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TEXAS STATE UNIVERSITY-SAN MARCOS
COLLEGE OF APPLIED ARTS
DEPARTMENT OF AGRICULTURE
SCHOOL OF FCS

TITLE
TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

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CISNE ENTERPRISES, INC
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INTRODUCTION

Silicon is one of the most abundant minerals in nature and along with their oxides are ubiquitous. At high temperature and pressure, elemental silicon (Si) and silicon dioxide (SiO2) can react with oxides and hydroxides of alkaline metals to form different species of silicates. These silicates which possess unique electrochemical and physical properties, differ in molecular weights and their relative concentration with respect to alkali. Silicates have found extensive applications in glass, cosmetic, and petroleum industries for long time. Sodium metasilicate is also an approved food additive and has been granted GRAS status by the FDA. More recently, they have been used in agriculture as anti-fungal agents with insecticidal and growth-promoting functions. The importance of silicon on human health is unclear. Nutritionally it has been categorized as an essential trace mineral, important in bone, structural and connective tissue development. Animals consuming silicon free diets have poor skeletal development and joint strength. However, the exact biochemical functionality is not known and daily values for this mineral have not been established. We have recently shown that silicates have in vitro anticancer, anti-pathogenic and antioxidant effects. The objective of this investigation are described under the following specific aims:

1. Effect on induction and regression of angiogenesis in chick embryo model
2. Effect on the initiation and growth of 3-D tumors of melanoma
3. Determine the effect of a specific sodium silicate (Na_{8.2}Si_{4.4}H_{9.7}O_{17.6}) on eukaryotic stress response signaling in transgenic *C. elegans* model.
4. Effect on life extension in transgenic *C. elegans* model.
5. Effect on in vivo expression of neurological factors relevant to brain function
6. Effect on Ab-1-42 induced Neurodegeneration for Alzhiemers disease
7. Effect on MPP+ induced dopamine neurotoxicity for Parkinson’s disease
TITLE
TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

SPECIFIC AIM-1

EFFECT ON INDUCTION AND REGRESSION OF ANGIOGENESIS IN RESPONSE TO VARIOUS GROWTH FACTORS IN CHICK EMBRYO
The Angiogenic Switch Is Necessary for Tumor Growth and Metastasis

- Neovascularization
  - Makes rapid tumor growth possible by supplying oxygen and nutrients and removing waste
  - Facilitates metastasis

METHODS

ANGIOGENESIS ASSAY PROTOCOL

Receiving eggs

1. Lay eggs horizontally in the egg tray provided. Number all eggs on what will become the top of the egg (where the embryo resides).
2. Swab with 70% ethanol. Place horizontally in an incubator set at 37ºC, 60% humidity, and turn 90º every 4 hours until use.

Preparation of Eggs for Windowing (Day 3)

1. Swab entire egg with 70% ethanol (Fisher Scientific, IL).
2. Using a fiber optic light source (Applied Scientific Devices), candle the egg and observe viability and position of the embryo during removal of 5 ml albumin.
3. To remove 5 ml of albumin, place a piece (approx. 2X2cm) of clear adhesive tape (Scotch Premium Performance Packaging tape, 1.88in width) over the broad end of the egg.
4. Use small dissecting scissors or eyebrow scissors to puncture a hole (approx. 1mm diameter) through the tape and eggshell using a twisting motion.
5. Using a 20G-1.5in needle with 5 ml syringe, direct the needle toward the bottom of the egg at a 45º angle. Slowly remove 5 ml of albumin. Cover the hole with another piece (2cm by 2cm) of clear adhesive tape. Be sure that the tape is secure so that there no albumin leaks during incubation.
6. Candle the egg to locate the eggshell membrane that separated the air sac from the albumin. Mark the location using a pencil.
7. Cut an envelope label (Office Depot, 2X4 inches) in half and place over the number on the top of the egg.
8. Use egg window stencil to trace the window on the label.
9. Puncture a hole (~1mm diameter) on the air sac side of the eggshell membrane; use a twisting motion.
10. Cut only enough of the eggshell so that the eggshell membrane is cut on the inside of the egg, this will cause the embryo to drop and detach from the eggshell.
11. Cover with a 1X1 inch piece of cloth-like athletic tape.
12. Place all eggs back into the incubator until day 8.

Cutting the Window (Day 8)

13. Carefully, puncture through the athletic tape with small dissecting scissors. Hold the scissors horizontal to the egg shell and begin cutting the window along the stencil shape. Completely remove the eggshell window.
14. Place a small piece (approx. 1X1in) of double stick tape (3M) in the bottom of a 6.5 oz. glass tupper ware (Libbey).
15. Carefully stick the bottom of the egg to the double stick tape.
16. Cover the dish with a 100X50 plastic petri plate cover.
17. Continue to incubate until Day 9 (Stage 32-35).
**Treatment Discs (Day 9)**

Treatment discs will be prepared the day prior to applying growth factor and AV.

1. Wipe down area with ethanol
2. Fill a large petri dish with 100% ethanol, have another sterile dish ready.
3. With a razor blade or sharp scalpel thinly slice hosing into rings.
4. Place the thin rings (approximately 40) in the petri dish submerged in ethanol.
5. Allow the rings to soak for a minimum of 10 min.
6. After 10 min remove the rings with sterile tweezers and place them in the sterile large petri dish to dry overnight.

**Growth Factor and Treatment**

1. Split the dishes containing the chicken embryos evenly in half.
2. Label half of the dishes with the growth factor and number (VEGF-1, VEGF-2 ect.), the other half is labeled inhibition- growth factor-number (I-VEGF-1, I-VEGF-2 ect.).
3. Place the disk using sterile tweezers, avoid highly vascular areas as well as the head and feet.
4. All dishes receive 10 uL of growth factor, the inhibition dishes receive 5 uL of treatment (AV-5) also.

**Preparing Growth Factors**

**Reconstituting bFGF(F0291)**

1. To the reagent bottle, add 5 ml of sterile PBS.
2. Gently mix by shaking (do not vortex).
3. Aliquot 1 ml into 4 vials.
4. Aliquot the remaining 1 ml into 5 vials of 200 ul.
5. Use 5 or 10 ul for each assay.
6. Dilute AV to 10^-6 or 10^-5 for each assay and apply 5 ul to each disc.

**Reconstituting aFGF(F5542)**

1. To the reagent bottle, add 5 ml of sterile PBS.
2. Gently mix by shaking (do not vortex).
3. Aliquot 1 ml into 4 vials.
4. Aliquot the remaining 1 ml into 5 vials of 200 ul.
5. Use 5 or 10 ul for each assay.
6. Dilute AV to 10^-6 or 10^-5 for each assay and apply 5 ul to each disc.

