UC Irvine

UC Irvine Previously Published Works

Title

Dexamethasone and zinc in combination inhibit the anchorage-independent growth of S-91 Cloudman murine melanoma.

Permalink

<https://escholarship.org/uc/item/7s15q7g4>

Journal

Life sciences, 36(9)

ISSN

0024-3205

Authors

Kreutzfeld, KL
Lei, KY
Bregman, MD
et al.

Publication Date

1985-03-01

DOI

10.1016/0024-3205(85)90205-x

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at
<https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

DEXAMETHASONE AND ZINC IN COMBINATION INHIBIT THE ANCHORAGE-INDEPENDENT
GROWTH OF S-91 CLOUDMAN MURINE MELANOMA

Kristie L. Kreutzfeld¹ and Kai Y. Lei¹
Marvin D. Bregman² and Frank L. Meyskens, Jr.²

¹Department of Nutrition and Food Science
University of Arizona, Tucson, Arizona 85721

²Department of Internal Medicine and the Cancer Center
Arizona Health Science Center, University of Arizona, Tucson, Arizona 85724

(Received in final form December 13, 1984)

Summary

Zinc inhibited the colony formation of Cloudman S-91 murine melanoma cells in a dose dependent manner with an ID₅₀ of 3.4 ug/ml. Total inhibition of the melanoma colony-forming units occurred at a zinc concentration of 4.42 ug/ml. In the presence of dexamethasone the ID₅₀ for zinc inhibition was reduced by 49% and total inhibition of anchorage-independent growth occurred at the achievable *in vivo* zinc concentration of 3.0 ug/ml. Dexamethasone and zinc in combination effected a greater than additive inhibition of the murine melanoma colony-forming units. Statistical evaluation of these results showed that zinc and dexamethasone interacted synergistically to inhibit the formation of murine melanoma colonies.

Zinc is an essential dietary trace element (1) and zinc deficiency as well as zinc supplementation have both been shown to modulate the *in vivo* growth of various transplantable rodent tumors (2-6). Zinc deficiency lowered the growth rate of carcinoma, ascites and other tumor types (2-4). High zinc diets reduced tumor initiation by sarcoma 180 cells (6). Supplemental zinc inhibited the *in vivo* growth of L1210 leukemia cells, but not solid BMW 5147 lymphatic tumors (5). Melanin containing tissues have a high zinc content (7). Harding-Passey, B-16 and Cloudman S-91 murine melanomas preferentially and actively incorporated zinc over other tissues and the zinc was localized to the melanosome (8,9). Zinc at a concentration of 7 ug/ml was cytotoxic on the anchorage-dependent growth of S-91 Cloudman murine melanoma. Similar concentrations of zinc were noncytotoxic to guinea pig keratinocyte and human lung fibroblast cell lines (10). The proliferation of fibroblasts was not affected by zinc up to a concentration of 17 ug/ml (11). These studies suggest murine melanoma is preferentially sensitive to zinc toxicity.

Glucocorticoids stimulate zinc uptake and amount bound to metallothionein in various cell types (12-16). Rodent melanoma cell growth was sensitive both *in vitro* and *in vivo* to inhibition by glucocorticoids (17-20). Because murine melanoma is an adrenocorticoid-responsive and zinc-sensitive transformed cell type, this study was designed to investigate the interaction of zinc and dexamethasone on the anchorage-independent growth of murine melanoma cells.

Materials and Methods

Zinc acetate was obtained from MCB. Dexamethasone, biotin, vitamin B₁₂, and vitamin coenzyme B₁₂ were purchased from Sigma Chemical Co. Dulbecco's Modified Eagle's Medium, penicillin/streptomycin and fetal calf serum

originated from GIBCO Laboratories. Horse serum was obtained from KC Biological, Inc.

Cloudman S-91 3960 (CCL 53.1) melanoma cells were routinely cultured in our laboratory by a combination of serial transplantation in syngeneic (DBA/2J) mice followed by less than ten subcultures on plastic. *In vitro* monolayer cell cultures were maintained in Dulbecco's Modified Eagle's Medium supplemented with 0.1 ug/liter biotin, 1.3 ug/liter B_{12} and coenzyme B_{12} as well as containing 100 IU penicillin and 100 ug streptomycin per milliliter.

A bilayer agar system was used as previously described (21). A bilayer of agar with Dulbecco's Modified Eagles Medium supplemented with ten percent horse serum and two percent fetal calf serum was constructed in 35 mm diameter Falcon petri dishes, with five thousand cells plated into the 1 ml, 0.3% agar overlay. Colonies were counted after ten days of incubation (37°C , 5% CO_2 , 95% air) based on a colony diameter of greater than 60 microns, utilizing the Omicon FAS-II (Bausch and Lomb), an optical image analyzer (22).

A sterile stock solution of one gram Zn acetate- $2\text{H}_2\text{O}$ /100 ml distilled H_2O (pH 5.5-6.0) was maintained and 300 to 400 ul of this stock was added to 100 ml medium (7 to 9 ug/ml) from which dilutions were made. Samples of all media and stock solutions were assayed for zinc by flame atomic absorption spectroscopy (Hitachi model 180-70; standard conditions; integrated standard curve). Zinc containing medium was used to construct both agar layers. Dexamethasone was added with the cells in the top layer.

