction

Selenium (Se) is a trace element that is fundamental to human health. While its therapeutic use in the cancer setting has mainly focused on chemoprevention [26], there is also evidence to support its use in established cancer [4]. Se exists in different chemical forms but organic, rather than inorganic, Se compounds are preferred as the latter are genotoxic [20]. Regarding diffuse large B-cell lymphoma (DLBCL), our department has reported that serum Se at diagnosis is predictive of outcome in patients with B-cell non-Hodgkin lymphoma, the majority of whom had DLBCL [18]. In addition, in vitro studies have demonstrated that high concentrations of Se are cytotoxic [17], while non-toxic concentrations can sensitise cells to the cytotoxic effects of chemotherapeutic agents [14]. The precise mechanism of Se action is not clear and differs based on the chemical form, dose/concentration and tumour type. In DLBCL cell lines, Se has been shown to inhibit the activation of NF-κB [14]. These pre-clinical data have led to funding for a multi-centre clinical trial of high-dose Se supplementation in the form of methylselenocysteine (MSC),

in combination with immunochemotherapy, in patients with relapsed/refractory DLBCL.

Metabolism of organic Se compounds, such as MSC, is necessary for their anti-tumour activity, with the metabolite methylselenol thought to be crucial. This metabolite is highly reactive and volatile and therefore cannot be directly measured or quantified [13]. However, its methylation products, dimethylselenide and dimethyldiselenide, can be quantified by LC–MS methods [10]. MSC is directly converted to methylselenol in vivo by $\beta$ and $\gamma$ elimination reactions, mediated by lyase enzymes found mainly in the liver and kidney [5]. As MSC is therefore mainly a prodrug, the compound methylseleninic acid (MSA) has been developed for in vitro studies. This is a water soluble, stable Se species that is readily taken up into cells and upon reaction with cellular thiols, such as glutathione, is reduced to methylselenol [13].

Recently, it has been reported that the organic Se compounds selenomethionine (SLM), and MSC can also undergo transamination reactions that generate α-keto acid Se metabolites [19, 23]. These metabolites have a similar structure to the known histone deacetylase (HDAC) inhibitor butyrate and were shown to inhibit HDAC activity in human prostate [19] and colon [23] cancer cell lines. HDACs regulate a large number of cellular processes through the deacetylation of both histone and non-histone proteins. One such process is angiogenesis, in which the expression of HIF-1α, a key pro-angiogenic transcription factor, is partly regulated by HDACs [7], and the activity of the protein itself is dependent on deacetylation by HDAC4 and 6, as acetylation results in ubiquitination and degradation [25].

As MSA is not an amino acid-containing Se species (unlike SLM and MSC) it is not a substrate for transaminases and cannot readily be converted to an a-keto acid, instead generating the volatile species methylselenol and more stable methylation products dimethylselenide and dimethyldiselenide [10]. This study reports for the first time that MSA inhibits HDAC activity in DLBCL cell lines, most likely attributable to the modification of cystine residues by methylselenol, and that this HDAC inhibition is associated with a decrease in cellular HIF-1α and VEGF secretion under hypoxic conditions.

## Methods

### Cell lines, cell culture and treatment of cell lines

Four DLBCL cell lines were used. DoHH2, RL and SUD4 cells were obtained from Cancer Research UK cell services, and the DHL4 cell line was obtained from the Dana Farber Cancer Institute (kind gift from Dr Margaret Shipp). Cells were maintained in RPMI-1640 culture medium (Sigma–Aldrich, Poole, UK) supplemented with 10% foetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere with 5% $CO_2$. Cells were also cultured under hypoxic conditions (1% $O_2$, 5% $CO_2$, 94% $N_2$) at 37°C in a humidified atmosphere. Stock solutions of MSA and MSC 10 mmol/l (PharmaSe® Inc., TX, USA) were prepared in deionised water and stored at −80°C prior to dilution in medium for use.

