Smoking habit and genetic factors associated with lung cancer in a population highly exposed to arsenic

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Abstract

In order to find some relationship between genetic differences in metabolic activation and detoxification of environmental carcinogens and host susceptibility to chemically induced cancers, we have investigated the distribution of the GSTM1 null genotype and CYP450 *1A1 MspI polymorphism in lung cancer patients and healthy volunteers of the second region in the north of Chile highly exposed to arsenic. The main sources of environmental arsenic exposure in Chile are copper smelting and drinking water, specially in the second region, the most important copper mining region in the world that shows the highest lung cancer mortality rate in the country (35/100.00). The population of Antofagasta, the main city of the region was exposed between 1958 and 1970 to arsenic concentrations in drinking water of 860 $\mu g/m^3$, presently declining to 40 $\mu g/m^3$. For men the MspI CYP1A1 *2A genotype was associated with a highly significant estimated relative lung cancer risk (O.R. = 2.60), but not GSTM1 by itself. The relative lung cancer risk for the combined 2A/null GSTM1 genotypes was 2.51, which increased with the smoking habits (O.R. = 2.98). In the second region the cancer mortality rate for As associated cancers, might be related at least part to differences in As biotransformation. In this work we demonstrate that genetic biomarkers such as CYP1A1 2A and GSTM1 polymorphisms in addition to DR70 as screening biomarkers might provide relevant information to identify individuals with higher risk for lung cancer, due to arsenic exposure.

Keywords: Arsenic; Polymorphism; Susceptibility biomarkers; Lung cancer

1. Introduction

Arsenic (As) is a recognized human carcinogen (Goering et al., 1999; Huff et al., 2000; Pott et al., 2001; Waalkes et al., 2003). The main sources of environmental As exposure in Chile are copper smelting and

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drinking water. In Chile the cancer mortality rate for all cancers is 118 per 100,000 inhabitants. The second region shows highly statistically differences of cancer mortality (rates/100,000 for 1990) for the As associated cancers: lung (46.3) (Chile: 15.5), bladder (9.6) (Chile: 2.2), kidney (8.5) (Chile: 3), skin (5.2) (Chile: 1.5), liver (4.8) (Chile: 2.2), and larynx (3.0) (Chile: 1.4) (Rivara et al., 1997). According to the Chilean Health Ministry the lung cancer mortality rate for 1996 was of 35/100,000, higher than the national lung cancer rate (13/100,000). The second region is the most important copper mining in the world, with Antofagasta as the greatest city in the region (Censo Nacional de Población y Vivienda, 2002). The main sources of drinking water in the region are the rivers which originate in the Andean mountains and that contain naturally high concentrations of As. As concentrations in drinking water of Antofagasta between 1958 and 1970 were 860 $\mu g/m^3$, declining presently to 40 $\mu g/m^3$ (Ferreccio et al., 2001; Smith et al., 1998). Smith et al. (1998) analyzed tobacco smoking (survey) data and cancer mortality rates (bladder, lung, kidney and skin) and concluded that smoking did not contribute to the increased mortality from these cancers and that As in drinking water was indeed a cause of the increased lung cancer rates in the second region. In 1999, the lung cancer mortality rate (both sex) in the region was 28.5/100.000, in the year 2000 was 34.4/100.00 and unofficial date indicates that in 2001 the rate increased to 36.5/100.00 (Goycolea M, Unidad de Profesiones Médicas y Paramédicas, Servicio de Salud de Antofagasta, personal communication, 2002). Usually, the incidence is higher in men than in women with a relationship of 3:1.