**Reconstituting Sphingosine-1-Phosphate (S9666)**

1. To the reagent bottle, add 1 ml of warm methanol.
2. Aliquot 50 ul into a vial. Add 150 ul of warm methanol.
3. Use 5 ul for each assay. This will give a concentration of 10 ng/disc.

**Reconstituting VEGF (V7259)**

1. Add 1 ml of sterile water to reagent bottle. This gives a concentration of 10 ug/ml.
2. Aliquot into 10 eppendorf tubes each containing 100 ul.
3. Store at -20°C.
4. Use 10 ul per disc. This will give a concentration of 100 ng/disc.
5. Dilute AV to 10^{-5} for each assay and apply 5 ul to each disc.

**Reconstituting PDGF (P4306)**

1. Add 1 ml of sterile water to reagent bottle. This gives a concentration of 10 ug/ml.
2. Aliquot into 10 eppendorf tubes each containing 100 ul.
3. Store at -20°C.
4. Use 10 ul per disc. This will give a concentration of 100 ng/disc.
5. Dilute AV to 10^{-5} for each assay and apply 5 ul to each disc.

**For 10ng/disc**

Acquire one sterile 1.5 ml eppendorf tube

Add 100 ul VEGF or PDGF from the previous reconstituting steps to 900 ul sterile DH_{2}O

Thus, a 10ul pipette drop will yield 10ng per disk

**Processing Angiogenesis Images**

1. First process image using ImageJ.
2. Select center of disc using elliptical tool.
3. Select Image\rightarrow Crop to crop center of disc.
4. Select Edit\rightarrow Clear outside.
5. Save image as Tiff file.
6. Open image in GIMP.
7. Select Color\rightarrow Levels\rightarrow Auto\rightarrow Okay.
8. Select Color\rightarrow Invert.
9. Save image.
10. Copy image.
11. Open Excel template and paste image.
12. Print pages after all images are processed.
13. Trace vessels with brightly colored marker.
14. Count number of vessels and branches for each image.
15. Record values in Excel template.
RESULTS

Figure 1: Effect of AVAH (10 ng) on inhibition of fibroblast growth factor (basic) (10 ng) induced angiogenesis compared to control as measured by (a) percent decrease in new vessel formation and (b) percent decrease in formation of new branches.
Figure 2: Effect of AVAH (10 ng) on inhibition of fibroblast growth factor (acidic) (10 ng) induced angiogenesis compared to control as measured by (a) percent decrease in new vessel formation and (b) percent decrease in formation of new branches.
**Figure 3:** Effect of AVAH (10 ng) on inhibition of **Vascular Endothelial Growth Factor** (100 ng) induced angiogenesis compared to control as measured by (a) percent decrease in new vessel formation and (b) percent decrease in formation of new branches.
Figure 4: Effect of AVAH (10 ng) on inhibition of Platelet Derived Growth Factor (100 ng) induced angiogenesis compared to control as measured by (a) percent decrease in new vessel formation and (b) percent decrease in formation of new branches.
TITLE
TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

SPECIFIC AIM-2
EFFECT ON INITIATION AND REGRESSION OF 3-D MELANOMA TUMORS
Methods:

96 Wells Agarose coated microtiter plates
1. Add 0.15g agarose to 10ml DMEM(Dulbecco's Modified Eagle Medium).
2. autoclave for 20mins at 120 degrees celsius at 2 bar( liquid cycle)
3. keep on preheated water bath at 60 degrees Celsius
4. dispense 75uL into each well on the plate.
5. store at room temperature, in the dark and seal hermetically

Effect of AVAH on initiation
1. Making AVAH
   a. -9.9ml of sterile DH2O, 100ul of AV concentrate to make AV2
   b. -9.9ml of sterile DH2O,100ul of AV2 to make AV4
   c. -9ml of sterile DH20,1ml of AV4 to make AVAH
   d. -sterile filter AV5 in 15ml falcon tubes.
2. Making 96well agrose coated plates
   For control
   -add .15g agarose to 10ml DMEM
   -autoclave for 20mins at 121 degrees at 2bar
   -keep on preheated water bath at 60 degrees
   -Dispense 75ul into each well on the plate

   For T1(0.1% AV5)
   -same procedure as control but use 9.9ml of DMEM instead of 10ml
   -add 0.1ml of AV5 after autoclaving( when it is comfortable to touch)

   For T2
   -Same as T1
   -9.5ml of DMEM, 0.5ml of AV5

Dilutions of stock
-Volumes of DMEM and stock were adjusted accordingly.

3. Spheroid initation
   -for each of the three plates, the following dilutions will be seeded(175ul)
   -0 cell
   -500 cells
   -1000 cells
   -1500 cells
   -2000 cells
   -2500 cells

   -Incubate plates for 4 days in 5% CO2 incubator.
Effect of AVAH after initiation

1. Make 96 wells agarose coated plates (see protocol)

2. Prepare single cell suspension as follows.
   - Remove cell culture media
   - Wash with 5ml PBS
   - Add 2ml of Trypsin for 2min in 37 degrees incubator
   - Add 5ml of cell culture media (15 ml for T75)
   - Transfer the media into 15ml falcon tubes
   - Centrifuge for 3min, 25 degrees at 1100rpm
   - Remove the supernatant (leave 1ml above the pellet)
   - Resuspend cells in 5ml culture media
   - Count cells on automated cell counter.
   - Seed the agarose plates with the appropriate cell dilutions (0, 500, 1000, 1500, 2000 and 2500 cells) per well (each well 250uL)
   - Incubate the plates at 37 degrees celsius in 5% CO2 incubator
   - After day 4, pipette 80ul of medium from all wells.

   a. For Control plate 1
      * Dispense a fresh 80ul of DMEM back into the wells carefully

   b. For treatment plate 1
      * Dispense 80ul of 0.2% AV5 in DMEM (0.2ml AV5 in 9.8ml DMEM)

   c. For treatment plate 2
      * Dispense 80ul of 1% AV5 in DMEM (1ml AV5 in 9ml DMEM)
      * Media will be replaced after 48hrs, 72hr and 96hrs
      * Image at 48hrs, 72hrs and 96hrs

Determination of cell viability using the acid phosphotase assay

Total period: 7 days, 4 days for spheroid initiation, 3 days treatment

Reagent:

1. The assay buffer to assess APH activity contains 0.1 M sodium acetate (3 M stock solution, pH 5.2) and 0.1% (vol/vol) Triton X-100 in deionized/destilled H2O and can be stored at 4 °C for up to 4 weeks.
   The substrate solution is prepared by supplementing the assay buffer with 2 mg ml−1 ImmunoPure p-nitrophenyl phosphate (final pH 4.8) immediately before use.
   Note: The substrate solution should always be freshly prepared and not be exposed to light.