### Western blotting

For whole cell protein extraction, cells were lysed using CelLytic™ MT cell lysis reagent (Sigma–Aldrich), and protein content determined using the BCA™ assay (Pierce, IL, USA). The method for nuclear protein extraction is given in 'Supplementary Data'. Twenty micrograms of lysate was resolved on pre-cast gels (Invitrogen, CA, USA) and then transferred to a polyvinylidene fluoride membrane using the iBlot™ gel transfer device (Invitrogen™). The following primary antibodies were used: acetylated histone H3 (Millipore, MA, USA), acetylated α-tubulin (Sigma–Aldrich), total histone H3, α-tubulin, GAPDH (Cell Signaling, MA, USA) p21 (Santa Cruz, CA, UK) and HIF-1α (Abcam, Cambridge, UK) followed by HRP-conjugated secondary antibodies (Dako Ltd, Cambridge, UK). Bands were visualised using an enhanced chemiluminescence method (ECL reagent, GE Healthcare, Little Chalfont, UK).

### Measurement of histone deacetylase activity

HDAC activity was measured using 96-well HDAC fluorimetric/discovery assay and HDAC fluorimetric cellular activity kits (Biomol, Plymouth Meeting, PA, USA) according to the manufacturers' instructions. The assay measures fluorescence emitted by the *Fluor de Lys*™ substrate when its acetylated lysine side chain is deacetylated. For the fluorimetric assay/discovery assay, HeLa nuclear extract (provided in the assay kit) was used as the source of HDAC activity and trichostatin A (TSA) was used as a positive control. For the HDAC fluorimetric cellular activity assay, $1 \times 10^5$ cells were plated in phenol-red-free medium in a 96-well plate followed by the addition of MSA or TSA and the cell-permeable *Fluor de Lys*™ substrate. Control wells consisted of medium, without cells, to which substrate, developer and/or MSA were subsequently added. Fluorescence (excitation 350–380 nm, emission 440–460 nm) was measured on a POLARstar OPTIMA plate reader (BMG Labtech, Offenburgh, Germany). The values from the control wells were deducted from the test wells, and the results expressed relative to the no inhibitor control. The addition of MSA to control wells did not significantly alter the fluorescence value.