Assuming that As exposure in individuals of Antofagasta might be added to that of other compounds with carcinogenic potential such as PAHs for smokers, we may ask: what is the contribution of the genetic factors in the metabolism of these compounds and its incidence in lung cancer? First of all it is well recognized that inorganic As is methylated to monomethyl arsenic acid (MMA) and dimethyl arsenic acid (DMA) with a methyl group from *S*-adenosylmethionine, after to be reduced by endogenous thiols such as glutathione (GSH) or by As^V reductases (Kala et al., 2000; Radabaugh and Aposhian, 2000; Vahter, 2000; Zakharyan et al., 2001; Radabaugh et al., 2002). Genetic differences in metabolic activation and detoxification of environmental carcinogens may partially explain host susceptibility to chemically induced cancers (Daly et al., 1994; Kala et al., 2000; London et al., 2000). Chiou et al. (1997) have demonstrated that methylation of As in people exposed to arsenic in drinking water in Taiwan was associated with genetic polymorphism of glutathione-*S*-transferases GSTM1 and GSTT1. On the other hand, polycyclic aromatic hydrocarbons (PAHs) are present in incomplete combustion process including vehicles exhaust, chimney emissions, smoked foods, cigarette smoke and indoor (heating and cooking) systems (Adonis and Gil, 2000, 2001; Adonis et al., 2003a, 2003b; Gil et al., 2000, 2003). PAHs are metabolized to reactive DNA binding diols epoxides by phase I enzymes as cytochrome P450 1A1 (CYP1A1) and detoxified by phase II enzymes as GSTs, before reaching their target. We might wonder, what is the contribution of individual variations in metabolic activities of each or both phases, in regulating the clearance of DNA toxic metabolites and are at least partially related to the individual host susceptibility to PAHs? Several polymorphisms have been described in CYP1A1, however, 3′ noncoding region (Msp1, CYP1A1*2A) has been the most studied.

2. Materials and methods

2.1. Study population

In this work we investigated in healthy subjects (103) and in lung cancer patients (57), resident for at least 20 years in the city of Antofagasta, how the associated risk due to smoking and As exposure in drinking water is modified by the CYP1A1 Msp1 and GSTM1 as single genotypes or by both polymorphisms combined. Lung cancer patients and healthy volunteers were recruited from the city of Antofagasta in the Hospital de Antofagasta and in the Corporación Nacional del Cancer (CONAC).

All samples were obtained following informed written consent, previously approved by the ethics committee of the Faculty of Medicine of the University of Chile. Extent of tobacco smoke exposure was assessed by smoking index (SI) (cigarettes/day × 365), considering as smoker a person with a SI of 800. Both healthy volunteers and cancer patients were interviewed regarding their years of residence in the city,

smoking habits, alcohol drinking, use of oral contraceptives or hormones, incidence of past records of cancer in family members, occupational, outdoor and/or indoor carcinogenic pollutants exposure. Lung cancer patients were previously diagnosed histologically as well as radiologically in the Hospital Regional de Antofagasta. Additionally, DR70 immunoassay (with an 83.3% sensitivity for lung cancer) was used as screening test for lung cancer (manufacturer AMDL Inc., Tustion, CA, USA) (Ding et al., 1999), giving a 94% of positive cases, among the lung cancer patients. The DR70 levels of the lung cancer patients without treatment were (average: 10.41 μg/mL) significantly higher than those of the healthy group (1.77 ± 1.32 μg/mL), those of patients with benign tumours (average: 2.58 μg/mL) and lung cancer patients with chemotherapy, radiotherapy and surgery (1.74 ± 1.48 μg/mL).

2.2. Genotyping methods

Genomic DNA was submitted to separate amplification followed by digestion with appropriate restriction enzymes. PCR based restriction fragment length polymorphisms (RFLP) was used to examine the polymorphisms of interest. For the Msp1 site of CYP1A1, PCR amplification was carried out using primers C44 and C47 described by Hayashi et al. (1991) yielding a fragment of 340 pb. The MspI restriction enzymes were used for detection of T3801C base change present in CYP1A1*2A allele (m2). GSTM1 genetic polymorphism was determined simultaneously with MSP1 primers as an internal control of amplification (Ambrosone et al., 1995). GSTM1 null genotype was assessed by the absence of a 273 bp fragment, using agarose electrophoresis. The PCR products were subjected to restriction enzyme digestion at 37 °C for 1 h with Msp1 for CYP1A1 (GIBCO BRL, Life Technologies Inc., Daithersburg, MD, USA).