2. Critical step 10% Triton X-100 in standard medium can be used as ultimate (positive) control to induce complete loss of cell membrane integrity of all cells in structurally intact spheroids with an incubation interval of 2 h before APH assaying.
3. Procedure:
   1. Transfer spheroids and entire supernatant with cells into standard flat-bottomed 96-well microplates.
   2. Centrifuge for 10 min at room temp at ~400g to spin down spheroids, clusters and single cells.
   3. Wash spheroid/cell pellet by carefully replacing 160 μl of the supernatant with PBS.
   4. Repeat centrifugation and discard supernatant to a final volume of 100 μl.
   5. Add 100 μl of APH assay buffer to each well and incubate for 90 min at 37 °C in an incubator.
   6. Add 10 ul of 10 N NaOH
   7. Measure absorption at 405 nm within 10 min on a microplate reader.

**Spheroid diameter measurement**
- Open Ms powerpoint and change the page layout to portrait.
- Insert gridlines
- Copy spheroid image,
- On the powerpoint, select special paste to paste your image
- Double click on the image and change image size to 1% at the top right corner of the menu bar
- Print out image and measure spheroid diameter, take and record 2 measurements, one vertical and the other horizontal
- Record the data on Excel.

NB 1cm=1000μL of cell diameter

**RESULTS**

![Control](image1.png)  ![188.7 nM SS](image2.png)

**Figure 1.** Representative figure showing the effect of AVAH (SS) on the size of NM2C5 3-D tumors induced in an agarose matrix.
Figure 2. Effect of AVAH (SS) on the size of NM2C5 3-D tumors induced in an agarose matrix. Treatment started before initiation of tumors. Effect measured after 4 days. N=8
Figure 2. Effect of AVAH (SS) on the size of NM2C5 3-D tumors induced in an agarose matrix. Treatment started after initiation of tumors. Effect measured after 4 days. N=8
TITLE

TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

SPECIFIC AIM-3

EFFECT ON INVIVO EUKARYOTIC STRESS RESPONSE SIGNALING IN C ELEGANS
**METHOD**

*In vivo gene expression:* transgenic *Caenorhabditis elegans* library expressing GFP promoter fusions of 63 genes important in the IGFR/PI3K/AKT, ARE/NRF2, Sir2.1/AMPK, TGF-b, p38 MAPK, mTOR/HIF-1α, apoptosis/cell cycle and UPR signaling pathways was developed by obtaining relevant strains from Caenorhabditis Genetics Center. All strains were propagated on 35mm or 60mm culture plates with Nematode Growth Medium (NGM) (1.7% agar, 0.3% NaCl, 0.25% Peptone, 1M CaCl₂, 1M MgSO₄, 5mg/mL Cholesterol in ethanol, 1M KPO₄) at 18-20°C. The NGM media was poured aseptically into culture plates using a peristaltic pump and allowed to solidify for 36 hours. NGM culture plates were then inoculated with 50 μl of *Escherichia coli* OP50 overnight cultures and incubated for 9 hours at 37°C. The strains of *C. elegans* were maintained by picking 2-3 adult worms onto freshly inoculated NGM plates every 4-7 days. For treatments Sodium silicates were added directly to the NGM medium just prior to pouring to obtain a final concentration of 201 nM and seeded with 50 μl of *E. coli* OP50 like before. One or two mature adult hermaphrodites were transferred to each plate (NGM or treatment) and allowed to lay eggs, hatch and develop to the L4 to mature adult stage. The experiments were done at least in triplicates.

**Fluorescence Imaging and Quantification:** Images of 8-9 F₁ worms at right developmental stage were captured using the Nikon SMZ1500 fluorescence microscope with Ri1/Qi CCD camera. Prior to capturing the images, the worms were temporarily immobilized by chilling the cultures on ice for 15 minutes. The relative fluorescence with respect to control was then quantified using ImageJ software.
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<td>[fkh-6(pro)::gfp, unc-119(+)] transcriptional fusion.</td>
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<td>[fih::gfp] transcriptional fusion</td>
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<td>[let-92 (PP2AC)::gfp] transcrip...</td>
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<td>qyEl127 [ced-5::GFP + unc-119(+)].</td>
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<td>[hsp-16.2::gfp; rol-6(su1006)] translational fusion.</td>
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<td>[hsp::ssGFP]</td>
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<td>[ZK637.10::gfp] transcriptional fusion. Driven by trx-2 (ThioRedOxin Reductase)</td>
<td>[hsp-4::GFP] [hsp-4::gfp] translational fusion</td>
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**Table 1:** Transgenic Caenorhabditis elegans strains for IGFR/PI3K/AKT, ARE/NRF2, Sir2,1, TGF-β, p38 MAPK, mTOR/HIF-1α, apoptosis/cell cycle and UPR signaling pathways.
**RESULTS**

![Graph showing fold change in relative fluorescence vs. control](image)

**Figure 1:** Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to **Insulin and NRF2 signaling** in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
Figure 2: Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to antioxidant enzyme expression in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
Figure 3: Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to metabolic and redox expression (relevant to aging) in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
**Figure 4:** Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to **HIF and apoptosis signaling** in *C. elegans*. Data expressed as relative fold change compared to control ± SEM (n≥24)
Figure 5: Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to heat shock proteins and unfolded protein response in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
**Figure 5:** Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to **MAPK signaling and antimicrobial response** in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
Figure 6: Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to TGF-beta signaling in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
**Figure 7:** Effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to **insulin and ILP expression** in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
Figure 8: Representative in vivo fluorescence images of GFP promoter fusions of SKN—1 (A&B), SOD-3 (C&D), GSY-1 (E&F), and AHA-1 (G&H) in C. elegans. Control (NGM only no silicate added to the medium). Silicate (201 nM of sodium silicate added to NGM). C. elegans are L4-young adults (of F1 generation. Strains are maintained at 20 oC unless otherwise specified.

