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

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Summary

Zinc inhibited the colony formation of Cloudman S-91 murine
melanoma cells in a dose dependent manner with an ID_{50} 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 ID_{50} 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 combina-
tion 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 low-
ered 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 con-
tent (7). Harding-Passey, B-16 and Cloudman S-91 murine melanomas preferen-
tially 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 transform-
ed 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 B_{12},
and vitamin coenzyme B_{12} 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 Biologi-
cal, 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 supple-
mented 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
overlayer. 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-$2H_2O$/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 later.

Statistical Analysis: Quantitation of in vitro inhibitions was deter-
mined 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 yeild 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 forma-
tion was 3.4 ug/ml.

The effect of dexamethasone ($5X10^{-9}$ M) as a single agent was to reduce
colony formation by 21.8%. When dexamethasone ($5X10^{-9}$ 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_{-9} 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 ($5X10^{-9}$ M) reduced the zinc ID_{50}
by 49% (Fig. 1).

Dexamethasone at a higher concentration ($5X10^{-8}$ M) reduced melanoma
colony formation by 80% (Fib. 1). Again a greater than additive inhibition
was observed when dexamethasone and zinc were combined. The zinc concentra-
tion 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 inhibi-
tion 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 dexa-
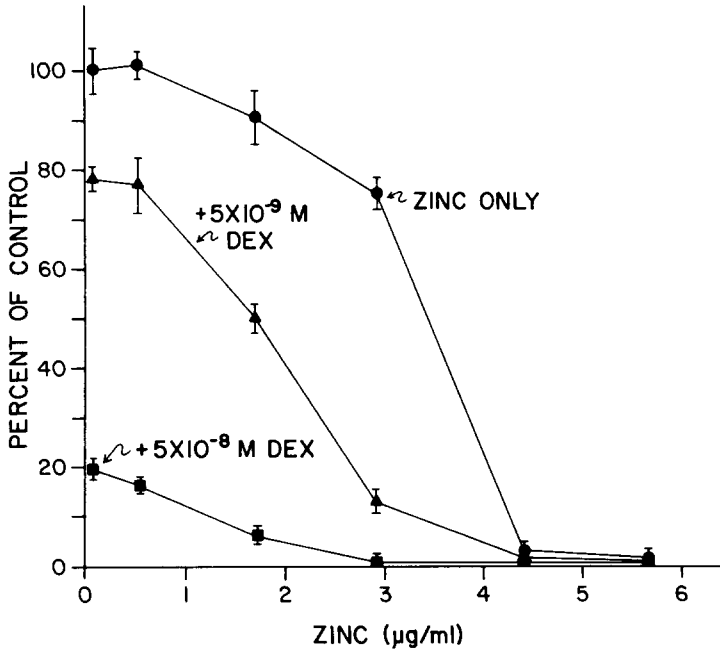methasone concentration. Highly significant Z statistics (p < 0.001) were

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) Z	P	Dexamethasone (50 nM) 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 gener-
ated 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 cellu-
lar zinc uptake, metallotionein synthesis and zinc-metallotionein intracellu-
lar 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 dexametha-
sone synergistically interacted with zinc to suppress the anchorage-independ-
ent growth of these murine melanoma cells. The addition of dexamethasone at
a low concentration ($5X\ 10^{-9}$ M) shifted the zinc inhibitory dose response
curve into an achievable pharmacological range (25). Glucocorticoid stimula-
tion of zinc uptake by induction of metallotionien may be responsible for the
increased zinc activity. Measurement of metallotionein 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 deter-
mine if these cells would be sensitive to reasonable levels of dexamethasone
and zinc in combination.

Acknowledgement

References

1. Food and Nutrition Board: Recommended Dietary Allowances; 8th Rev Ed (Na-
    tional Academy of Science, Washington, D.C. 1974).
2. J.T. MCQUITTY, 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-Seylers 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. Opthalm. 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. STUHLDREIER, 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).