3. Results

Genotype frequencies for CYP1A1 and GSTM1 were consistent with the Hardy–Weinberg equilibrium model. In the healthy group, the CYP1A1 *2A allele frequency for MspI was 0.41, whereas for lung can-

Table 1
Genotype distribution and allele frequencies of CYP1A1 and GSTM1 in healthy controls and lung cancer patients stratified by smoking status

	N	Group		Genotype	Allele	Frequency
Smokers						
CYP1A1 (Msp1)			*1A/*1A	*1A/*2A	*2A/*2A	
				N (%)		
	50	Healthy control	24 (48.0)	16 (32)	10 (20)	*1A = 0.64; *2A = 0.36
	34	Lung cancer	10 (29.4)	17 (50)	7 (20.6)	*1A = 0.54; *2A = 0.46
*1A*2A + *2A*2A/*1A*1A: O.R. = 2.30 (95% CI = 0.95–5.26, $p = 0.0736$)						
GSTM1				GSTM1 (+)	GSTM1 (−)	
	50	Healthy control		34 (68)	16 (32)	(+) = 0.68; (−) = 0.32
	34	Lung cancer		21 (61.7)	13 (38.3)	(+) = 0.62 (−) = 0.38
Null GSTM1: O.R. = 1.31 (95% CI = 0.57–3.01, $p = 0.5575$)						
Nonsmokers						
CYP1A1 (Msp1)			*1A/*1A	*1A/*2A	*2A/*2A	
				N (%)		
	53	Healthy control	20 (36.5)	17 (32.6)	16 (30.7)	*1A = 0.54; *2A = 0.46
	21	Lung cancer	5 (21.7)	14 (65.2)	2 (13)	*1A = 0.55; *2A = 0.45
*1A*2A + *2A*2A/*1A*1A: O.R. = 1.93 (95% CI = 0.70–4.76, $p = 0.2576$)						
GSTM1				GSTM1 (+)	GSTM1 (−)	
	53	Healthy control	33 (62.2)		20 (37.7)	(+) = 0.62; (−) = 0.38
	21	Lung cancer	12 (57.1)		9 (42.9)	(+) = 0.57; (−) = 0.43
Null GSTM1: O.R. = 1.23 (95% CI = 0.47–3.27, $p = 0.6861$)						

*1A refers to the wild type, *1A/*2A heterozygous, and *2A/*2A homozygous; *N*: number of subjects.

cer group was 0.46. Nonstatically significant difference was observed between the healthy group and lung cancer group ($p = 0.437$, CI = −0.224 to 0.124). However, the CYP1A1 *2A genotype was associated with an increased relative lung cancer risk O.R. = 2.08 (95% CI = 1.04–4.03, $p = 0.04$). In addition, 35% of healthy group and 39% of the lung cancer group were homozygote for the null variant allele of GSTM1.

When the sample was stratified by gender, in the healthy and cancer groups no statically significant differences were observed between males and females in the genotype frequencies for the CYP1A1 *2A allele of CYP1A1 (healthy: 0.39 males, 0.44 females; cancer: 0.46 males, 0.47 females) and null allele of GSTM1 (healthy: 0.32 males, 0.32 females; cancer: 0.38 males, 0.41 females). However, for men the CYP1A1 *2A genotype was associated with a highly significant estimated relative lung cancer risk O.R. = 2.60 (95% CI = 1.07–5.94, $p = 0.0334$). The sample stratified by smoking habits showed no statically significant differences for smokers in the genotype CYP1A1 *2A for CYP450 and for the null GSTM1 (Table 1). In the healthy smokers group, the allele frequency for CYP1A1 *2A was 0.36, whereas in the lung cancer smokers group was 0.46 ($p = 0.2532$, CI 95% = −0.338 to 0.138). In the healthy nonsmokers group, the allele frequency for CYP1A1 *2A was 0.46, whereas in the lung cancer nonsmokers group was 0.45 ($p = 0.2532$, CI 95% = −0.338 to 0.138). A non-significant relative lung cancer risk was observed for either smokers or nonsmokers group for CYP1A1 allele CYP1A1 *2A and for GSTM1 null (Table 1).

