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4-Hydroxynonenal inhibits cell proliferation and alters differentiation pathways in human fetal liver hematopoietic stem cells

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Abstract

During fetal development, the liver serves as the primary hematopoietic organ in which hematopoietic stem cells (HSC) comprise a large proportion of hepatic cell populations. Because HSC are capable of initiating long-term hematopoiesis, injury to these cells may have ramifications with regard to the etiology of blood-borne diseases. In the current study, we examined the effects of 4-hydroxynonenal (4-HNE), a mutagenic α,β-unsaturated aldehyde that can be produced in utero, on HSC proliferation, differentiation, viability and apoptosis. Exposure of HSC to acute single doses of 4-HNE as low as 1 nM inhibited HSC proliferation. Because 4-HNE rapidly disappears from culture media, a multiple dosing régime was also employed to approximate short-term steady state 4-HNE concentrations relevant to physiological oxidative stress. 4-Hydroxynonenal steady state concentrations as low as 1 μM altered HSC differentiation pathways, but did not affect apoptosis or cause cell death. In contrast, exposure to steady state 5 μM 4-HNE elicited a loss in viability, and increased the rate of apoptosis in total HSC populations. Collectively, our data indicate that cellular levels of 4-HNE associated with a low level of oxidative stress cause a loss of proliferation and viability and alter differentiation pathways in human fetal HSC.

Keywords: 4-Hydroxynonenal; Human fetal hematopoietic stem cells; Differentiation; Apoptosis

1. Introduction

4-Hydroxynonenal (4-HNE) is a mutagenic α,β-unsaturated aldehyde that is produced by peroxidation of 6ω polyunsaturated fatty acids [1]. A factor contributing to the mutagenic ability of 4-HNE is its relative stability and ability to easily pass among subcellular compartments and to interact with a multitude of different cellular proteins [2]. Increased cellular production of 4-HNE and related aldehydes has been linked to pathophysiological effects associated with oxidative stress [3]. For example, high nanomolar concentrations of 4-HNE have been shown to inhibit DNA, RNA, and protein synthesis, [4,5] and adversely affect membrane fluidity [6]. At the tissue level, production of 4-HNE has been associated with cell injury linked to carcinogenesis [7,8], atherosclerosis [9], Alzheimer's Disease [10], Parkinson's Disease [11] and pre-eclampsia [12].

In addition to disorders of adulthood, there is evidence to suggest that production of reactive aldehydes in utero are important in the pathogenesis of several prenatal diseases including chronic lung disease [13], perinatal hypoxia [14] and liver injury [15]. Studies in rodents have demonstrated that 4-HNE can be generated at high levels in utero as a result of maternal alcohol consumption [15,16]. During development, the fetal liver is the primary site of hematopoiesis and is in a continuous dynamic process of cell turnover [17]. The pluriopotency of the fetal liver is maintained by a number of HSC, which can differentiate along multiple lineages. Because of these aforementioned factors, and due to the fact that the fetal liver receives a substantial proportion of maternal blood flow, it is possible that fetal liver HSC may be potential targets for transplacental chemicals that may elicit oxidative injury. In this regard, Robinson and Seelig [18] demonstrated that maternal ethanol consumption in rats has a direct effect on hematopoietic cell development by decreasing early B and T cell populations post-conception. Maternal alcohol consumption can also affect hematopoiesis in mice by

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reducing the number of lymphocytes and macrophages, respectively [19,20]. Interestingly, Chen et al. [15] demonstrated that maternal alcohol consumption can actually result in higher levels of 4-HNE in fetal liver than in adults. Collectively, these studies suggest a role of the reactive metabolite 4-HNE in the etiology of hematopoietic disorders.

We had previously demonstrated that cultured HSC do not efficiently detoxify 4-HNE, and that this poor detoxification capacity leads to rapid 4-HNE protein adducts formation in vitro [21]. What is not known is if low, but physiologically relevant concentrations of 4-HNE can adversely affect HSC proliferation, viability, differentiation and apoptosis. Ultimately, HSC injury or altered HSC differentiation may be a causative agent in the etiology of hematopoietic disorders that develop after birth. The present study was initiated to determine if levels of 4-HNE associated with a low level of oxidative stress may alter differentiation pathways and increase apoptosis in human fetal HSC. Our model system uses cultured second trimester human fetal liver $CD34^+$ cells, which are a unique cell model highly relevant to addressing toxicological mechanisms of relevance to the in utero human environment.

