HAIR HEAVY METAL AND PROTEIN ANALYSIS IN OCEANIA

Effects of mining and lifestyle changes

by

Sitwat Lubna Khawar

M.Sc. (Pakistan), M.Phil (Pakistan)

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Dedicated to Iqbal and Hasan

Declaration

The whole of the experimental work presented in this thesis was carried out by myself in the Department of Biochemistry/Microbiology. The conclusions are my own, reached after numerous critical discussions with Dr. Graham Jones and Dr. Ken Watson. The substance of this thesis has not been submitted for any other degree or qualification, and any help received in the preparation of this thesis and all sources used or literature cited have been acknowledged.

SITWAT LUBNA KHAWAR

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SITWAT LUBNA KHAWAR

Abbreviations

1D	One-dimensional
2D	Two-dimensional
ANSTO	Australian Nuclear Science and Technology Organisation
APS	Ammonium persulfate
As_2O_3	Arsenic trioxide
ATP	Adenosine triphosphate
CdH	Cadmium in hair
CSR	Commonwealth Sugar Refineries
dl	Decilitre
DMAA	Dimethyl arsinic acid
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
HC	High cysteine
HPLC	High performance liqiud chromatography
IAA	Iodoacetic acid
ICPMS	Inductively coupled argon mass spectrometry
IEF	Isoelectric focussing
IFs	Intermediate filaments
KCl	Potassium chloride
LC	Low cysteine
M_r	Relative molecular mass
NaOH	Sodium hydroxide
ppb	Part per billion
ppm	Part per million
SCMC	S-carboxymethylcysteine
SCMK	S-carboxymethylkerateine

SCMKs	S-carboxymethylkerateines
SEM	Standard error of mean
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TEMED	N, N, N', N, -tetramethylenediamine
Tris	Tris (hydroxymethyl) diaminomethane
TTD	Trichothiodystrophy
VHC	Very high cysteine
VLC	Very low cysteine

Abstract

Hair has advantages over other tissues as a biological sampling milieu since it can be easily and atraumatically collected, stored and analysed. Hair preserves a mid to long range record of exposure to many metals (some, like lead, toxic in high levels) that can be studied and analysed. In this thesis, a group of Melanesian people living close to a copper and gold mine (Ok Tedi) in Papua New Guinea and another group from the same racial background living far from the mine site have been examined to obtain evidence for enhanced exposure to metals that may have occurred as a consequence of mining operations. For comparative purposes, hair samples from Australian Europeans (predominantly Anglo-Celtic origin) living in a non-industrial rural environment (Armidale, NSW) and Australian Aboriginals living close to a manganese mine (Groote Eylandt) were also analysed. Results showed that each group displayed a characteristic pattern of metal distribution in hair. Distressingly high levels of copper, lead and mercury analysed in the Aboriginal group may be related to environmental pollution and/or maladaptive lifestyle practices among that group while the levels shown by test sites (Ok Tedi) were found to be largely well below the previously published values concerning Westernised urban populations. It was concluded that the elevated heavy metal levels among the Melanesian Ok Tedi population by comparison with the Melanesian control group may be correlated less with the mining activity and more with a changed life style and diet. A longitudinal analysis of key heavy metals (Fe, Zn, Cu, Pb, Cd and Hg) from 1983 (premining) to 1990 (6 years post mining) showed generally that the Ok Tedi group changed from a profile strongly resembling the Melanesian controls to one much closer to the Australian European control group in Armidale. Given the extensive rapid Westernisation and current, almost total, dependence of the Ok Tedi population on processed food provided by the mining consortium, this transition is perhaps not too surprising.

For some time now the levels of different metals in hair have been thought to partly depend on the matrix proteins. This has lead to the idea that the matrix proteins of human hair belonging to different racial groups may absorb metals differentially. A central idea of this thesis is to explore possible relationships between the presence of metallic elements and the nature and disposition of matrix proteins in the different groupings studied. Relatively minor changes in hair proteins may affect the rate of heavy metal accumulation in hair and therefore impact significantly on group to group comparisons. The long term aim of the work of which this thesis is a part is to search for a possible relationship between hair heavy metal accumulation and hair proteins within and between species.

Hair samples from several hundred different individuals representing several human racial groupings (Australian European, Australian Aboriginals, Melanesians, Africans and Chinese) were analysed for keratins (major hair proteins) by one and two-dimensional polyacrylamide gel electrophoresis. The use of ^{14}C-iodoacetic acid to label S-carboxymethylatedkerateines allowed the assignment on autoradiographs of cysteine containing proteins. Polymorphic variation of hair proteins in the mid-range low cysteine kerateine zones was confirmed. In addition to these variations, further variations in high cysteine zones (molecular masses, 45-30 kDa) were also noted. Furthermore, homologous proteins from mammalian (both placental and marsupial) species were compared with respect to their molecular masses and cysteine content using laser densitometry of gels. The relative cysteine content of Coomasssie stained gels (as an estimate of proteins) were compared with autoradiographs (as an estimate of cysteine). These results may be quite useful forensically as an adjunct to fibre analysis since they yield more information per analysis than previous procedures using more laborious two-dimensional electrophoretic separation. Finally, a simple and rapid two-dimensional electrophoretic method for peptide mapping of human and animal hair and feather proteins has been developed. The protease digestion pattern was reproducible and characteristic of each sample.

Publications arising from this thesis

1. Khawar, S. L., Watson, K. and Jones, G. L. (1995) **High resolution one-dimensional electrophoretic separation and partial characterisation of human head hair proteins.** Electrophoresis, 16: 110-115.

2. Khawar, S. L., Watson, K. and Jones, G. L. **Changes in the hair heavy metal levels in populations near the Ok Tedi mine site, Papua New Guinea.** Poster presented at International Environmental Biometrics Conference Sydney, Australia. 14-15 December, 1992.

3. Khawar, S. L., Watson, K., Taufa, T., Lourie, J. A. and Jones, G. L. **Improved separation and partial characterisation of S-carboxymethylated human hair proteins.** Poster presented at 38th Annual Conference of Australian Society for Biochemistry and Molecular Biology, Gold Coast, Australia. 26-29 September, 1994.

4. Khawar, S. L., Watson, K. and Jones, G. L. **An electrophoretic comparison of hair and feather proteins from various species of mammals and birds.** Poster presented at 38th Annual Conference of Australian Society for Biochemistry and Molecular Biology, Gold Coast, Australia. 26-29 September, 1994.

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5. Khawar, S. L., Watson, K. and Jones, G. L. **A comparative electrophoretic analysis of mammalian hair and avian feather proteins.**

6. Khawar, S. L., Watson, K. and Jones, G. L. **Peptide mapping of S-carboxymethylated hair and feather proteins using two-dimensional electrophoresis.**

List of Tables

List of Figures

TABLE OF CONTENTS