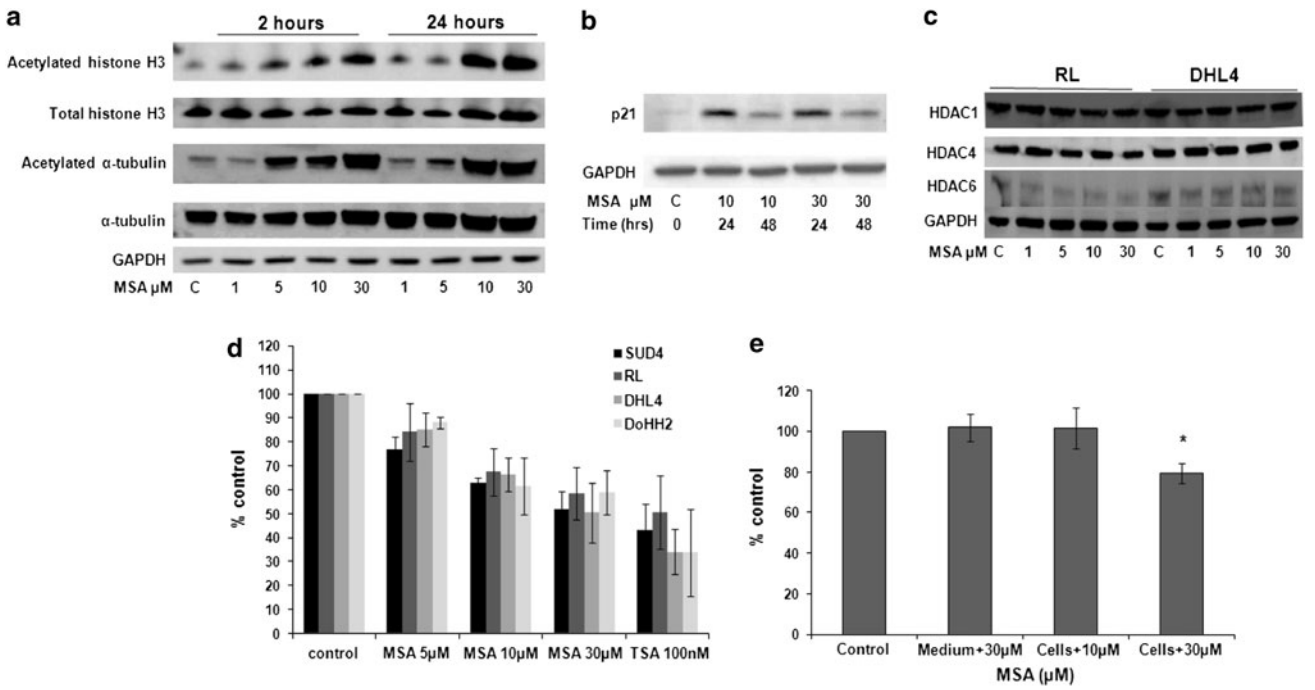


**Fig. 1** **a** Protein acetylation in SUD4 cells exposed to MSA for 2 and 24 h; **b** p21 expression in DHL4 cells exposed to MSA; **c** HDAC expression in RL and DHL4 cells exposed to MSA for 24 h; **d** HDAC activity in 4 DLBCL cells after exposure to MSA or TSA for 2 h; **e** Inhibition of HeLa nuclear HDAC activity by medium from DHL4 cells exposed to MSA for 2 h. $*P < 0.05$

### Measurement of vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) levels in cellular supernatants were measured using the MSD® electrochemiluminescent cytokine assay (Meso Scale Discovery, Maryland, USA) according to the manufacturer's instructions. Twenty-five microlitres of the supernatant was added, in duplicate, to the wells of a custom-made 96-well microtitre plate, which was pre-coated with VEGF antibodies in addition to having an integrated electrode. The plate was incubated at room temperature for 1 h with vigorous shaking. Following this, 25 µl of the detection antibody solution (1 µg/ml) was added to each well and the plate again incubated at room temperature for 1 h with vigorous shaking. Following three wash steps, the plate was analysed on a SECTOR™ Imager (Meso Scale Discovery) and light emitted at 620 nm was measured. A standard curve was prepared from the cytokine calibrator solution provided with the assay kit. Measurements were corrected for cell number and expressed relative to control (supernatant from cells cultured under normoxic conditions).

### Determination of intracellular Se species

Following the exposure of cell lines to MSA, cells were lysed as for western blotting. Cell lysates were then analysed by reverse-phase high-performance liquid chromatography (Agilent 1200, Agilent technologies, Palo Alto, CA, USA) coupled to an Agilent 7500i inductively coupled plasma mass spectrometer (ICP-MS) for element-specific detection. For chromatographic and ICP-MS conditions, see 'Supplementary Data Table S1'.

## Results

### The effect of MSA on protein acetylation and HDAC proteins in short-term culture

Time- and concentration-dependent experiments revealed similar effects in all four cell lines (data for SUD4 cells shown in Fig. 1a). MSA concentrations as low as 1 µM increased the acetylation of histone H3 and α-tubulin as early as 2 h, an effect sustained until 24 h. The induction of acetylated histone H3 continued to increase out to 24 h, while acetylated α-tubulin was typically maximal at 2 h, showing a slight decrease at 24 h. In addition, MSA induced the expression of the cyclin-dependent kinase inhibitor p21, a known HDAC-regulated gene, at 24 h but by 48 h the effect was less marked (Fig. 1b). MSA did not alter the expression of class I (HDAC1), class IIa (HDAC4) or class IIb (HDAC6) HDAC proteins (Fig. 1c).