2. Materials and methods

2.1. Chemicals

4-HNE was purchased from Cayman Chemical (Ann Arbor, MI). Iscove's Modified Dulbecco's Medium (IMDM), penicillin, streptomycin and heat-inactivated fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). Recombinant human interleukin 3 (IL-3), recombinant human granulocyte colony stimulating factor (G-CSF) and recombinant human stem cell factor (SCF) were obtained from Research Diagnostics Inc. (Flanders, NJ). Second trimester human fetal liver derived cryopreserved $CD34^+$ cells (>95% purity) were purchased from Cambrex–Biowhittaker (Camarillo, CA). Flow cytometry antibodies CD33-mouse anti-human $IgG1,_K$-fluorescein isothiocyanate (FITC), CD34-mouse anti-human $IgG1,_K$-R-Phycoerythrin (PE), CD-38-mouse anti-human $IgG1,_K$ allophycocyanin (APC), isotype controls: mouse $IgG1,_K$-FITC, mouse $IgG1,_K$-PE and mouse $IgG1,_K$-APC and the APO-BRDU^{TM} kit were purchased from BD Biosciences (San Diego, CA). Vented culture flasks and 96-well plate were purchased from Corning Inc. (Corning, NY). Trypan blue was purchased from Sigma Chemical Company (St. Louis, MO) and Alamar blue was purchased from BioSource International (Camarillo, CA).

2.2. Cell culture

Human fetal liver derived cryopreserved $CD34^+$ cells were thawed in accordance with the manufacturers instructions and seeded at approximately 6250 cells/ml of IMDM containing 15% FBS, 2 ng/ml IL-3, 1 ng/ml G-CSF, 20 ng/ml SCF, 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were maintained in culture over 8–16 days (a timeframe which allows for moderate increases in cell number while maintaining a relatively primitive undifferentiated status) in a humid chamber at 37 °C in 95% O_2/5% CO_2. HSC under these conditions undergo moderate proliferation and differentiate as a mixed population of several hematopoietic cell lineages ($CD34^+$), monocytes ($CD14^+$), neutrophils ($CD15^+$) and megakaryocytes ($CD41^+$) [22]. Cell counts and viability were determined over the culture period using a hemocytometer and Trypan blue exclusion, respectively. Basal levels of 4-HNE in cultured HSC were determined by ELISA using a polyclonal antibody directed against 4-HNE protein adducts (EnVirtue Biotechnologies, Inc., Winchester, VA) and ranged from 0.16 to 0.50 μM.

2.3. Effects of 4-HNE on HSC proliferation

HSC were cultured as described above. After 7 days in culture, cell counts and viability were determined. Cells were triturated and 25 μl of cell suspension (3000 cells) was transferred to each well of a 96-well plate. 4-Hydroxynonenal was added to media to achieve the final concentrations of (1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM). Vehicle controls consisted of EtOH (0.15%, v/v). The effects of 4-HNE on HSC proliferation were determined by addition of 10% Alamar blue and fluorescent measurement of Alamar blue reduction at 554 nM excitation and 590 nM emission using a 96-well fluorescence microplate reader [23].

2.4. Effects of 4-HNE on HSC differentiation and apoptosis

In order to determine if physiologically relevant doses of 4-HNE alter HSC differentiation pathways, fluorescent labeled antibodies were employed to identify and follow different hematopoietic lineages. Following 7 days in culture and prior to each flow cytometry experiment, cell counts and viability were determined. Cells were pooled to obtain similar seeding densities with equal volumes of the cell suspension transferred to each experimental culture flask. It has previously been reported that 4-HNE added to cells in culture media is undetectable after 45–60 min [24]. Therefore, a repeated 4-HNE steady-state exposure protocol involving the addition of multiple doses of 4-HNE (or vehicle) every 45 min for 4 h (6 doses) was employed [25]. Cells were exposed to vehicle (EtOH), 1 or 5 μM 4-HNE under steady state conditions and harvested at 24, 48, 72 and 96 h post-exposure for flow cytometry analysis. A set of control reactions was conducted in parallel with the experiment treatments. The control reactions consisted of (1) unstained HSC; (2) HSC incubated with the appropriate lineage antibody; and (3) monoclonal antibody isotypes

that do not bind to HSC. Cells (10^6) were resuspended in 1 ml 1× PBS containing 0.1% sodium azide, pH 7.4. Cells were centrifuged at 200 × *g* at 4 °C for 5 min, supernatants removed and the cell pellets incubated on ice with the appropriate antibody for 30 min in the absence of light according to manufacturer instructions. The cells were then rinsed in 1× PBS (containing 0.1% sodium azide), incubated with 0.5% paraformaldehyde solution, and prior to flow cytometry analysis, the paraformaldehyde was removed and replaced with 1× PBS containing 0.05% sodium azide.

The effect of 4-HNE exposures on HSC apoptosis was determined 24 h post-exposure using APO-BRDU kit, which is based upon the TUNEL assay [26]. Control reactions were conducted as described above for flow cytometry. After determination of cell counts and viability, cells were incubated with the appropriate antibody for 30 min in the absence of light at room temperature. The cells were centrifuged, rinsed and fixed on ice for 30 min. Following fixation, the cells were rinsed twice in 5 ml 1× PBS and incubated with 1 ml 70% ice-cold ethanol and stored at −20 °C. Samples were centrifuged, rinsed and the cell pellets incubated with DNA labeling solution for 1 h at 37 °C. Cells were stained with anti-BrdU and controls with mouse-PE for 30 min at room temperature and analyzed within 3 h by flow cytometry.