However, the combined relative lung cancer risk analyses for the CYP1A1 *2A allele and null GSTM1 genotypes were significantly higher than the individuals' genotypes (Table 2). The relative lung cancer risk for the total sample with the CYP1A1 *2A/null GSTM1 genotype was 2.51 (O.R. = 2.51, CI = 1.07–5.40, $p = 0.0322$), which one increased when the sample was stratified by smoking habit (O.R. = 2.98, CI = 1.10–7.10, $p = 0.0497$).

Table 2
Risk analysis for combined CYP1A1 *2A/GSTM1 'null' genotypes and smoking habit for healthy voluntaries and lung cancer patients

Group	O.R.	CI (95%)	*p*-value
Total (smokers and nonsmokers)	2.51	1.07–5.40	0.0322
Smokers	2.98	1.10–7.10	0.0497

4. Discussion

Several studies suggest that methylation of As is an activation rather than a detoxification pathway. Thus, the role of the oxidation state of arsenic is determinant in the reactivity and the toxicity of this element (Moore et al., 1997; Styblo et al., 2000). Much of the potentially toxic compounds are taken up or removed from the cell by GSH-mediated pathway. Chiou et al. (1997) have demonstrated that methylation of As in people exposed to arsenic in drinking water in Taiwan was associated with genetic polymorphism of glutathione-*S*-transferases GSTM1 and GSTT1. Subjects having the GSTM1 null genotype have a significantly higher percentage of inorganic As in urine, while those with the null genotype of GSTT1 had an increased percentage of DMA in urine. The mechanism behind such association is not clear, besides that GSH is involved in the reduction of As^{V} to As^{III}. Since detoxification of electrophilic compounds by GSH requires GSTs activity in humans, a low level of GSTs and of its activity, might decrease the detoxification function of GSH. Thus, humans with null genotypes of GSTM1 and GSTT1 may have As methylation capability and body retention different from those with non-null genotypes.

We investigated the null genotype of GSTM1, in order to define some relationship between this genotype and lung cancer. The study shows non-statistical significant differences for the frequency of the GSTM1 null genotype between the healthy subjects and the lung cancer patients stratified by gender or smoking habit. Same results were observed for the MspI CYP P450 *1A1 polymorphism, even though the CYP1A1 *2A genotype represented an estimated relative lung cancer risk O.R. = 2.08 ($p = 0.04$).

However, the combined relative lung cancer risk analyses for the CYP1A1 *2A allele and null GSTM1 genotypes were significant higher than those associated with the individual genotypes. The relative lung cancer risk for the total sample with the 2A/null GSTM1 genotype was 2.51 (O.R. = 2.51, CI = 1.07–5.40, $p = 0.0322$), which one increased when additionally the sample was stratified by smoking habit (O.R. = 2.98, CI = 1.10–7.10, $p = 0.0497$). As most chemicals that initiate lung cancer require bioactivation in the lung to their 'ultimate' genotoxic metabolites that interact with DNA, the genetic differences observed in

this study in metabolic activation and detoxification of environmental carcinogens may partially explain host susceptibility to chemically induced cancers (Daly et al., 1994).