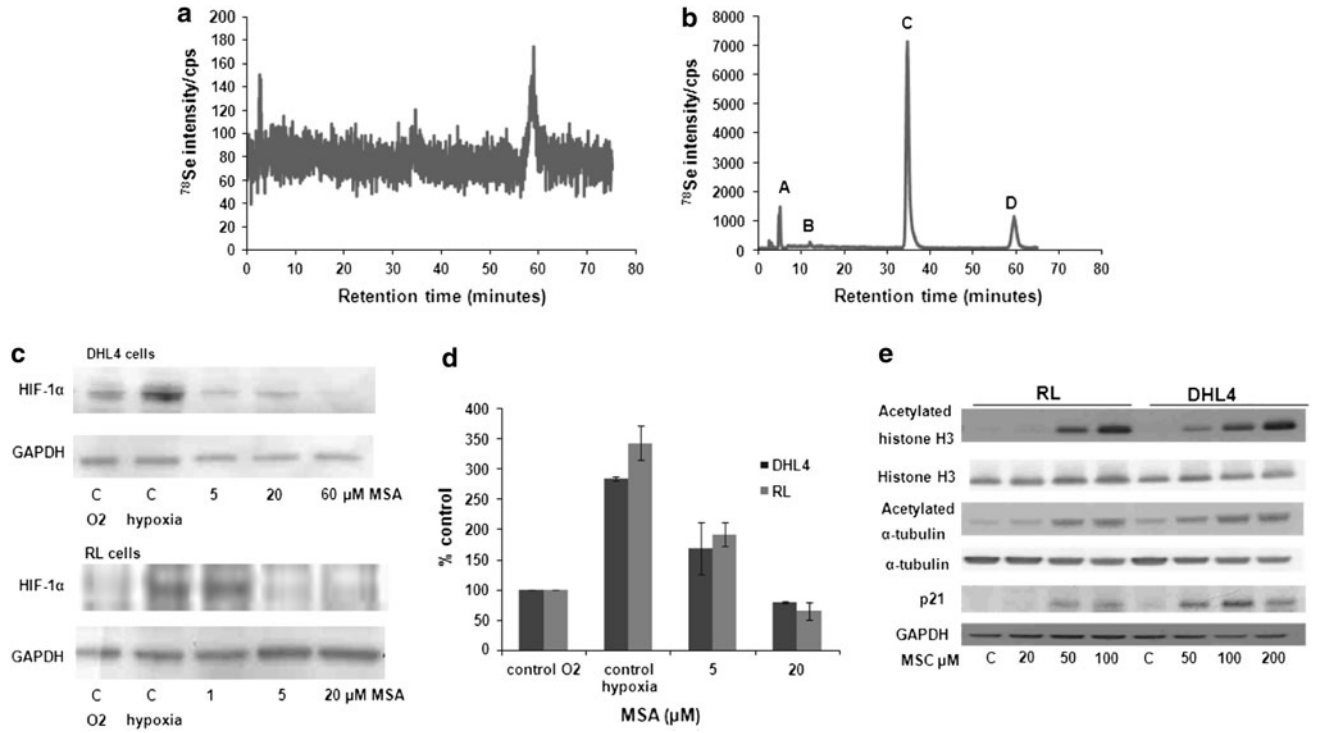


**Fig. 2** **a** Representative chromatogram of control DHL4 cells; **b** Representative chromatogram demonstrating intracellular Se species generated after exposure of DHL4 cell to 20 μM MSA for 2 h Peak A is MSC, peak B is SLM, peak C is dimethylselenide and peak D is S-methylselenoglutathione; **c** HIF-1α expression in DHL4 ann RL cells exposed to hypoxia and MSA for 24 h; **d** VEGF levels in the supernatant of DHL4 and RL cells exposed to hypoxia and MSA for 24 h; **e** protein acetylation and p21 expression in RL and DHL4 cells exposed to MSC