2.5. Statistical analysis

All viability and cell proliferation data represent the mean ± S.E.M. of three experiments with incubations for each experiment performed in triplicate. Flow cytometry data are from experiments conducted using triplicate incubations. The effects of 4-HNE on HSC viability and differentiation were assessed using two way analysis of variance (ANOVA) followed by Tukey's post-hoc test (SigmaStat 2.0, Jandel Scientific, Chicago, IL). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. HSC proliferation and differentiation in culture

As observed in Fig. 1, HSC maintained in culture underwent marked proliferation resulting in exponential growth after initial seeding on day 0. Specifically, 12-fold to 250-fold increases in HSC cell growth were observed between days 4 and 10. HSC viability exceeded 91% during the culture period (data not shown). As observed in Fig. 2, HSC rapidly differentiate and occupy both myeloid and lymphoid lineages in culture and the percentage of cells occupying each lineage does not remain constant. For example, cells occupying the $CD34^+$ lineage (HSC progenitor cells) remain more consistent over the culture period, occupying 17% and 5% of the total cell population

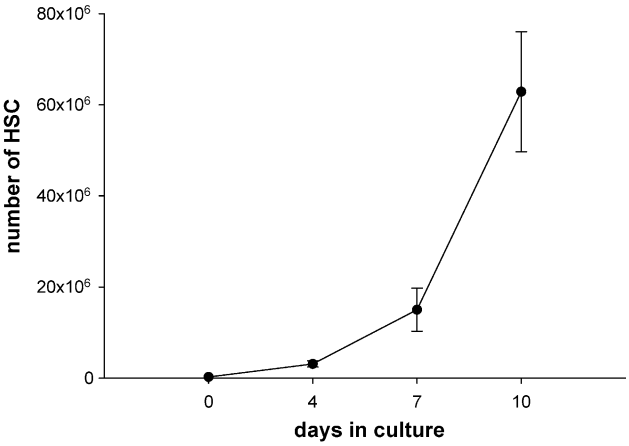

Fig. 1. HSC proliferation rate during 10 days of cell culture. HSC viability at the four times analyzed were 91% on day 4, 93% on day 7, and 99% on day 10, respectively. Data represents mean ± S.E.M. of three experiments.

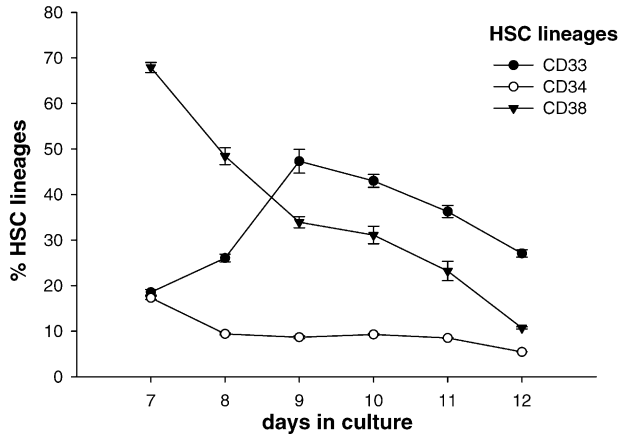

Fig. 2. Percentage of HSC occupying CD33, CD34 or CD38 lineages as determined by flow cytometry during 7–12 days of culture. Data represents the mean ± S.E.M. of three experiments.

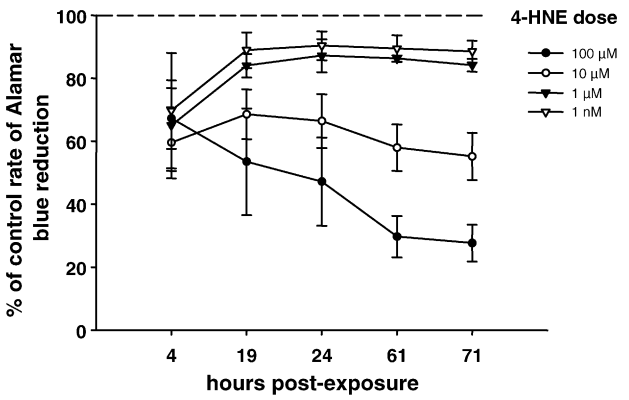

Fig. 3. Effects of single exposure of 4-HNE on HSC proliferation. Cell proliferation rates were determined by Alamar blue reduction using a range of single acute 4-HNE doses from 1 to 100 μM 4-HNE. 4-Hydroxynonenal exposures elicited a significant reduction ($p < 0.05$) in the rate of HSC proliferation at all time points and doses. Data is expressed as percentage control rate of proliferation and represents the mean ± S.E.M. of three experiments.