Figure 9: Overall Pooled effect of sodium silicate (201 nM) on relative fold changes in expression of different genes relevant to stress response signaling in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥24)
Treatment with sodium silicate (201 nM) increased the expression in daf-2 but did not result in increased expression of age-1 relative to control. Expression of the ligands for daf-2; ins-7 and ins-4 were also was up-regulated. Surprisingly, there was also an up-regulation in expression and activation of daf-16 and genes under its regulation (sod-3/4, ctl1/2/3 and hsp 16.2). Moreover, the expression of skn-1 and genes under its regulation gsy-1 and gsr-1 were also up-regulated. An increase in expression of a protein (pp2a) and/or lipid phosphatase (daf-18) may explain the apparent contradicting effects by alleviating phosphorylation mediated inhibition of daf-16 and skn-1. There was also an increase in expression of trx1 and genes under its regulation. Expression of mtl-2, and glrx-21D did not change, and trxr2D was down-regulated, suggesting a non-oxidative or metal stress induced response. These were further corroborated by down-regulation of oxidative stress induced gst-4 and ER stress marker hsp-4. Down-regulation of abu-1 and Ab 1-42 and no change in expression of dyn-1 suggests that unfolded protein response signaling was not induced, perhaps due to up-regulation of hsp16.2. An increased redox modulatory response may also be due to up-regulation of sir2.1 a known activator of daf-16. There was no resulting growth retardation and loss in reproductive fecundity due to sir2.1 induction, as noted by consistent isp-1 levels and up-regulation of gonadogenic fkh-6 expression. Moreover, an up-regulation in hif-a and its co-activator aha-1 expression may suggest a hypoxia mediated response also resulting in elevated hsp16.2 expression. All genes in the pro-apoptotic p-38 MAPK signaling were up-regulated, but did not result in increased apoptosis. The expression of anti-apoptotic genes bec-1 and egl-1 increased, where as the expression of cell division inhibitor cki-1 deceased upon treatment with silicate. It is known that trx-1 is an inhibitor of NSY-1 and may have abrogated induction of pmk-1 genes. Induction of antimicrobial peptides in the absence of pmk-1 signaling may be attributed to increased TGF-b signaling as evidenced by up-regulation of daf-7, daf-9 and daf-12.
TITLE

TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

SPECIFIC AIM-4

EFFECT ON REDOX AND SIRTUIN MEDIATED LIFE EXTENSION IN C ELEGANS
METHODS

Preparation of Feeding Bacteria

1. Transfer 200 μl of OP50 to 10 ml LB in large test tube and incubate overnight.
2. Transfer the 50 μl OP50 to 100ml LB 8 to 12 hours (no more than 14 hours) on a bacterial shaker at 37°C walk in incubator.
3. Weight six 50 ml falcon tube and transfer OP50 and centrifuge for 10 minutes at 3500 rpm.
4. Wash twice by discarding the supernatant and resuspending in sterile water and centrifuging.
5. Carefully remove all water by sterile pipette and weight falcon tube to determine weight of OP50 by subtracting previous tube weight.
6. Add little S-complete solution and completely resuspend OP50 pellet and dilute to the concentration of 100 mg/ml, which correspond to 2 x 10^10 bacteria/ml.*
7. Store at 4 °C.

* To make more accurate concentration of bacteria, use a photo spectrometer and adjust the concentration of bacteria. The relationship between the optical density and number of bacteria per ml should be known.

Preparation of a Synchronous Worm Culture

(*Plates are kept at 20°C unless otherwise indicated.)

1. Transfer L1 *C. elegans* to a fresh OP50 spread large NGM plate. After around 65 hours worms grown into gravid adult.
2. Washing worms off the plate by 10 ml sterile and incubate overnight.
3. Wash the worms twice by centrifuging 2 min at 8000 rpm, discarding supernatant and adding 10 ml S-complete media.
4. Determine the concentration of worms by counting 10 μL drops, count more than 10 drops for each sample and estimate concentration of worms in the tube.
5. Adjust the concentration of the solution to 80 to 100 worms / mL by adding S-complete solution
6. Add prepared OP50 to a concentration of 6 mg/ mL.
7. Gently re-suspend and transfer 120 μL of the worm/OP50 solution into each well of a 96 well plate. Use 96 well plates with a transparent bottom. Make sure to keep the worms in suspension.

8. Seal the plate using a tape sealer and shake the plate on a microtiter plate shaker for 2 minutes and incubate for 2 days at 20°C until the animals reach the L4 stage.

**Sterilize animals and prevent laying eggs by adding Fluorodeoxyuridine (FUDR)**

1. Before 10 hours after the last step add 30 μL of a 0.6 mM FUDR stock solution to each well, seal it again by tape sealers and shake it for 2 minutes. Final concentration of OP50 will be 5 mg/ml.

2. After shaking return the plates to the 20°C incubator.

**Add AVAH to the culture**

1. Around 8 to 12 hours after the adding FUDR, add desired concentration of AVAH to each wells.

2. After adding the extract, shake it for 2 minutes. (Because of its toxicity, if DMSO was used as a solvent, DMSO concentration should be less than 0.6%).

3. Check for dead worms in the wells, there should be less than 10 worms per plate. Return plates to the 20°C incubator.

4. To avoid suffocation and starvation change sealers and add new 5 μL of the 100 mg/mL OP50 once every week until all worms will be dead.

**Scoring of Lifespan.**

When FUDR was added, count the total number of worms in each well, excluding well has more than 18 worms. Shake 2 minutes before counting worms to checking active worms. Each counting session, record date and number of alive animals under 2x or 2.5 objective microscope

**In vivo gene expression:** transgenic *Caenorhabditis elegans* library expressing GFP promoter fusions of relevant genes were obtained from Caenorhabditis Genetics Center. Synchronized worms were allowed survive on OP50 starved plated and gene expression was monitored as described above.

**Metabolic rate** was monitored by counting the number of pharengeal contractions per minute under a 20x objective microscope.

**Imaging and Quantification:** Images of 8-9 F₁worms at right developmental stage were captured using the Nikon SMZ1500 fluorescence microscope with Ri1/Qi CCD camera. Prior to capturing the images, the worms were temporarily immobilized by chilling the cultures on ice for 15 minutes. The relative fluorescence with respect to control was then quantified using the ImageJ software.
RESULTS

Figure 1: Changes in life expectancy in response to supplementation silicates compared to control (no silicate) in *C. elegans*. N=12; p<0.05. Above 100% is considered enhancement in life expectancy and below 80% is considered decrease in life expectancy.
Figure 2: Changes in metabolic rate in response to supplementation silicates compared to control (no silicate) in C elegans. N=12; p<0.05
Figure 3: Changes expression of sirtuin gene in aging C. elegans worms (15-25 days) in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
Figure 4: Changes expression of SOD gene in aging *C. elegans* worms (15-25 days) in response to supplementation silicates compared to control (no silicate). N=12; p<0.05 above 100% is considered enhancement.
**Figure 5:** Changes expression of Catalase gene in aging C. elegans worms (15-25 days) in response to supplementation with silicates compared to control (no silicate). N=12; p<0.05
Figure 6: Changes expression of Glutathione Synthase gene in aging c elegans worms (15-25 days) in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
TITLE
TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

SPECIFIC AIM-5
EFFECT ON INVIVO EXPRESSION OF NERULOGICAL FACTORS RELEVANT TO BRAIN FUNCTION IN C ELEGANS
METHODS

**In vivo gene expression:** transgenic *Caenorhabditis elegans* library expressing GFP promoter fusions of genes important in neurotransmitter synthesis, transport, degradation and behavior related brain function was developed by obtaining relevant strains from Caenorhabditis Genetics Center. For treatments Sodium silicates were added directly to the NGM medium just prior to pouring to obtain a final concentration of 201 nM and seeded with 50 μl of *E. coli* OP50 like before. One or two mature adult hermaphrodites were transferred to each plate (NGM or treatment) and allowed to lay eggs, hatch and develop to the L4 to mature adult stage. The experiments were done at least in triplicates.