PAHs are metabolized to reactive DNA binding diols epoxides by phase I (CYP1A1) and detoxified by phase II enzymes (GSTs) before reaching their target. It is possible that individual variations in metabolic activities in each phase or in coordination of these two phases regulate the clearance of DNA toxic metabolites and might be partially responsible for individual host susceptibility to PAHs. Thus, someone exposed to compounds with carcinogenic potential as the PAHs in cigarette smoke and carrying the CYP1A1 *2A allele, might have high levels of DNA-binding species (diol epoxides PAHs) able to produce adducts and or mutations, additionally, if it is also expose to As and carry the null GSTM1 genotype, it might be expected an increase in the PAHs derivatives that are not conjugated with glutathione and might also affect the detoxification and clearance of arsenic at the cellular level.

In conclusion, our results suggest that the lung cancer people included in this study with a previous history of As exposure as the Antofagasta population, and with smoking habit might have an additional risk factor related with genetic susceptibility to lung cancer associated to the ethnicity. Individuals with the CYP1A1*2A or the combined CYP1A1*2A and GSTM1 null genotype might have a greater capacity to metabolically activate PAHs and lower to conjugate with glutathione and clearance of As, which may result in a higher risk of lung cancer or respiratory tract illness. Thus, our results are contradiction to those of Smith et al. (1998), which suggested that smoking habit do not play a role in arsenic risk to lung cancer in the population of the second region of Chile. According our results, the smoking habit has an important impact in the lung cancer mortality in the second region of Chile, specially in people with the CYP1A1*2A and GSTM1 null genotype.

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References

Adonis, M., Gil, L., 2000. Polycyclic aromatic hydrocarbons levels and mutagenicity of inhalable particulate matter in Santiago, Chile. Inhal. Toxicol. 12 (12), 1173–1183.

Adonis, M., Gil, L., 2001. Indoor air pollution in a zone of extreme poverty area of metropolitan Santiago. Indoor + Built Environ. 10, 138–146.

Adonis, M., Riquelme, R., Ríos, C., Rodríguez, L., Rodríguez, E., Martínez, V., Gil, L., 2003a. PAHs and mutagenicity of inhalable and respirable particulate matter from diesel exhaust in Santiago, Chile. Polycyclic Aromatic Compd. 23 (5), 495–514.

Adonis, M., Martínez, V., Riquelme, R., Ancic, P., González, G., Tapia, R., Castro, M., Lucas, D., Berthou, F., Gil, L., 2003b. Susceptibility and exposure biomarkers in people exposed to PAH-diesel exhaust. Toxicol. Lett. 144 (1), 3–15.

Ambrosone, C., Freudenheim, J., Oraham, S., Marshall, J., Vena, J., Brasure, J., Laughlin, R., Nemoto, T., Michalek, A., Harrington, A., Ford, T., Shields, P., 1995. Cytochrome P4501A1 and glutathione *S*-transferase (Ml) genetic polymorphisms and postmenopausal breast cancer risk. Cancer Res. 55, 3483–3485.

Chiou, H.Y., Hsueh, Y.M., Hsieh, L.L., Hsu, L.I., Hsu, Y.H., Hsieh, F.I., Wei, M.L., Chen, H.C., Yang, H.T., Leu, L.C., Chu, T.H., Chen-Wu, C., Yang, M.H., Chen, C.J., 1997. Arsenic methylation capacity, body retention, and null genotypes of glutathione *S*-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutat. Res. 386 (3), 197–207.

Daly, A.K., Cholerton, S., Armstrong, M., Idle, J.R., 1994. Genotyping for polymorphisms in xenobiotic metabolism as a predictor of disease susceptibility. Environ. Health Perspect. 102 (Suppl. 9), 55–61.

Ding, L., Ping, S., Jingmei, Y., 1999. Application of tumor marker of DR-70 in the diagnosis of malignant tumors. Chongqing Med. J. 28 (5).

Ferreccio, C., Gonzalez, C., Milosavjlevic, V., Marshall, G., Sancha, A.M., Smith, A.H., 2001. Lung cancer and arsenic concentrations in drinking water in Chile. Epidemiology 12 (2), 283.