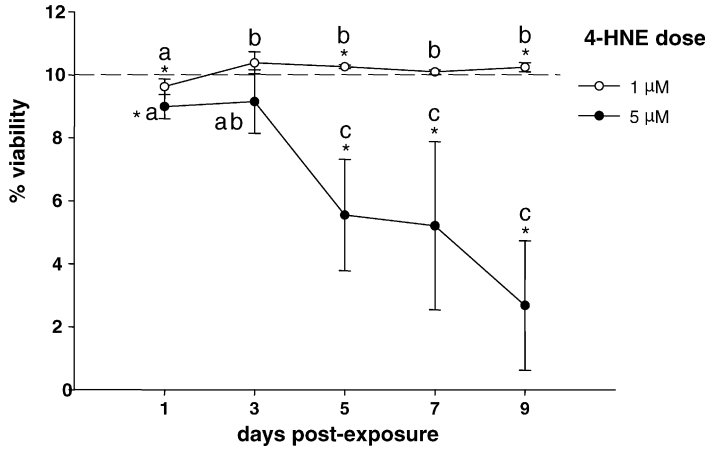

Fig. 4. Effects of steady state exposure (4 h) to 1 or 5 μM 4-HNE on HSC viability. Treatment effects that elicited a significant loss in viability at $p < 0.05$ are denoted by (*) and common letters between and within treatments do not significantly differ. Data represents the mean ± S.E.M. of three experiments.

on days 7 and 12, respectively (Fig. 2). Cells within the CD33 lineage (lymphoid cells) occupy 19% of total HSC on day 7, 47% on day 9, and 27% of the cell population on day 12 (Fig. 2). The total number of cells within CD38 lineage (myeloid lineage) was 68% on day 7 steadily declining to 11% of the total cell population on day 12 (Fig. 2).

3.2. Effect of 4-HNE on HSC proliferation and viability

Exposure to single doses of 4-HNE from 1 nM to 100 μM elicited a dose-dependent decrease in the rate of proliferation of HSC at all time points post-exposure (Fig. 3). Specifically, 4-HNE concentrations of 1 nM, 1 μM, 10 μM, and 100 μM elicited 10–12%, 13–16%,

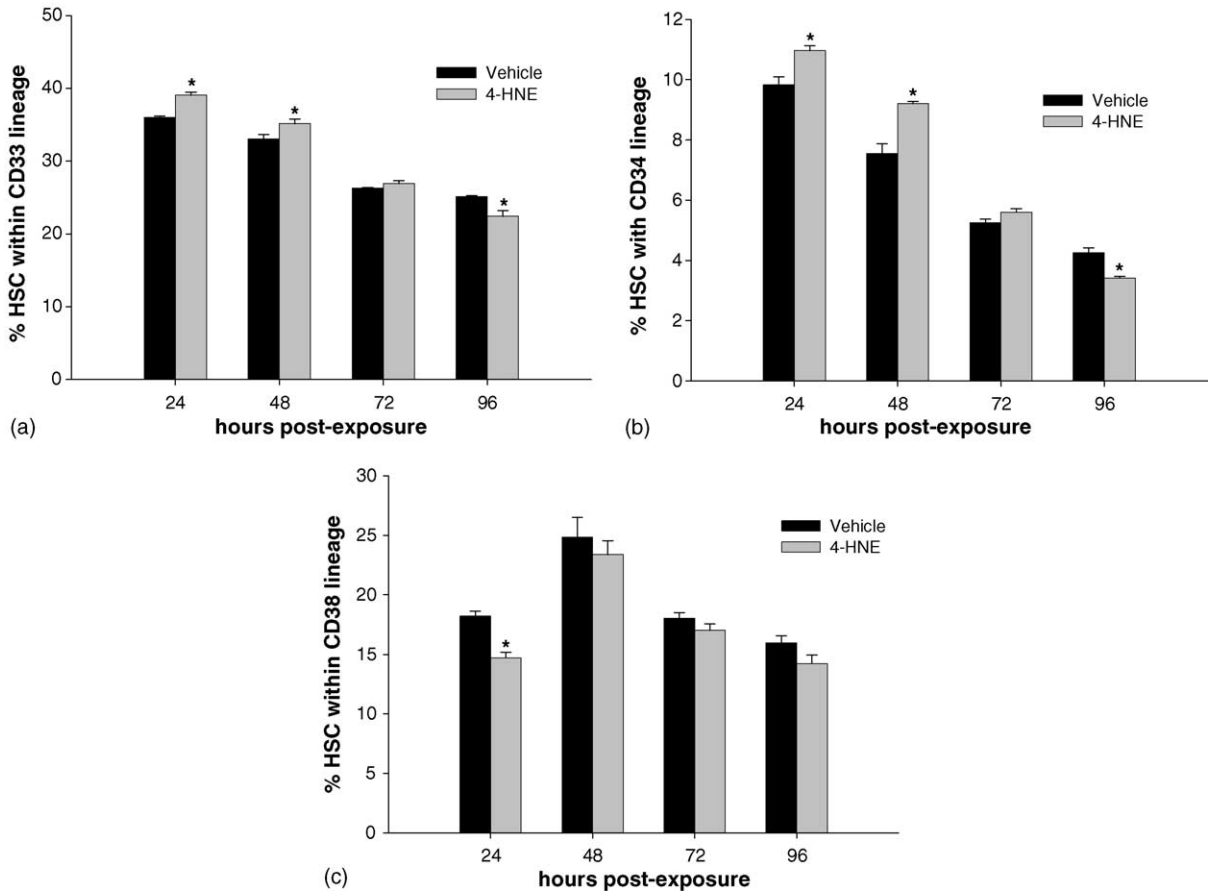

Fig. 5. Effects of 4 h steady state exposure to 1 μM 4-HNE on HSC differentiation as determined by flow cytometry on (a) CD33, (b) CD34 and (c) CD38 lineages. Data expressed as lineage percentage of total HSC populations. (*) Denotes significant differences between treatment groups at $p < 0.05$. Data represents mean ± S.E.M. of three replicates.