Statistical Analysis: Quantitation of *in vitro* inhibitions was determined by the methods of Drewinko et al (23). Briefly, the observed minus expected surviving fractions, yields an estimator which when divided by the square root of the estimator's variance is a standard normally distributed Z statistic with corresponding p-values of a two-tailed test. A completely additive effect would yield a Z statistic of zero. Synergistic inhibitory responses are indicated by a negative Z value, and antagonistic responses yield a positive Z value, as observed survival is greater than expected. A Z score was computed for each of the results across the zinc dose-responses curves for both dexamethasone concentrations and was averaged for purposes of dose comparison (average Z). The end of the dose response curves was defined to be the first point at which less than 5% of the control population survived.

Results

The dose-dependent effect of zinc ions on the anchorage-independent growth of CCL 53.1 murine melanoma is presented in Fig. 1. Zinc inhibited colony formation over a concentration range from 0.55 to 4.42 ug/ml. The number of murine melanoma colonies was reduced 98% by the addition of zinc at a concentration of 4.42 ug/ml. The ID_{50} for zinc inhibition of colony formation was 3.4 ug/ml.

The effect of dexamethasone (5×10^{-9} M) as a single agent was to reduce colony formation by 21.8%. When dexamethasone (5×10^{-9} M) and increasing concentrations of zinc were added together an enhanced inhibition of colony formation occurred. The combination results were statistically analyzed and a series of negative Z_g statistics were generated (Table 1), which indicated the growth of melanoma colony-forming units. This was also evident by a shift in the zinc inhibition curve. Dexamethasone (5×10^{-9} M) reduced the zinc ID_{50} by 49% (Fig. 1).

Dexamethasone at a higher concentration (5×10^{-8} M) reduced melanoma colony formation by 80% (Fig. 1). Again a greater than additive inhibition was observed when dexamethasone and zinc were combined. The zinc concentration needed to inhibit 90% of the colony formation was reduced from > 3 ug/ml to 1.5 ug/ml at the higher dexamethasone concentration. Almost total inhibition of colony formation was obtained at a zinc concentration of 3 ug/ml, which was significantly less than the zinc level needed with the lower dexamethasone concentration. Highly significant Z statistics ($p < 0.001$) were

Figure 1. The effect of dexamethasone and increasing concentrations of zinc on the anchorage-independent growth of CCL 53.1 murine melanoma cells. Control plates contained 2800 colonies greater than 60 microns in diameter by day 10.

TABLE I. Zinc and Dexamethasone Synergistic Inhibition of Colony Formation Z Statistics

Zinc (ug/ml)	Dexamethasone (5 nM)		Dexamethasone (50 nM)	
	Z	P	Z	P
0.09	0.0	1.0	0.0	1.0
0.55	-0.65	.5157	-3.56	.0004
1.73	-8.26	<.0001	-13.29	<.0001
3.00	-24.78	<.0001	-23.73	<.0001
4.40	-2.23	.0257	---	---
Average				
Z-Stat.	-7.18	<.0001	-13.53	<.0001

obtained (Table 1) in the zinc concentration range of 0.55 ug/ml to 3 ug/ml. The p values obtained with the higher dexamethasone concentration and zinc were indicative of a larger synergistic interaction than those p values generated at the lower dexamethasone concentration. The average Z statistics for the low and high doses of dexamethasone in combination with zinc were -7.18 and -13.53 respectively and confirm that biological synergism was increased with a ten-fold increase in dexamethasone concentration.

Discussion

Zinc cytotoxicity of anchorage-dependent murine melanoma cell growth at zinc levels greater than 7 ug/ml has been observed (10). Evaluation of the zinc effect on the anchorage-independent growth of CCL 53.1 murine melanoma cells indicated a similar sensitivity, although total inhibition of colony formation was achieved at the lower zinc concentration of 4.42 ug/ml. The soft agar results imply serum zinc levels of 4.4 ug/ml would have to be achieved before a significant effect on murine melanoma tumor growth would be observed. Zinc inhibition of the growth of rodent melanoma tumors has not been achieved (24), although neither the blood or tissue zinc levels were obtained to verify if effective zinc levels were achieved in situ.

Based upon experimental evidence that glucocorticoids can increase cellular zinc uptake, metallothionein synthesis and zinc-metallothionein intracellular storage in other cell types (12-16), we combined dexamethasone and zinc to see if they could enhance each other's inhibitory effects on the anchorage-independent growth of murine melanoma. Our data demonstrated that dexamethasone synergistically interacted with zinc to suppress the anchorage-independent growth of these murine melanoma cells. The addition of dexamethasone at a low concentration (5×10^{-9} M) shifted the zinc inhibitory dose response curve into an achievable pharmacological range (25). Glucocorticoid stimulation of zinc uptake by induction of metallothionein may be responsible for the increased zinc activity. Measurement of metallothionein levels in the presence of dexamethasone and zinc in this cell line are needed to verify this implied mechanism. Also, because glucocorticoids decrease zinc-albumin interactions in vivo (26) dexamethasone could both increase the amount of free zinc ions, enhance the zinc sensitivity of the murine melanoma cell and exert its own inhibitory effects on murine melanoma cell growth. Thus the net effect of using zinc and dexamethasone in combination may be even more promising than the in vitro results might indicate. Recently human melanoma has been shown to contain glucocorticoid receptors (27,28) and respond in vitro (19,29). Investigations with human melanoma cell lines should be undertaken to determine if these cells would be sensitive to reasonable levels of dexamethasone and zinc in combination.