Gil, L., King, L., Adonis, M., 2000. Trends of polycyclic aromatic hydrocarbons levels and mutagenicity in Santiago's inhalable airborne particles in the period 1992–1996. Inhal. Toxicol. 12 (12), 1185–1204.

Gil, L., Martínez, V., Riquelme, R., Ancic, P., González, G., Rodríguez, L., Adonis, M., 2003. Occupational and environmental levels of mutagenic PAHs and respirable particulate matter associated with diesel exhaust in Santiago. Chile J. Occup. Environ. Med. 45 (9), 984–992.

Goering, P.L., Aposhian, H.V., Mass, M.J., Cebrian, M., Beck, B.D., Waalkes, M.P., 1999. The enigma of arsenic carcinogenesis: role of metabolism. Toxicol. Sci. 49 (1), 5–14.

Hayashi, S., Watanabe, J., Nakachi, K., Kawajiri, K., 1991. PCR detection of an A/G polymorphism within exon 7 of the CYP1A1 gene. Nucl. Acid Res. 19, 4797.

Huff, J., Chan, P., Nyska, A., 2000. Is the human carcinogen arsenic carcinogenic to laboratory animals? Toxicol. Sci. 55 (1), 17–23.

Kala, S.V., Neely, M.W., Kala, G., Prater, C.I., Atwood, D.W., Rice, J.S., Lieberman, M.W., 2000. The MRP2/cMOAT transporter and arsenic-glutathione complex formation are required for biliary excretion of arsenic. J. Biol. Chem. 275 (43), 33404–33408.

London, S.J., Smart, J., Daly, A.K., 2000. Lung cancer risk in relation to genetic polymorphisms of microsomal epoxide hydrolase among African–Americans and Caucasians in Los Angeles county. Lung Cancer 28 (2), 147–155.

Moore, M.M., Harrington-Brock, K., Doerr, C.L., 1997. Relative genotoxic potency of arsenic and its methylated metabolites. Mutat. Res. 386 (3), 279–290.

Pott, W.A., Benjamin, S.A., Yang, R.S., 2001. Pharmacokinetics, metabolism, and carcinogenicity of arsenic. Rev. Environ. Contam. Toxicol. 169, 165–214.

Radabaugh, T.R., Aposhian, H.V., 2000. Enzymatic reduction of arsenic compounds in mammalian systems: reduction of arsenate to arsenite by human liver arsenate reductase. Chem. Res. Toxicol. 13 (1), 26–30.

Radabaugh, T.R., Sampayo-Reyes, A., Zakharyan, R.A., Aposhian, H.V., 2002. Arsenate reductase II purine nucleoside phosphorylase in the presence of dihydrolipoic acid is a route for reduction of arsenate to arsenite in mammalian systems. Chem. Res. Toxicol. 15 (5), 692–698.

Rivara, M.I., Cebrián, M., Corey, G., Hernandez, M., Romieu, I., 1997. Cancer risk in an arsenic contaminated area of Chile. Toxicol. Ind. Health 13 (2/3), 321–338.

Smith, A.H., Goycolea, M., Haque, R., Biggs, M.L., 1998. Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. Am. J. Epidemiol. 147 (7), 660–669.

Styblo, M., Del Razo, L.M., Vega, L., Germolec, D.R., LeCluyse, E.L., Hamilton, G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch. Toxicol. 74 (6), 289–299.

Vahter, M., 2000. Genetic polymorphism in the biotransformation of inorganic arsenic and its role in toxicity. Toxicol. Lett. 15 (112–113), 209–217.

Waalkes, M.P., Ward, J.M., Liu, J., Diwan, B.A., 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol. Appl. Pharmacol. 186 (1), 7–17.

Zakharyan, R.A., Sampayo-Reyes, A., Healy, S.M., Tsaprailis, G., Board, P.G., Liebler, D.C., Aposhian, H.V., 2001. Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-*S*-transferase superfamily. Chem. Res. Toxicol. 14 (8), 1051–1057.