34–45% and 53–72% decreases, respectively, in the overall rates of HSC proliferation by 24–72 h post-exposure (Fig. 3). As observed in Fig. 4, exposure to 1 μM 4-HNE under 4 h steady state conditions, did not markedly affect overall HSC viability, using trypan blue exclusion as an indicator of viability. In contrast, exposure to 5 μM 4-HNE elicited a marked decrease in HSC viability at 1, 5, 7, and 9 days (Fig. 4).

3.3. Effects of 4 h steady state 4-HNE exposure on HSC differentiation

Exposure to steady state 1 μM 4-HNE for 4 h elicited an initial increase (6–8% increase) in the percentage of HSC within the CD33 lineage at 24 and 48 h post-exposure, followed by a reduction in CD33 cells by 96 h (Fig. 5a). Similarly, the percentage of HSC within the CD34 lineage significantly increased by 23% by 48 h after exposure to steady state 1 μM 4-HNE, followed by a loss of CD34 cells in the 4-HNE-treated group by 96 h (Fig. 5b). In contrast, exposure to steady state 1 μM 4-HNE for 4 h resulted in an initial 17% decrease in the percentage of HSC within the CD38 lineage that was generally sustained over 96 h (Fig. 5c).

3.4. Effect of 4-HNE on apoptosis in HSC

As observed from Fig. 6, exposure of HSC to 5 μM 4-HNE resulted in a 13-fold increase in the extent of apoptosis. In contrast, no measurable increase in the rate of apoptosis was detected at 1 μM 4-HNE concentrations (data not shown).

4. Discussion

HSC and progenitors grown in suspension cultures offer several experimental advantages relative to cells cultured on substrate. For example, cells grown in suspension cultures do not require enzymatic digestion prior to performing cell counts or determining cell viability and in addition permit culture of greater number of cells. Furthermore, the phenotype of cells grown in a suspension culture is preserved, which is not always the case with adherent cell culture systems [22]. The fact that HSC can differentiate into the blood cell lineages renders these cells particularly useful in studies of the effects of drugs or chemicals on hematopoietic differentiation. In this regard, human fetal HSC in culture can be driven to yield hematopoietic myeloid and lymphoid cell lineages while maintaining their primitive nature [22,27]. Under our culture conditions, HSC growth increased exponentially and remained at a high level of viability at up to 16 days in culture. The employment of fluorescent antibodies to specific cell surface markers confirmed that under our culture conditions, HSC differentiated into early hematopoietic lineages that are important in the development of hematopoiesis.

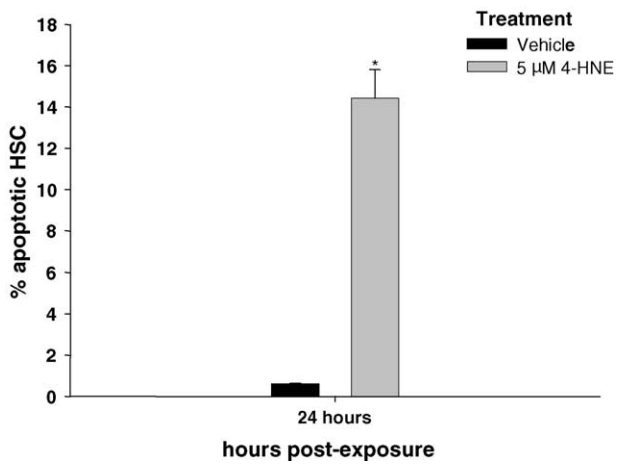

Fig. 6. Effects of steady state 5 μM 4-HNE exposures on the level of apoptosis in total HSC 24 h following 4-HNE exposure. Data represents mean ± S.E.M. of three replicates.

Collectively, toxicological experiments of HSC suspension cultures have been carried out using cells from several sources, including bone marrow, peripheral blood, cord blood and fetal liver [21,22,27,28]. However, significant differences in gene expression and phenotype may exist among $CD34^+$ cells isolated from adult human bone marrow and human fetal liver [29–31]. Accordingly, extrapolation of data using bone marrow-derived $CD34^+$ cells for studies relevant to the in utero environment may not be appropriate. Therefore, employment of cultured human fetal HSC as a developmental toxicology research model offers an attractive alternative for studies addressing perturbation to the in utero environment, which may have ramifications on hematopoiesis.