Acknowledgement

Supported in part by grants from the National Cancer Institute (CA 17094, CA 27502 and CA 34689).

References

1. Food and Nutrition Board: Recommended Dietary Allowances; 8th Rev Ed (National Academy of Science, Washington, D.C. 1974).
2. J.T. MCQUITY, W.D. DEWYS, L. MONACO, W.H. STRAIN, C.G. ROB, J. APGAR and W.J. PORIES, Cancer Res. 30 1387-1390 (1970).
3. W. DEWYS and W.J. PORIES, J. Natl. Cancer Inst. 48 375-381 (1972).
4. D.T. MINKEL, P.J. DOLHUN, B.L. CALHOUN, L.A. SARYAN and D.H. PETERING, Cancer Res. 39 2451-2456 (1979).

5. A.D. WOSTER, M.L. FAILLA, M.W. TAYLOR and E.D. WEINBERG, *J. Natl. Cancer Inst.* 54 1001-1003 (1975).
6. J.L. PHILLIPS and P.J. SHERIDAN, *J. Natl. Cancer Inst.* 57 361-363 (1976).
7. J. HORCICKO, J. BOROVANSKY, J. DUCHON and B. PROCHAZKOVA, *Hoppe-Seyler's Z. Physiol. Chem.* 354 203-204 (1973).
8. J. BOROVANSKY, P.R. HEARN, S.S. BLEEHAN and R.G. RUSSELL, *Neoplasma* 27 247-252 (1980).
9. J.F. O'ROURKE, H. PATTON and R. BRADLEY, *Amer. J. Ophthalm.* 44 190-197 (1957).
10. J. BOROVANSKY and P.A. RILEY, *Eur. J. Clin. Oncol.* 19 91-99 (1983).
11. J. EPSTEIN, *J. Cell. Physiol.* 110 17-22 (1982).
12. M. L. FAILLA and R.J. COUSINS, *Biochem. Biophys. Acta* 543 293-304 (1978).
13. R.P. COX and A. RUCKENSTEIN, *J. Cell. Physiol.* 77 71-82 (1971).
14. M. KARIN and H.R. HERSCHMAN, *Eur. J. Biochem.* 113 267-272 (1981).
15. P.J. GILES and R.J. COUSINS, *Cancer Res.* 42 2-7 (1982).
16. K.E. MAYO and R.D. PALMITER, *J. Biol. Chem.* 256 2621-2624 (1981).
17. D. HORN and R.L. BUZARD, *Cancer Res.* 41 3155-3160 (1981).
18. K. ADACHI, S. KONDO and F. HU, *Nature* 220 1132-1133 (1968).
19. M.D. BREGMAN, E. PETERS, D. SANDER and F.L. MEYSKENS Jr., *J. Natl. Cancer Inst.* 71 927-932 (1983).
20. H.S. BHAKOO, N.S. PAOLINI, R.J. MILHOLLAND, R.E. LOPEZ and F. ROSEN, *Cancer Res.* 41 1695-1701 (1981).
21. M.D. BREGMAN, D. SANDER and F.L. MEYSKENS Jr., *Biochem. Biophys. Res. Commun.* 104 1080-1086 (1982).
22. B.E. KRESSNER, R.A. MORTON, A.E. MARTENS, S.E. SALMON, D.D. VON HOFF and B. SOEHNLEN, In: *Cloning of Human Tumor Stem Cells*, (S.E. Salmon, ed) Vol. 48, pp 179-193. New York: Alan Liss, Inc. (1980).
23. B. DREWINKO, T.L. LOO, B. BROWN, J.A. GOTTLIEB and E.J. FREIREICH, *Cancer Biochem. Biophys.* 1 187-195 (1976).
24. J. BOROVANSKY, P.A. RILEY, E. VRANKOVA and E. NECAS, *International Pigment Cell Conference, Abstract 18*, Giessen, W. Germany (1983).
25. J.C. SMITH Jr., J.A. ZELLER, E.D. BROWN, and S.C. ONG, *Science* 193 496-498 (1976).
26. J.D. BOYETT and J.F. SULLIVAN, *Metabolism* 19 148-157 (1970).
27. R. BECHER, H. BOJAR and B. STUHLBREIER, *Anticancer Res.* 2 245-250 (1982).
28. H.S. BHAKOO, R.J. MILHOLLAND, R. LOPEZ, C. KARAKOUSIS and F. ROSEN, *J. Natl. Cancer Inst.* 66 21-25 (1981).
29. D.M. DISORBO, B. MCNULTY and L. NATHANSON, *Cancer Res.* 43 2664-2667 (1983).