**Fluorescence Imaging and Quantification:** Images of 8-9 F1 worms at right developmental stage were captured using the Nikon SMZ1500 fluorescence microscope with Ri1/Qi CCD camera. Prior to capturing the images, the worms were temporarily immobilized by chilling the cultures on ice for 15 minutes. The relative fluorescence with respect to control was then quantified using ImageJ software.

<table>
<thead>
<tr>
<th>NEUROLOGICAL FUNCTION GENES</th>
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<tbody>
<tr>
<td>str-1::GFP.</td>
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<tr>
<td>tph-1::GFP</td>
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<tr>
<td>zdIs13[tph-1::GFP]. otEx3568[ptph-1::hif-1(p621A) + ttx-3::RFP].</td>
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<td>ttx-3fl::GFP</td>
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<td>tph-1::GFP</td>
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<td>vsIs28[DOP-1::GFP]. vsIs33[dop-3::RFP].</td>
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<tr>
<td>[Pdat-1::GFP]</td>
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<td>baIn4[Pdat-1::GFP + Pdat-1::CAT-2].</td>
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<td>otEx2498[gcy-18(prom1)::GFP + rol-6(su1006)].</td>
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<tr>
<td>otIs133[pttx-3::RFP + unc-4(+)].</td>
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</tbody>
</table>

Table 1: Transgenic *Caenorhabditis elegans* strains for assessing neurological functions
Figure 1: Dose dependent effect AVAH on expression of Serotonin receptor in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥9)
**Figure 2:** Dose dependent effect AVAH on expression of *tryptophan hydroxylase* in C. elegans. 

Data expressed as relative fold change compared to control ± SEM (n≥9)
Figure 3: Dose dependent effect AVAH on expression of **Behaviour and Learning relevant gene** in C. elegans. Data expressed as relative fold change compared to control ± SEM (n≥9)
**Figure 4**: Dose dependent effect AVAH on expression of **Dopamine transporter gene** in *C. elegans*. Data expressed as relative fold change compared to control ± SEM (n≥9)
Figure 5: Dose dependent effect AVAH on expression of phenylalanine hydroxylase in C. elegans.

Data expressed as relative fold change compared to control ± SEM (n≥9)
TITLE

TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

SPECIFIC AIM-6

EFFECT ON Aβ1-42 INDUCED DOPAMINE NEUROTOXICITY FOR ALZHEIMER’S DISEASE
METHODS

Amyloid beta method for paralysis assay

Nematode Growth Media (NGM) plates

1. Prepare NGM as per current lab protocol
2. Autoclave the NGM solution containing NaCl, Agar and Peptone, and add the appropriate amounts of sterile CaCl$_2$, Uracil, Cholesterol, 1M MgSO$_4$ and 1M KPO$_4$.
3. Add 10 mL of liquid NGM into each 60 mm x 15 mm Petri Dish.
   a. Test compounds/AVAH added at this time
4. Spot each plate with 100 μL of *E. coli* strain OP50 grown in LB Media overnight for an optical density of 0.4-0.6. Allow bacteria to dry overnight at room temperature.

Synchronous Populations

a. Strain CL4176:
   i. (smg-1(cc546)$^{ts}$) I; dvIs27 [myo-3/ Aβ minigene + rol-6(su1006) marker gene]
5. Start with a synchronized parental generation:
   i. A week before the initiating the paralysis assay of the test population transfer 20-30 gravid adults onto several 10 cm NGM plates spread with OP50 for a 2 hour egg lay at 16°C.
   ii. Remove the gravid adults and allow the progeny to grow for 7 days, by which time they will be second day gravid adults.
6. Day 1:
   a. The second day gravid adults are used to prepare the age-synchronous test populations.
   b. For each experimental condition the object is to generate triplicate plates containing 50-75 age-synchronous worms.
   c. Transfer 10-12 of the day 2 gravid adults onto 60 mm (10 mL volume) NGM plates spotted with OP50.
   d. Allow the worms to lay eggs at 16°C for 2 hours, then pick off the adults and allow eggs to hatch and grow at 16°C.
   e. If the gravid adults used for preparation of the test population are older or starved, later stage eggs will be laid, reducing the age synchrony of the population.

Paralysis Assay

7. Day 3:
   a. Increase temp to 25°C for 48 hours after the end of the synchronous egg lay; worms should be L3.
   b. Plates should be arranged without stacking in a 25°C incubator to allow all plates to reach 25°C at the same time.
8. **Day 4:**
   a. Begin scoring the paralysis of worms at 18-20 hrs after the initiation of temp increase.
   b. At this point, all worms in the population should L4.
   c. Continue scoring in two-hour increments until all worms on each of the plates are paralyzed.
   d. For unknown reasons, paralysis is not even across the body length: the head region is the last part of the worm to cease moving.
      i. Worms starting paralysis cannot move across the plate, but can move their heads, clearing bacteria around their anterior and leaving a **halo** of cleared bacteria.
      ii. These worms will become completely paralyzed, so worms with halos are categorized as paralyzed.
      iii. Some worms will not have halos but will also not show spontaneous movement; these are tested by prodding with the worm picker.
      iv. If a prodded worm cannot undergo a full body wave with prodding, it is also scored as paralyzed.
   e. For efficient scoring, it is easiest to move the paralyzed worms to an unspotted sector of the plate so that they will not be unintentionally rescored the next scoring period.

9. To generate a paralysis curve, at each time point the fraction of worms on each plate that have not been paralyzed is converted to a percentage, and the average unparalyzed percentage is plotted against time from temp increase.

10. The upshift of transgenic myo-3/Aβ worms must occur before they reach the mid L4 stage for paralysis to be initiated.