Basal 4-HNE concentrations in cells typically range between 0.3 and 3.0 μM [3]. In contrast, our cultured fetal HSC populations maintained intracellular levels of 4-HNE in the 0.16–0.50 μM range, which places HSC at the low range of cells with regards to basal 4-HNE concentrations in the absence of oxidative stress. Interestingly, addition of single acute doses of 4-HNE as low as 1 nM inhibited the overall rate of HSC proliferation in assays using 96-well plate. Thus, the potency of 4-HNE with respect to inhibition of proliferation of HSC was dramatic and somewhat unexpected. Others have reported that the potency of 4-HNE as an inhibitor of cell proliferation is dependent upon the cell density in culture, as well as initial concentrations of 4-HNE in media [3,32]. In addition to the effects on HSC proliferation, exposure to 1 μM 4-HNE significantly altered the percentage of HSC within the myeloid, lymphoid and CD34 cells. In light of the fact that 4-HNE potency is dependent on cell number and density, it is possible that 4-HNE at a steady state concentration of 1 μM in culture flask experiments (containing 3×10^7 cells) is acting more as a physiological messenger [1] compared to similar doses in 96-well plate (containing

3×10^3 cells) experiments, where it is acting as a toxicant and causing a loss of cell proliferative capacity.

Certainly, 4-HNE constitutes one of the most potent and reactive aldehydes that can be produced during oxidative injury. For example, Esterbauer et al. [3], demonstrated that of six aldehydes tested on human fibroblasts, 4-HNE proved to be the most potent toxicant, with 4-HNE concentrations between 1 and 10 μM resulting in a 70% decrease in cell survival. Furthermore, acute exposure to 1 μM 4-HNE has been shown to decrease the rates of proliferation of HL-60 human leukemic cells [33]. It is readily apparent from our study that fetal liver HSC constitute one of the most sensitive cell types with regards to sensitivity to 4-HNE injury. The extreme sensitivity of these HSC to 4-HNE may indeed be a reflection of a poor ability to detoxify α,β unsaturated aldehydes during oxidative stress, as suggested by our previous work [21]. It has been previously shown that other cell lines exposed to high levels of 4-HNE have elevated levels of certain phase I and II biotransformation enzymes [34,35], including some of those enzymes involved in 4-HNE metabolism. The aforementioned induction of 4-HNE protective enzymes may serve as an adaptive response to 4-HNE injury by facilitating 4-HNE metabolism and excretion at high concentrations. However, the concentrations of 4-HNE shown to induce protective enzymatic pathways in other cells are certainly well above those 4-HNE concentrations tolerated by HSC, which further supports our hypothesis that the sensitivity of HSC to 4-HNE is at least partially dependent upon the expression and activities of enzymatic pathways involved in 4-HNE detoxification.

We observed marked differences in HSC injury depending upon the 4-HNE dosing régime employed. Typically, cell toxicity studies involve single doses of a test agent, which may not be representative of physiological exposure. In the case of 4-HNE, a single acute application of 4-HNE to cell culture media can result in the disappearance of the aldehyde within 60 min [24,32]. The disappearance of 4-HNE in culture may be a result of degradation, metabolism, or nucleophilic adduction to proteins within cells. Because 4-HNE can be generated in vivo following exposure to pro-oxidant drugs and alcohol, short-term steady state 4-HNE exposures may be more reflective of human physiological exposures. In this regard, a single acute 4-HNE exposure to cells is not representative of alcohol consumption, which can occur over multiple hours.

Stem cells in culture can be driven to differentiate into different terminal cell types as a result of incubation with different combinations of growth factors and cytokines. For example, human fetal myeloid cells cultured in the presence of alcohol exhibit alterations in cytokine levels [36], whereas alcohol exposure also disrupts the ability of mouse fetal liver B-lineage intermediates to progress to maturity in utero [37]. Evidence has emerged demonstrating that 4-HNE has the capacity to alter the activity of growth factors, affect cell signaling [38], granulocytic differentiation and monocyte migration [39,4], respectively. Collectively, these studies suggest that altered cytokine levels during early gestation may have adverse effects on immune system development and hematopoiesis. The fact that exposure to low levels of 4-HNE can alter the differentiation of HSC, as well as affect overall rates of proliferation, suggest that this compound has the potential to affect hematopoiesis. This is of particular significance in that alterations in hematopoiesis have been linked to blood-borne diseases manifested in later life [40].

The fact that exposure to 5 μM 4-HNE elicited a significant increase in the induction of apoptosis at 24 h post-exposure, whereas no detectable levels of apoptosis were observed at 1 μM 4-HNE, suggests a threshold of 4-HNE mediated activation of apoptosis in the low micromolar range. The presence of an adequate compliment of detoxification pathways by cells has also be shown to moderate the ability of 4-HNE to induce apoptosis [41]. Furthermore, Awasthi et al. [42] demonstrated that overexpression of hGSTA4-4, a GST isoform with a high 4-HNE conjugation capacity, protects HL-60 cells from 4-HNE-mediated apoptosis. Impairment of hematopoiesis due to increased rates of apoptosis in HSC lineages have been linked to aplastic anemia and childhood leukemia [43,44], respectively. In this regard, it is apparent from our studies that a window for 4-HNE-mediated apoptosis in HSC exists between 1 and 5 μM 4-HNE concentrations. However, what is not known from our studies is if the sensitivity of HSC to 4-HNE is lineage-specific. Further studies employing FAC sorted cells and fluorescent antibodies to specific cell surface markers will elucidate those particular lineages that may be especially susceptible to 4-HNE-mediated growth perturbations.