## The effect of MSA on HDAC activity

When MSA was incubated with HeLa isolated nuclear extract for 30 min and 2 h, there was no inhibition of *Fluor de Lys*™ substrate deacetylation, suggesting that MSA, in this cell-free assay system, was not able to inhibit HDAC activity (data not shown). However, when HDAC activity was investigated in a cell-based assay, MSA did result in inhibition of HDAC activity in a concentration-dependent manner in all four cell lines, with 30 μM MSA inhibiting HDAC activity by between 40 and 50% (Fig. 1d). This suggests that cellular metabolism of MSA is required to generate an active metabolite.

To further investigate whether a cellular metabolite of MSA was responsible for this effect, the HDAC inhibitory activity of medium from DHL4 cells incubated with MSA for 2 h was tested in the cell-free HDAC assay. Medium from DHL4 cells incubated with 10 μM MSA was not able to inhibit *Fluor de Lys*™ substrate deacetylation, but medium from cells incubated with 30 μM MSA had a significant HDAC inhibitory effect (20.6 ± 4.7%; Fig. 1e) relative to control. Medium incubated with 30 μM MSA for 2 h in the absence of cells had no effect on HDAC activity. These data support the hypothesis that a cellular metabolite of MSA is responsible for the HDAC inhibitory effect seen in cell-based assays.

## Determination of the intracellular Se metabolites formed after exposure of cells to MSA

RL and DHL4 cell lines were exposed to 20 μM MSA for 1 and 2 h. After incubation with MSA, three main Se-containing peaks were detected, the most abundant, identified as dimethylselenide, with a retention time of around 35 min (Fig. 2b, peak C), a peak eluting at 60 min (peak D; subsequently identified as S-methylselenoglutathione [9]) and MSC (peak A, 5 min). A very small peak eluting at 11 min (peak B) was identified as SLM. In the RL cell line, the same peaks were detected with the exception of MSC (data not shown) and the peak with the highest intensity was again dimethylselenide.

## Effect of MSA on HIF-1α expression and VEGF secretion

Inhibition of HDAC activity has been shown to inhibit angiogenesis through effects on HIF-1α [7]. Therefore, the effect of MSA on HIF-1α expression and VEGF production was investigated after culture of RL and DHL4 cells under

hypoxic conditions. Western blotting for HIF-1α in nuclear extracts showed that hypoxia induced the expression of HIF-1α, but this induction was suppressed by MSA (Fig. 2c). Hypoxia also increased the production of VEGF by RL and DHL4 cell lines but this was inhibited by MSA in a concentration-dependent manner (Fig. 2d).

The effect of MSC on protein acetylation

MSC will be the Se compound used in the forthcoming clinical trial. Figure 2e shows that in RL and DHL4 cells, MSC increased the acetylation of histone H3 and α-tubulin and the expression of p21 in a concentration-dependent manner, suggesting that MSC, or a metabolite of MSC, also inhibits HDAC activity.

## Discussion

These experiments demonstrate for the first time that the organic Se species MSA can inhibit HDAC activity in DLBCL cell lines. The deacetylation of histone proteins is mainly mediated by class I HDACs, while α-tubulin deacetylation is mediated by HDAC6, a class II HDAC. The concentration-dependent increase in the acetylation of both histones and α-tubulin strongly suggests that MSA inhibits both class I and II HDACs, although possibly with a different time-course as the acetylation change was more apparent at an early time-point with a class II HDAC substrate. The HDAC inhibitory action of MSA was confirmed by an HDAC activity assay. However, in the cell-free assay, MSA was not able to inhibit the HDAC activity of HeLa nuclear extract. HDAC inhibition was only apparent when MSA was exposed to intact, viable cells and activity measured using a cell-based assay. This is likely to be due to the intracellular activation of MSA to methylselenol, which is the metabolite thought to be responsible for its antitumour effect [13]. Although medium from cells exposed to MSA was able to inhibit the HDAC activity of HeLa nuclear extract, the effect was small (30 μM MSA inhibited HDAC activity by 21%). This is probably due to the volatile nature of MSA metabolites which are not retained in the cell medium [10]. Medium incubated with MSA alone, in the absence of cells, had no effect on HDAC activity in a subsequent cell-based assay, confirming the Se species responsible for the effects observed is a cellular metabolite and not a breakdown product.