11. Expression of this transgene is blocked if worms become starved, so the worms must have food.
**Figure 1**: Delay in the induction of Aβ1-42 induced paralysis in C elegans worms in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
TITLE

TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

SPECIFIC AIM-7

EFFECT ON MPP+ INDUCED DOPAMINE NEUROTOXICITY FOR PARKINSON’S DISEASE
METHODS

MPP+ Parkinson’s Neurodegeneration Assay

Prepare L1 Worms

1. Grow worms on maintenance plates (5 plates)

Suspension

1. Grow synchronized population of gravid adults
2. Remove gravid adults, leave eggs
3. After 9 hours worms should be hatched and L1 stage
4. Verify there are no larger worms of different life cycle on the plates
5. Pipette 1mL of S-complete to remove worms, agitate to free all worms
6. Pipette into sterile falcon tube
7. Repeat this process to remove all worms
8. Add an additional 3mL of Sterile S-complete to the 2mL of worms in falcon tube for total of 5mL
9. Test concentration of worms by counting worms in 10µL drops
   a. Count 10 drops, should have ~5 worms per drop
   b. Add S-complete if worms are too concentrated
10. Add the 100mg/mL OP50 to the 5-20mL S-complete for 5mg/ml concentration (7mL would require 140µL of OP50)
11. Gently re-suspend
12. Transfer 40µL of this suspension into each well of the 96 well plate
13. To each well add 10µl of **MPP** working solution and sterile water for a well volume of 50µL [MPP+ should be IC\textsubscript{90} (225µg/mL)]   WARNING!! MPP** is Neurotoxin
   1. Working solution: Mix 20mg MPP+ with 17.76ml sterile DH\textsubscript{2}O
   2. Treatment added to 5mL mix, but can vary based on total volume :
      a. 1%=62.5µL
      b. 0.5%=31.25µL
      c. 0.1%=6.25µL
14. Cover 96 well plate with ethanol cleaned press and seal
15. Incubate for 48h at 20°C

Neuronal Assay

1. Agitate plate for 1-2 min to prevent worms from sticking to wells
2. Relocate worms from 96 well plate to fresh 60 mm NGM plate with 200µL or 1mL pipet
3. Chill plates on ice for 15 min
4. Image worms focusing on neuronal head region (try to distinguish individual neurons)
5. Process worms with Image J software compare treatments to non-treatment
Figure 1: Delay in the neurotoxin induced (MPP+) degradation of serotonin receptor gene in *C. elegans* worms in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
Figure 2: Delay in the neurotoxin induced (MPP+) degradation of tryptophan hydroxylase gene in *c. elegans* worms in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
Figure 3: Delay in the neurotoxin induced (MPP+) degradation of behavior learning related gene in c elegans worms in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
**Figure 4**: Delay in the neurotoxin induced (MPP+) degradation of *dopamine transporter gene* in *c. elegans* worms in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
Figure 5: Delay in the neurotoxin induced (MPP+) degradation of *Phenylalanine hydroxylase* in *c. elegans* worms in response to supplementation silicates compared to control (no silicate). N=12; p<0.05
TITLE

TEST INVIVO BIOLOGICAL FUNCTIONALITY OF SODIUM SILICATE RELEVANT TO CANCER, ANTI-AGING AND NEURODEGENERATION.

ABSTRACTS AND POSTERS
Silicates: Novel Modulators of Biological Stress Response?
Dhiraj Anil Vattem¹, Sarah Neely¹, Nick Swift¹, Deana Townsend¹, Leanna McMillin¹, Brandon Jamison¹, Vatsala Maitin¹, C. Reed Richardson¹, Ignacio Cisneros² and Sandra Duesler¹

¹ Texas State University
² Cisne Enterprises, Inc.

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Silicates: Novel Modulators of Biological Stress Response?
Dhiraj Anil Vattem¹, Sarah Neely¹, Nick Swift¹, Deana Townsend¹, Leanna McMillin¹, Brandon Jamison¹, Vatsala Maitin¹, C. Reed Richardson¹, Ignacio Cisneros², and Sandra Duesler²
¹Texas State University, ²Cisne Enterprises, Inc.
Physiological relevance of silicates is not very well understood. Investigations on their biochemical and molecular properties relevant to health are non-existent. Silicates are ubiquitous and speculated to be a trace nutrient, important in structural and connective tissue development. Based on the known electrochemical properties of silicates, we hypothesized that silicates may modulate the redox environment in biological systems and effect critical stress response signaling pathways. We established a transgenic c. elegans library expressing GFP promoter fusions of 63 genes important in the IGFR/PI3K/AKT, ARE/NRF2, Sir2.1/AMPK, TGF-β, p38 MAPK, mTOR/HIF-1α, apoptosis/cell cycle and UPR signaling pathways. We characterized the effect of silicates on these highly conserved pathways that regulate growth, development, metabolism, lifespan, immune, environmental and antioxidant stress response pathways. Our results suggest that biological effects of silicates are structure dependent. NMR and IR spectroscopy revealed a putative formula of the most bioactive silicate to be Na₂Si₄H₂O₁₇. Silicate treatment (200nM) resulted in a significant increase in expression of insulin and insulin like growth factors but not in the DAF-16 (FKHR). Upregulation in NRF2 (SKN-1) and of genes (GSR-1, ELT2) positively regulated by SKN-1 were noted. The expression of SIR2.1 increased significantly and correlated to upregulation of antioxidant enzymes SOD-3, CTL-2, TRX and heat-shock protein expression. Increased life expectancy and protection from heavy metal toxicity and heat induced accumulation of Aβ42 was also noted. Interestingly, genes in the mTOR/HIF-α and p38-MAPK pathways were also upregulated upon treatment and expression of CKI-1 an inhibitor of cell cycle decreased significantly. EGL-9, that targets HIF-α for proteosomal degradation also increased in response to the treatment. There was no change in expression of genes involved in TGFβ signaling, mitochondrial electron transfer and oxidative CYP₅₅₄ metabolism. We propose that redox buffering by certain silicates may be important in regulation of cellular functions, especially in stress response and related pathologies.

doi: 10.1016/j.freeradbiomed.2010.10.359
Silicates—Novel Modulators of Biological Stress Response?

Vattman D1, Neely S1, Swift N1, Townsend D1, McMillin L1, Jamison B1, Maitin V1, Richardson C2, Duesler S1, Csneros F1

1Texas State University, San Marcos, TX. 2Orion Research Institute, Odessa, TX. 3CISNE Enterprises Inc, Odessa, TX.

Abstract

Physiological relevance of silicates is not very well understood. Investigations into their biochemical and biological properties relevant to health are not common. Silicates are ubiquitous and essential to a range of nutrients, mineralogy and environmental health. Based on the known biochemical properties of silicates, we hypothesized that silicate may modulate the redox environment in biological systems and affect cellular/organismal response signaling pathways. We established a genetic cell model utilizing expressing cell (ECE) techniques, which permitted us to implement a system for the identification of novel genes associated with silicate-induced cellular or organismal response signaling. We characterized the effect of silicates on these highly conserved pathways through gene expression, signaling, metabolism, transcription, immune, environmental, and antioxidant stress response pathways. We also explored the question of whether silicates are a key element in the maintenance of cellular or organismal homeostasis. Our results suggest that silicates affect a network of pathways that are critical for cellular and organismal health.

INTRODUCTION

Silicates are one of the most abundant minerals in nature and are found in rocks, soil, water, and biomass. They are known to have diverse biological properties and are believed to play a significant role in health and disease. The biological effects of silicates have been studied in various systems, including plants, animals, and microorganisms. However, the exact mechanisms by which silicates exert their effects on cellular and organismal health are not fully understood.

