Hexavalent Chromium Cytotoxicity Is Organ and Species Specific for Steller Sea Lion and Mink Cells

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Abstract

The western population of the Alaskan Steller sea lion is continuing to decline for unknown reasons. The fact that the decline is limited to this population strongly suggests that there may be an environmental factor involved. Environmental contaminants, particularly metals, may be one factor playing a role in this decline. We are examining the toxicity of a range of metals to cell lines established from major organ systems of the Steller sea lion. Currently, we have established cultures of testes, skin, liver, kidney and lung cells. Our initial metal toxicity experiments indicate that hexavalent chromium Cr(VI) is variably cytotoxic to Steller sea lions depending on the organ involved. For example, 2.5, 5, 10 and 25 uM sodium chromate induced 100, 71, 44 and 2 % relative survival respectively in skin fibroblasts, but 38, 18, 3 and 0 % relative survival in lung fibroblasts and no survival at any of these concentrations in liver fibroblasts. Thus, skin is more resistant to Cr(VI) than lung cells, and liver cells are the most sensitive of the three. It has been proposed that mink are a suitable surrogate model for studying contaminant effects on the Steller sea lion. To compare the response of the two species to metal toxicity, we isolated a primary mink skin fibroblast cell line and tested it. We found that 2.5, 5, 10 and 25 uM sodium chromate induced 28, 37, 0.8 and 0.1 percent relative survival respectively in mink cells. Thus mink cells were 20-55 times more sensitive at moderate to high doses and 3.4 times more sensitive at relatively low doses. This suggests that mink are a suboptimal model for studying the effects of metals in Steller sea lions. This work was supported by grant NA16FX1412.

Introduction

The western population of the Alaskan Steller sea lion is continuing to decline for unknown reasons. The fact that the decline is limited to this population strongly suggests that there may be an environmental factor involved. Environmental contaminants, particularly metals, may be one factor playing a role in this decline. Currently our laboratory is working on establishing cell lines from various tissue types from the Steller sea lion to use as model systems for the effects of metals. We have successfully cultured and maintained cells from the testes, skin, liver, kidney, and lung. Our goal is to test a variety of soluble metal compounds on these cell types. In this report, we present data concerning the cytotoxicity of hexavalent chromium (VI) in Steller sea lion lung, skin, and liver cells. In addition we show data that compares the cytotoxicity of Cr(VI) and arsenic in Steller sea lion lung cells. Finally because mink have been proposed as a model system for the Steller sea lion we present data that compares the cytotoxicity of Cr (VI) in Steller sea lion skin cells to mink skin cells.

Methods

- **Cell Culture:** Steller sea lion and mink cell lines were isolated from tissue explants. The cells were cultured in a 50:50 mixture of Dulbeccos's minimal essential medium and Ham's F12 medium plus 15% cosmic calf serum, 1% L-glutamine and 1% penicillin/streptomycin. All cells were maintained in a 37°C, humidified incubator with 5% CO_2.
- **Chemical Preparations:** $NaCrO_4$ and $NaAsO_2$ were dissolved in water as previously described (Wise et al., 2002).
- **Cytotoxicity:** Cytotoxicity was measured with a clonogenic assay using standard methods (Wise et al., 2002).

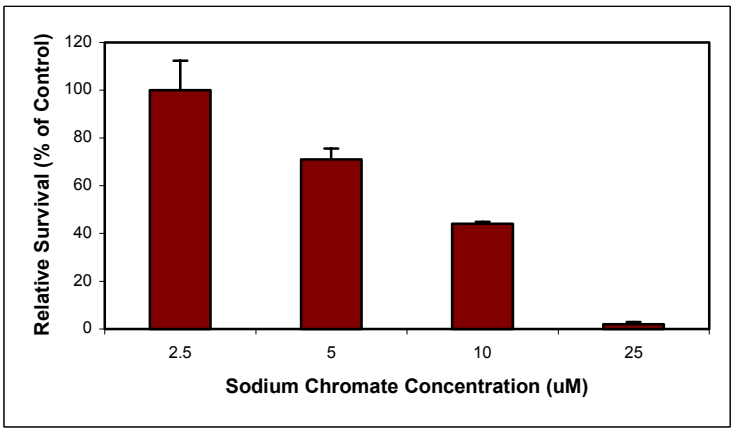

Figure 1. Cytotoxicity of Sodium Chromate in SSL1 Skin Fibroblasts

Sodium chromate induced concentration-dependent cytotoxicity in Steller sea lion skin cells. Sodium chromate concentrations of 2.5, 5, 10, and 25 uM induced 100, 71, 44, and 2% relative survival in skin cells. Error bars = standard error of mean.

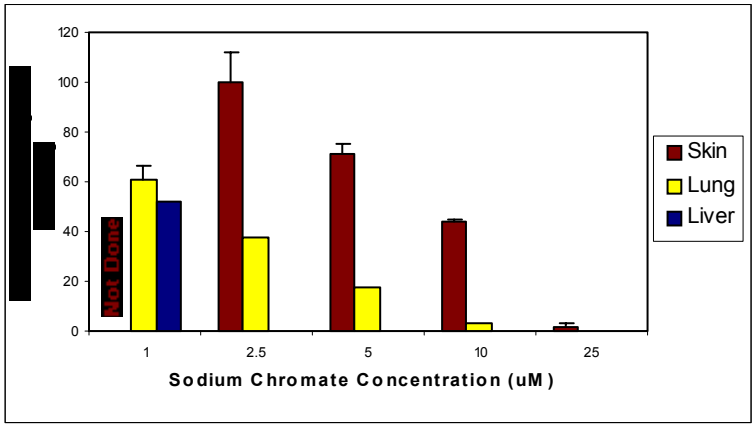

Figure 2. Cytotoxicity of Sodium Chromate in Steller Sea Lion Lung, Skin and Liver Fibroblasts

Sodium chromate induced concentration-dependent cytotoxicity in Steller sea lion lung and skin cells. Specifically, sodium chromate concentrations of 2.5, 5, 10 and 25 uM induced 100, 71, 44 and 2% relative survival in skin cells cells respectively, while 1, 2.5, 5, 10, and 25 uM sodium chromate induced 60, 38, 18, 3 and 0% relative survival in lung cells respectively. There was 52% relative survival at 1 uM sodium chromate and no survival for the higher four doses in liver cells. Thus, skin is more resistant to sodium chromate than lung cells, and liver cells are the most sensitive of the three. Error bars= standard error of mean.