In summary, the results of our study indicate that fetal liver HSC are extremely sensitive to 4-HNE injury, with in vitro exposures in the low nanomolar range adversely effecting HSC proliferation. Based on viability, differentiation and apoptosis experiments, we conclude that a window of 4-HNE-mediated toxicity to HSC exists between concentrations of 1 and 5 μM, with 4-HNE concentrations of 1 μM 4-HNE altering differentiation of HSC lineages. These doses are highly relevant to the in vivo environment and at a threshold of oxidative injury. Due to the fact that fetal liver receives a substantial proportion of maternal blood flow during pregnancy, and given the critical functional role of fetal hematopoietic stem cells in the initiation of long-term hematopoiesis, injury to these cell types may have toxicological ramifications post-natally. Thus, of interest with regards to our future experiments is if the in vitro HSC alterations observed in the present study are associated with functional alterations in hematopoiesis in vivo.

Acknowledgments

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References

[1] Dianzani MU. 4-Hydroxynonenal from pathology to physiology. Mol Aspects Med 2003;24:263–72.

[2] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog Lipid Res 2003;42:318–43.

[3] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.

[4] Poot M, Verkerk A, Koster JF, Esterbauer H, Jongkind JF. Reversible inhibition of DNA and protein synthesis by cumene hydroperoxide and 4-hydroxy-nonenal. Mech Ageing Dev 1988;43:1–9.

[5] Hauptlorenz S, Esterbauer H, Moll W, Pumpel R, Schauenstein E, Puschendorf B. Effects of the lipid peroxidation product 4-hydroxy-nonenal and related aldehydes on proliferation and viability of cultured Ehrlich ascites tumor cells. Biochem Pharmacol 1985;34:3803–9.

[6] Chen JJ, Yu BP. Alterations in mitochondrial membrane fluidity by lipid peroxidation products. Free Radic Biol Med 1994;17:411–8.

[7] Zanetti D, Poli G, Vizio B, Zingaro B, Chiarpotto E, Biasi F. 4-Hydroxynonenal and transforming growth factor-beta1 expression in colon cancer. Mol Aspects Med 2003;24:273–80.

[8] Okamoto K, Toyokuni S, Uchida K, Ogawa O, Takenewa J, Kakehi Y, et al. Formation of 8-hydroxy-2′-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in human renal-cell carcinoma. Int J Cancer 1994;58:825–9.

[9] Palinski W, Yla-Herttuala S, Rosenfeld ME, Butler SW, Socher SA, Parthasarathy S, et al. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. Arteriosclerosis 1990;10:325–35.

[10] Markesbery WR, Lovell MA. 4-Hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. Neurobiol Aging 1998;19:33–6.

[11] Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc Natl Acad Sci USA 1996;93:2696–701.

[12] Morikawa S, Kurauchi O, Tanaka M, Yoneda M, Uchida K, Itakura A, et al. Increased mitochondrial damage by lipid peroxidation in trophoblast cells of preeclamptic placentas. Biochem Mol Biol Int 1997;41:767–75.

[13] Ogihara T, Hirano K, Morinobu T, Kim HS, Hiroi M, Ogihara H, et al. Raised concentrations of aldehyde lipid peroxidation products in premature infants with chronic lung disease. Arch Dis Child Fetal Neonatal Ed 1999;80:F21–5.

[14] Schmidt H, Grune T, Muller R, Siems WG, Wauer RR. Increased levels of lipid peroxidation products malondialdehyde and 4-hydroxynonenal after perinatal hypoxia. Pediatr Res 1996;40:15–20.

[15] Chen JJ, Schenker S, Henderson GI. 4-Hydroxynonenal levels are enhanced in fetal liver mitochondria by in utero ethanol exposure. Hepatology 1997;25:142–7.

[16] Chen JJ, Schenker S, Henderson GI. 4-Hydroxynonenal detoxification by mitochondrial glutathione S-transferase is compromised by short-term ethanol consumption in rats. Alcohol Clin Exp Res 2002;26: 1252–8.

[17] Morrison SJ, Weissman IL. Heterogeneity of hematopoietic stem cells: implications for clinical applications. Proc Assoc Am Physicians 1995;107:187–94.

[18] Robinson RS, Seelig Jr LL. Effects of maternal ethanol consumption on hematopoietic cells in the rat fetal liver. Alcohol 2002;28:151–6.

[19] Watson RR, Prabhala RH, Abril E, Smith TL. Changes in lymphocyte subsets and macrophage functions from high, short-term dietary ethanol in C57/BL6 mice. Life Sci 1988;43:865–70.

[20] MacGregor RR. Alcohol and immune defense. J Am Med Assoc 1986;25:1474–9.

[21] Gardner JL, Doi AM, Pham RT, Huisden CM, Gallagher EP. Ontogenic differences in human liver 4-hydroxynonenal detoxification are associated with in vitro injury to fetal hematopoietic stem cells. Toxicol Appl Pharmacol 2003;191:95–106.