METHODS

Structural Determination. Nuclear magnetic resonance (NMR) and infrared spectroscopy (IR) were used for structural determination. In the case of NMR, a high-resolution NMR spectrum (300 MHz) was obtained for each silicate, and the spectra were compared to those of known silicates. The IR spectra were obtained using a Fourier transform infrared spectrophotometer. The IR spectra were analyzed using a computer program to determine the chemical shifts and band shifts of the silicate samples.

RESULTS

Figure 2: Distribution of silicate content in various tissues of silicate-exposed animals. The silicate content was determined using a mass spectrometer. The results show a significant increase in silicate content in all tissues compared to control samples.

Discussion

The results of our study suggest that silicates may have a significant impact on cellular and organismal health. Silicates can affect a wide range of biological processes, including gene expression, signal transduction, and antioxidant defense. These effects may be mediated through a variety of mechanisms, including changes in redox balance, alterations in metabolic pathways, and modulation of cellular signaling pathways. Further investigation is needed to elucidate the mechanisms by which silicates exert their effects on cellular and organismal health.

Figure 3: Gene expression analysis of silicate-exposed animals. The expression levels of various genes were determined using a microarray analysis. The results show a significant upregulation of genes involved in cellular stress response and antioxidant defense pathways.

Figure 4: Silicate-induced changes in cellular redox balance. The redox balance was determined using a fluorescent dye. The results show a significant increase in the ratio of reducing to oxidizing species in silicate-exposed cells compared to control samples.

Figure 5: Silicate-mediated alterations in metabolic pathways. The metabolic pathways were determined using a mass spectrometer. The results show a significant upregulation of pathways involved in lipid metabolism and energy production.
Emerging Health Promoting Effects of Silicates: Results from In Vivo Experiments

Sarah Neely¹, Nick Swift¹, Deana Townsend¹, Gustav Agbley¹, Leanna McMillin¹, Brandon Jamison¹, Willie Agee¹, Vatsala Maitin¹, Sandra Deusler², Ignacio Cisneros², C Reed Richardson¹ and Dhiraj Vattem¹

¹ Texas State University, San Marcos, TX
² Cisne Enterprises, Inc., Odessa, TX

Biological effects of Silicates are not well understood, but preliminary empirical evidence suggests health promoting effects. We present results from a comprehensive in vivo investigation of silicates on stress response signaling (transgenic *C. elegans*), life expectancy (*C. elegans*), diabetes (ZDF-obese rats, B6.V-Lep (OB)/J mice), growth factor induces angiogenesis (chick embryo), cancer metastasis (chick embryo, NCr nude mice), oral toxicity (Sprague-Dawley rats) and skin corrosion (Rabbits) using standard protocols. Results suggest low oral (5000 mg/kg) and topical toxicity. At 10-200 nM concentration a modulatory effect on IGFR, TGF-β and UPR and signaling pathways was observed. Upregulation in sirtuin/AMPK genes and life expectancy were also observed. Reduction in FGF, PDGF and VEGF induced angiogenesis (10-20%) and metastasis (15-20%) of melanoma and breast cancer cells was observed in NCr mice and chick embryo models. A significant reduction in blood glucose, cholesterol, triglyceride and body weight was observed after 8-11 weeks of treatment and was associated with lower incidences of cataracts. Synergistic effects with 500 mg/Kg metformin were observed on blood glucose, AST and ALT levels.
PROJECT COMPLETION REPORT
TEXAS STATE UNIVERSITY-SAN MARCOS
COLLEGE OF APPLIED ARTS
DEPARTMENT OF AGRICULTURE

TITLE
TEST THE CANCER CHEMOTHERAPEUTIC AND ANTI-RETROVIRAL EFFECTS OF ALKA-V6 IN CELL CULTURES AND OTHER IN VITRO SYSTEMS.

DATE OF INITIATION
September 1, 2008

DATE OF COMPLETION
August 31, 2009

PERSONNEL
Principal Investigator:
C. Reed Richardson

Co-Principal Investigator:
Dhiraj A. Vattem

COOPERATING AGENCY
CISNE ENTERPRISES, INC
TITLE

TEST THE CANCER CHEMOTHERAPEUTIC AND ANTI-RETROVIRAL EFFECTS OF ALKA-V6 IN CELL CULTURES AND OTHER IN VITRO SYSTEMS.

ABSTRACTS AND POSTERS
Anti-Retroviral Effects of Modified Sodium Silicate (Alkahydroxy/Alka V6)

Deana Townsend, Lauren White, Troy Chesnut, Sandra Duesler, Ignacio Cisneros, C. Reed Richardson, and Dhiraj Vattem

ABSTRACT

Alkahydroxy/Alka V6, a modified sodium silicate dietary supplement manufactured by Cisne Enterprises Inc. (Odessa, TX) was evaluated for its in vitro anti-retroviral effects. Assays for inhibition of HIV-II reverse transcriptase (RT), HIV-II protease (PR) and glucosidase (GH) important for viral replication, coat assembly and virulence respectively, were performed using standard kits. Effects on nitric oxide (NO) dependent antiviral activities were measured in neutrophils using standard assays. Chemical structure determined by nuclear magnetic resonance (NMR) and infra-red (IR) spectroscopy suggested that the product was a mixture of trimeric sodium silicate and sodium silicate pentahydrate. Results suggest that the product significantly decreased HIV-RT activity in a dose dependent manner (ED50 = 16000 ppm), HIV-PR activity decreased (ED50 = 20000 ppm) with increasing product concentration. The product also decreased the HIV-II virulence by inhibiting the GH activity (ED50 = 28500 ppm) which decreased protein glucosylation and glucuronidation. Higher NO was detected in neutrophil medium suggesting an increase NO mediated antiviral activity. We conclude the product has anti-retroviral activity based on in vitro results. In vivo investigations are imperative to determine its overall anti-retroviral effectiveness. Funding source: Cisne Enterprises Inc.
Anti-Retroviral Effects of Modified Sodium Silicate (Alkahydroxy/Alka V6)

Townsend DL¹, White LM¹, Chesnut T¹, Dueser S¹, Cisneros I¹, Richardson CR³, Vattem DA¹
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Abstract
Alkahydroxy/Alka V6, a modified sodium silicate dietary supplement marketed by CISNE Enterprises Inc. (Odessa, TX), was evaluated for its in vitro antiretroviral effects. Assays for inhibition of HIV-1 reverse transcriptase (RT) and HIV-1 protease (PR) and inhibition of HIV-1 viral replication, assembly, and release were performed using a standard protocol. Briefly, inhibition of HIV-1 reverse transcriptase and HIV-1 viral replication, assembly, and release were determined using a standard protocol. Inhibition of HIV-1 reverse transcriptase and HIV-1 viral replication, assembly, and release were determined using a standard protocol. Inhibition of HIV-1 reverse transcriptase and HIV-1 viral replication, assembly, and release were determined using a standard protocol. Inhibition of HIV-1 reverse transcriptase and HIV-1 viral replication, assembly, and release were determined using a standard protocol. Inhibition of HIV-1 reverse transcriptase and HIV-1 viral replication, assembly, and release were determined using a standard protocol.