It is currently unclear whether this HDAC inhibitory activity is due to reversible binding of a Se species at the catalytic site on the enzyme, or an irreversible effect due to the redox modification of cysteine residues in HDAC proteins. The main species formed from MSA (methylselenol, dimethylselenide and dimethyldiselenide) do not contain the classical features of an HDAC inhibitor, requiring a side chain to reach into the active site pocket and a Zn binding group, typically a carboxylic or hydroxamic acid [22]. Moreover, MSA has been shown to inhibit other enzymes, notably PKC isoforms, through the redox modification of key cysteines [11]. Although similar effects have not been reported with Se compounds and HDACs, reactive carbonyl species have recently been shown to inactivate HDAC enzymes through the modification of two conserved cysteine residues ($Cys^{261}$ and $Cys^{273}$), possibly representing a redox signalling response that would result in the transcription of a subset of genes through HDAC inhibition [6]. There is therefore the potential for Se compounds to directly alter HDAC structure and thereby catalytic activity.

The intracellular Se metabolites formed after exposure of cells to MSA revealed that the major metabolite was dimethylselenide, the methylation product of methylselenol. As expected, methylselenol itself was not detected, due to its high volatility. However, the presence of dimethylselenide confirmed that methylselenol was the major metabolite formed and hence was likely to be responsible for HDAC inhibition. α-Keto acid Se metabolites, reported by others to show HDAC inhibitory activity [19, 23], were not detected by the ICP-MS method used. The assay was not optimised for the detection of these α-keto acid species, although given this is a robust, highly sensitive method for trace element and elemental speciation analysis, with an extremely low detection limit and shown by others to detect a wide range of organic Se compounds [1], it is likely that such metabolites would not have been detected if present.

Se compounds have also been reported to inhibit angiogenesis in xenograft models [3]. Although we have not demonstrated a direct causal link between the activity of HDACs and the expression or activity of HIF-1α, other evidence suggests the two are associated. As outlined earlier, the regulation of HIF-1α expression involves HDACs [7], while HIF-1α activity is dependent on deacetylation by HDACs 4 and 6, which maintains the stability of the protein [25]. HIF-1α expression is also regulated by NF-k$\beta$, itself subject to acetylation/deacetylation modifications of the p65, and possibly p52, subunits which affects DNA binding [2]. We have previously reported the NF-k$\beta$ inhibitory activity of MSA [14]. Given that in the experiments now reported MSA, at similar concentrations to those required for HDAC inhibition, was able to inhibit HIF-1α expression and VEGF secretion, suggests that HDAC inhibition may be a potential mechanism by which Se compounds inhibit angiogenesis in vivo.

HDAC inhibition by MSA requires relatively high concentrations, with 30 μM for 2 h resulting in 40–50% inhibition of activity across the four cell lines tested. HDACi currently in clinical trials inhibit activity in vitro at nanomolar concentrations [15]. However, this Se concentration

is potentially clinically achievable. In the phase I/II dose escalation study of SLM in patients with solid tumours, the highest dose level of 7,200 μM twice daily resulted in a mean plasma Se concentration after 8 days of 31 μM/l [8].