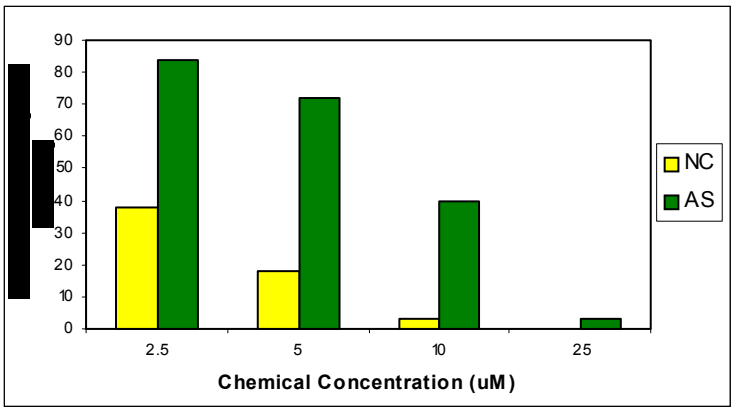

Figure 3. Cytotoxicity of Sodium Chromate and Sodium Metaarsenite in Steller Sea Lion Lung Fibroblasts

This figure shows that sodium chromate is more potent than sodium metaarsenite in Steller sea lion lung cells. Specifically, sodium chromate concentrations of 2.5, 5, 10 and 25 uM induced 38, 18, 3 and 0% relative survival in lung cells respectively, while sodium metaarsenite induced 82, 74, 40 and 3% relative survival at these same concentrations respectively.

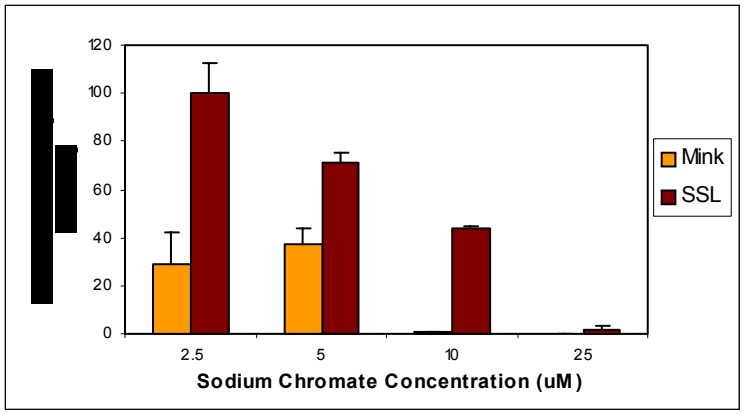

Figure 4. Cytotoxicity of Sodium Chromate in Mink and Steller Sea Lion Skin Fibroblasts

This figure shows that mink skin cells are more sensitive to sodium chromate than Steller sea lion skin cells. Sodium chromate induced 100, 71, 44 and 2% relative survival in Steller sea lion skin cells at concentrations of 2.5, 5, 10 and 25 uM respectively, while relative survival in mink skin cells was 29, 37, 0.8 and 0.1% at these same concentrations. Thus, mink cells were 20-55 times more sensitive at moderate to high doses and 3.4 times more sensitive at relatively low doses. Error bars = standard error of mean.

Conclusion

We have developed skin, liver, lung, testes and kidney cell culture models for the Steller sea lion. We found that hexavalent chromium (Cr (VI)) induced concentration-dependent cytotoxicity in lung and skin cells and concentration-associated cytotoxicity in the liver cells. Furthermore, we found that liver cells were the most sensitive to sodium chromate treatment and skin cells were the more resistant with lung cells exhibiting intermediate toxicity. This indicates that the potency of toxicants is organ specific in Steller sea lions and suggests that a toxicant concentration that is apparently harmless to one organ may be highly detrimental to another. It also suggests that to better determine the risk of a toxicant exposure to these animals it is necessary to have measures of both contaminant levels and toxicity in various organs. Further research is aimed at testing thirteen different soluble chemicals on all six cell types and extending the lifespan of these cells in culture by introducing telomerase.

We also found that arsenic was less cytotoxic than Cr (VI) to Steller sea lion cells. This indicates that contaminants will have varying toxicity and that a priority list for intervention measures can be developed. Further work is aimed at determining whether the difference in potency is consistent across organs.

Finally we found that mink skin cells were significantly more sensitive to Cr (VI) than Steller sea lion skin cells. Mink have been proposed as a surrogate model for Steller sea lion. This finding indicates that mink have a different response to contaminants than Steller sea lions. It suggests that from a risk assessment perspective using mink as a model may be more protective than using Steller sea lions cells themselves. However, from mechanistic, physiological and biochemical perspectives, mink are a suboptimal model for studying the effects of metals in Steller sea lions because their cells react differently.

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References

J.P. Wise, S.S. Wise, J.E. Little. The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human lung cell, Mutation Res. 517 (2002) 221-229.