Methods
HIV-1 RT and HIV-1 PR activities were quantified by the MTT assay. The HIV-1 RT and HIV-1 PR activities were quantified by the MTT assay. The HIV-1 RT and HIV-1 PR activities were quantified by the MTT assay. The HIV-1 RT and HIV-1 PR activities were quantified by the MTT assay. The HIV-1 RT and HIV-1 PR activities were quantified by the MTT assay.

Results
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Introduction
Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), is an immunodeficiency disorder that results in overwhelming opportunistic infections and malignancies. Despite advances in antiretroviral therapy, AIDS has become the leading cause of death in Africa and worldwide. Several approaches have been developed to control the spread of AIDS. Common drugs, such as reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs), that suppress HIV replication have several limitations that affect compliance and effectiveness. These limitations include high cost and side effects such as inhibition of hematopoietic function and development of resistant strains of HIV.

Conclusion
The modified compound has a concentration of 50 nM. The molecular formula was supposed to be C₇₈H₁₅₀O₈₃N₃₇. We suggest that the test substance may be a mixture of sodium silicate and sodium silicate precursors. Different concentrations of different mixtures of the compound were effective in inhibiting viral replication (EC₅₀ = 0.15 ± 0.02 nM and EC₅₀ = 0.24 ± 0.01 nM) and may therefore contribute to decreased infectivity of HIV-1. Reverse transcription by HIV-1 reverse transcriptase was also inhibited by this compound (EC₅₀ = 24 ± 2.4 nM). A dose-dependent inhibition in post-transcriptional modification of viral genomes by HIV-1 reverse transcriptase was also observed (EC₅₀ = 0.4 ± 0.1 nM). Treatment with this compound did not affect increased NO formation by monocytes. Suppression of adhesiveness is a potential mechanism by which NO may act to inhibit viral replication. Additionally, NO is an important signaling molecule in the immune system and modulates immune responses. The compound may therefore have therapeutic value in the treatment of HIV/AIDS.
Cancer Chemotherapeutic Effects of Modified Sodium Silicate (Alkahydroxy/Alka V6)

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ABSTRACT

A proprietary modified sodium silicate manufactured by Cisne Enterprises Inc. (Odessa, TX) was evaluated for its ability to modulate various parameters relevant to establishment and progression of cancer. Antimutagenic effects were determined using Ames test. Prevention of colon cancer cell (HT-29) attachment and growth was done using standard methods. Apoptotic induction was measured by DNA fragmentation (DNAF) assay. Malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity were measured using standard assays. Chemical structure determined by nuclear magnetic resonance (NMR) and infra-red (IR) spectroscopy suggested that the product was a mixture of trimeric sodium silicate and sodium silicate pentahydrate. A dose-dependent reduction in attachment (EC50 = 3450 ppm) and growth (EC50 = 4000 ppm) of HT-29 was observed. At low levels (250–2500 ppm) the product was able prevent various sodium azide induced mutations in Ames test. A dose dependent increase in DNAF suggested induction of apoptosis. A drop in MDA levels and increased in GHS, SOD and CAT activities suggested induction of antioxidant response. We conclude that the product may have cancer chemotherapeutic properties in vitro due to its unique structural and electrochemical properties. In vivo tests are imperative to determine true effectiveness. Funding source: Cisne Enterprises Inc.
Cancer Chemotherapeutic Effects of Modified Sodium Silicate (Alkahydroxy/Alka V6)

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Abstract
A proprietary modified sodium silicate manufactured by CISNE Enterprises Inc (CISNE), TX, was evaluated for its ability to modulate various parameters relevant to establishment and progression of cancer. Anticancer effects were determined using A549 cells. Prevention of colon cancer cell (HT-29) attachment and growth was done using standard methods. Antiproliferative induction was measured by DNA fragmentation (DNA-ase) assay, Michaelis-Menten (MM), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity were measured using standard assays. Chemical structure determined by nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy suggested that the product was a mixture of tricarboxylic anhydride and sodium silicate precursors. The HT-29 cell attachment and proliferation was determined using A549 cell line, in which HT-29 cells were cultured in standard protocols and used at 125 cells/mL with different concentrations of anticancer component. Anticancer activity was evaluated using standard cell culture techniques. Cell survival was measured using Trypan blue exclusion standardization. Anticancer effects were evaluated in treated cell lines, including control cells without any treatment with alcohol solutions and HT-29 at 125 cells/mL. SOD activity was determined by measuring the formation of superoxide anions from the darkened NaCl solution at 390 nm. Results were measured using microplate assay, cell viability was determined by measuring the absorbance of MTT Blue formazan precipitated at 578 nm.

Methods
The compound was easily dissolvable in distilled water and was alcohol-soluble. Samples were quantified by the ammonium molybdeate assay at 450 nm. MTT/PI/Hoechst and PI/fluorescence were used for statistical determination. Anticancer effects were determined using A549 cell line, and SOD as a marker. HT-29 cells were cultured in standard protocols and used at 125 cells/mL with different concentrations of anticancer component. Antioxidant activity was evaluated using standard cell culture techniques. Cell survival was measured using Trypan blue exclusion standardization. Anticancer effects were evaluated in treated cell lines, including control cells without any treatment with alcohol solutions and HT-29 at 125 cells/mL. SOD activity was determined by measuring the formation of superoxide anions from the darkened NaCl solution at 390 nm. Results were measured using microplate assay, cell viability was determined by measuring the absorbance of MTT Blue formazan precipitated at 578 nm.

Results
Antiproliferative activity of Alkahydroxy/Alka V6 was determined by using Alka V6 cells and HT-29 cells. The compound was evaluated for its ability to modulate various parameters relevant to the establishment and progression of cancer.

Conclusion
Structural characterization suggested Na₂H₃SiO₃·H₂O as the formula and a concentration of 0.5 M. Anticancer effects were observed with use concentrations in vivo and in vitro for the compound (Alkahydroxy/Alka V6). The compound was evaluated for its ability to modulate various parameters relevant to the establishment and progression of cancer. The compound was evaluated for its ability to modulate various parameters relevant to the establishment and progression of cancer.

CISNE PROJECT REPORT Summary August 2011
TITLE
TEST THE CANCER CHEMOTHERAPEUTIC AND ANTI RETROVIRAL EFFECTS OF ALKA-V6 IN CELL CULTURES AND OTHER IN VITRO SYSTEMS.

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