MSA cannot be used clinically due to its high reactivity, thus the naturally occurring organic Se compounds, SLM and MSC, have been used as precursors of methylselenol. Methylselenol is generated from MSC and SLM by the action of pyridoxal 5′-phosophate (PLP)-dependent $\beta$-lyases and $\gamma$-lyases, respectively, predominantly in the liver and kidney [24]. To date, SLM has been the most widely used compound in clinical trials [16, 21], however, its conversion to methylselenol is not as efficient as that of MSC, as SLM can also be non-specifically incorporated into body proteins in place of methionine [27]. In addition, MSC has been shown to have a more potent anti-tumour effect [12, 20], and therefore it is the compound that will be used in the forthcoming clinical trial. These experiments show that MSC can also inhibit HDAC activity in vitro, although only at relatively high concentrations. This is likely to be because tumour cells have only low $\beta$-lyase activity, thus generating only limited amounts of methylselenol. It is anticipated that when used in vivo much lower concentrations of MSC would be required to inhibit HDAC activity.

In summary, this work demonstrates that methylselenol can inhibit HDAC activity, an effect likely to induce other biological changes and potentially to contribute to the anti-tumour effects of Se in vivo.

**Acknowledgments** We thank the Medical Research Council and the Barry Reed Research Fund for supporting this work.

## References

1. B'Hymer C, Caruso JA (2006) Selenium speciation analysis using inductively coupled plasma-mass spectrometry. J Chromatogr A 1114:1–20
2. Bendinelli P, Matteucci E, Maroni P, Desiderio MA (2009) NF-kappaB activation, dependent on acetylation/deacetylation, contributes to HIF-1 activity and migration of bone metastatic breast carcinoma cells. Mol Cancer Res 7:1328–1341
3. Bhattacharya A, Seshadri M, Oven SD, Toth K, Vaughan MM, Rustum YM (2008) Tumor vascular maturation and improved drug delivery induced by methylselenocysteine leads to therapeutic synergy with anticancer drugs. Clin Cancer Res 14:3926–3932
4. Cao S, Durrani FA, Rustum YM (2004) Selective modulation of the therapeutic efficacy of anticancer drugs by selenium containing compounds against human tumor xenografts. Clin Cancer Res 10:2561–2569
5. Cooper AJ, Krasnikov BF, Niatsetskaya ZV, Pinto JT, Callery PS, Villar MT, Artigues A, Bruschi SA (2010) Cysteine S-conjugate beta-lyases: important roles in the metabolism of naturally occurring sulfur and selenium-containing compounds, xenobiotics and anticancer agents. Amino Acids
6. Doyle K, Fitzpatrick FA (2010) Redox signaling, alkylation (carbonylation) of conserved cysteines inactivates class I histone deacetylases 1, 2, and 3 and antagonizes their transcriptional repressor function. J Biol Chem 285:17417–17424
7. Ellis L, Hammers H, Pili R (2009) Targeting tumor angiogenesis with histone deacetylase inhibitors. Cancer Lett 280:145–153
8. Fakih MG, Pendyala L, Brady W, Smith PF, Ross ME, Creaven PJ, Badmaev V, Prey JD, Rustum YM (2008) A phase I and pharmacokinetic study of selenomethionine in combination with a fixed dose of irinotecan in solid tumors. Cancer Chemother Pharmacol 62:499–508
9. Goenaga-Infante H, Kassam S, Stokes E, Hopley C, Joel SP (2010) HPLC with APEX-Q nebulisation ICP-MS and ESI MS/MS to compare selenium uptake and speciation in non-malignant cells and B cell lymphoma lines. Anal Bioanal Chem 399:1789–1797
10. Goenaga Infante H, Joel S, Warburton E, Hopley C, Hearn R, Jüliger S (2007) Investigation of the selenium species distribution in a human B-cell lymphoma line by HPLC- and GC-ICP-MS in combination with HPLC-ESIMS/MS and GC-TOFMS after incubation with methylseleninic acid. J Anal At Spectrom 22:888–896
11. Gundimeda U, Schiffman JE, Chhabra D, Wong J, Wu A, Gopalakrishna R (2008) Locally generated methylseleninic acid induces specific inactivation of protein kinase C isoenzymes: relevance to selenium-induced apoptosis in prostate cancer cells. J Biol Chem 283:34519–34531
12. Ip C, Birringer M, Block E, Kotrebai M, Tyson JF, Uden PC, Lisk DJ (2000) Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. J Agric Food Chem 48:4452
13. Ip C, Thompson HJ, Zhu Z, Ganther HE (2000) In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. Cancer Res 60:2882–2886
14. Juliger S, Goenaga-Infante H, Lister TA, Fitzgibbon J, Joel SP (2007) Chemosensitization of B-cell lymphomas by methylseleninic acid involves nuclear factor-kappaB inhibition and the rapid generation of other selenium species. Cancer Res 67:10984–10992
15. Khan N, Jeffers M, Kumar S, Hackett C, Boldog F, Khramtsov N, Qian X, Mills E, Berghs SC, Carey N, Finn PW, Collins LS, Tumber A, Ritchie JW, Jensen PB, Lichenstein HS, Sehested M (2008) Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors. Biochem J 409:581–589
16. La Vecchia C, Franceschi S (2000) Nutrition and gastric cancer. Can J Gastroenterol 14(Suppl D):51D–54D
17. Last K, Maharaj L, Perry J, Strauss S, Fitzgibbon J, Lister TA, Joel S (2006) The activity of methylated and non-methylated selenium species in lymphoma cell lines and primary tumours. Ann Oncol 17:773–779
18. Last KW, Cornelius V, Delves T, Sieniawska C, Fitzgibbon J, Norton A, Amess J, Wilson A, Rohatiner AZ, Lister TA (2003) Presentation serum selenium predicts for overall survival, dose delivery, and first treatment response in aggressive non-Hodgkin's lymphoma. J Clin Oncol 21:2335–2341
19. Lee JI, Nian H, Cooper AJ, Sinha R, Dai J, Bisson WH, Dashwood RH, Pinto JT (2009) Alpha-keto acid metabolites of naturally occurring organoselenium compounds as inhibitors of histone deacetylase in human prostate cancer cells. Cancer Prev Res (Phila) 2:683–693
20. Li GX, Lee HJ, Wang Z, Hu H, Liao JD, Watts JC, Combs GF Jr, Lu J (2008) Superior in vivo inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite. Carcinogenesis 29:1005–1012
21. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, Parsons JK, Bearden JD III, Crawford ED, Goodman GE, Claudio J, Winquist E, Cook ED, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella

RM, Albanes D, Taylor PR, Probstfield JL, Jagpal TJ, Crowley JJ, Meyskens FL Jr, Baker LH, Coltman CA Jr (2009) Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the selenium and vitamin E cancer prevention trial (SELECT). JAMA 301:39–51

22. Marks PA (2010) Histone deacetylase inhibitors: a chemical genetics approach to understanding cellular functions. Biochim Biophys Acta 1799(10–12):717–725
23. Nian H, Bisson WH, Dashwood WM, Pinto JT, Dashwood RH (2009) Alpha-keto acid metabolites of organoselenium compounds inhibit histone deacetylase activity in human colon cancer cells. Carcinogenesis 30:1416–1423
24. Okuno T, Ueno H, Nakamuro K (2006) Cystathionine gamma-lyase contributes to selenomethionine detoxification and cytosolic glutathione peroxidase biosynthesis in mouse liver. Biol Trace Elem Res 109:155–171
25. Qian DZ, Kachhap SK, Collis SJ, Verheul HM, Carducci MA, Atadja P, Pili R (2006) Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1 alpha. Cancer Res 66:8814–8821
26. Rayman MP (2005) Selenium in cancer prevention: a review of the evidence and mechanism of action. Proc Nutr Soc 64:527–542
27. Rayman MP, Infante HG, Sargent M (2008) Food-chain selenium and human health: spotlight on speciation. Br J Nutr 100:238–253