[22] Warren MK, Rose WL, Beall LD, Cone J. CD34+ cell expansion and expression of lineage markers during liquid culture of human progenitor cells. Stem Cells 1995;13:167–74.

[23] Ahmed SA, Gogal Jr RM, Walsh JE. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H] thymidine incorporation assay. J Immunol Methods 1994;170:211–24.

[24] Barrera G, Di. Mauro C, Muraca R, Ferrero D, Farace MG. Induction of differentiation in human HL-60 cells by 4-hydroxy-nonenal, a product of lipid peroxidation. Exp Cell Res 1991;197: 148–52.

[25] Barrera G, Muraca R, Pizzimente S, Serra A, Rosso C, Saglio G, et al. Inhibition of c-myc expression induced by 4-hydroxynonenal, a product of lipid peroxidation, in the HL-60 human leukemic cell Lline. Biochem Biophys Res Commun 1994;203:553–61.

[26] Li X, Darzynkiewicz A. Labeling DNA strand breaks with BrdUTP detection of apoptosis and cell proliferation. Cell Prolif 1995;28: 571–9.

[27] Muench MO, Firpo MT, Moore MA. Bone marrow transplantation with interleukin-1 plus kit-ligand ex vivo expanded bone marrow accelerates hematopoietic reconstitution in mice without the loss of stem cell lineage and proliferative potential. Blood 1993; 81:3463–73.

[28] Sutherland HJ, Lansdorp PM, Henkelman DH, Eaves AC, Eaves CJ. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. Proc Natl Acad Sci USA 1990;87:3584–8.

[29] Lu SJ, Li F, Vida L, Honig GR. CD34CD38 Hematopoietic precursors derived from human embryonic stem cells exhibit an embryonic gene expression pattern. Blood 2004;103:4134–41.

[30] Ng YY, van Kessel B, Lokhorst HM, Baert MR, van den Burg CM, Bloem AC, et al. Gene-expression profiling of CD34+ cells from various hematopoietic stem-cell sources reveals functional differences in stem-cell activity. J Leukoc Biol 2004;75:314–23.

[31] Shojaei F, Gallacher L, Bhatia M. Differential gene expression of human stem progenitor cells derived from early stages of in utero human hematopoiesis. Blood 2004;103:2530–40.

[32] Siems W, Grune T. Intracellular metabolism of 4-hydroxynonenal. Mol Aspects Med 2003;24:167–75.

[33] Barrera G, Pizzimenti S, Laurora S, Moroni E, Giglioni B, Dianzani MU. 4-Hydroxynonenal affects pRb/E2F pathway in HL-60 human leukemic cells. Biochem Biophys Res Commun 2002;295: 267–75.

[34] Samprey BP, Korourian S, Ronis MJ, Badger TM, Petersen DR. Immunohistochemical characterization of hepatic malondialdehyde and 4-hydroxynonenal modified proteins during early stages of ethanol induced liver injury. Alcohol Clin Exp Res 2003;27:1015–22.

[35] Cheng JZ, Sharma R, Yang Y, Singhal SS, Sharma A, Saini MK, et al. Accelerated metabolism and exclusion of 4-hydroxynonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress. J Biol Chem 2001; 276:41213–23.

[36] Svinarich DM, DiCerbo JA, Zaher FM, Yelian FD, Gonik B. Ethanol-induced expression of cytokines in a first-trimester trophoblast cell line. Am J Obstet Gynecol 1998;179:470–5.

[37] Biber KL, Moscatello KM, Dempsey DC, Chervenak R, Wolcott RM. Effects of in utero alcohol exposure on B-cell development in the murine fetal liver. Alcohol Clin Exp Res 1998;22:1706–12.

[38] Zarkovic N. 4-Hydroxynonenal as a bioactive marker of patho-physiological processes. Mol Aspects Med 2003;24:281–91.

[39] Barrera G, Pizzimenti S, Muraca R, Barbiero G, Bonelli G, Baccino FM, et al. Effect of 4-hydroxynonenal on cell cycle progression and expression of differentiation-associated antigens in HL-60 cells. Free Radic Biol Med 1996;20:455–62.
[40] Belov L, Huang P, Barber N, Mulligan SP, Christopherson RI. Identification of repertoires of surface antigens on leukemias using an antibody microarray. Proteomics 2003;3:2147–54.
[41] Henderson GI, Chen JJ, Schenker S. Ethanol, oxidative stress, reactive aldehydes, and the fetus. Front Biosci 1999;4:D541–50.
[42] Awasthi YC, Sharma R, Cheng JZ, Yang Y, Sharma A, Singhal SS, et al. Role of 4-hydroxynonenal in stress-mediated apoptosis signaling. Mol Aspects Med 2003;24:219–30.
[43] Maciejewski JP, Risitano A. Hematopoietic stem cells in aplastic anemia. Arch Med Res 2003;34:520–7.
[44] McHale CM, Smith MT. Prenatal origin of chromosomal translocations in acute childhood leukemia: implications and future directions. Am J Hematol 2004;75:254–7.