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Purine nucleoside phosphorylase and the enzymatic antioxidant defense system in breast milk from women with different levels of arsenic exposure

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Abstract

Purine nucleoside phosphorylase (PNP) is an ubiquitous enzyme which plays an important role in arsenic (As) detoxification. As is a toxic metalloid present in air, soil and water; is abundant in the environment and is readily transferred along the trophic chain, being found even in human breast milk. Milk is the main nutrient source for the growth and development of neonates. Information on breast milk synthesis and its potential defense mechanism against As toxicity is scarce. In this study, PNP and antioxidant enzymes activities, as well as glutathione (GSH) and total arsenic (TAs) concentrations, were quantified in breast milk samples. PNP, superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) activities and GSH concentration were determined spectrophotometrically; TAs concentration ([TAs]) was measured by atomic absorption spectrometry. Data suggest an increase in PNP activity (median = 0.034 U mg protein^{-1}) in the presence of TAs (median = 1.16 g L^{-1}). To explain the possible association of PNP activity in breast milk with the activity of the antioxidant enzymes as well as with GSH and TAs concentrations, generalized linear models were built. In the adjusted model, GPx and GR activities showed a statistically significant (p<0.01) association with PNP activity. These results may suggest that PNP activity increases in the presence of TAs as part of the detoxification mechanism in breast milk.

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PURINA NUCLEÓSIDO FOSFORILASA Y EL SISTEMA DE DEFENSA ANTIOXIDANTE ENZIMÁTICO EN LECHE MATERNA DE MUJERES CON DIFERENTES NIVELES DE EXPOSICIÓN A ARSÉNICO

Resumen

Purina nucleósido fosforilasa (PNP) es una enzima ubicua que desempeña un papel importante en la desintoxicación del arsénico (As). As es un metaloide tóxico presente en el aire, el suelo y el agua; es abundante en el medio ambiente y se transfiere fácilmente a lo largo de la cadena trófica, encontrándose incluso en la leche materna humana. Información sobre la síntesis de la leche materna y su potencial mecanismo de defensa contra tóxicos es escasa. En este estudio, se cuantificó la actividad de PNP y de las enzimas antioxidantes así como la concentración de glutatión (GSH) y de arsénico total ([TAs]) en muestras de leche materna. La actividad de PNP, superóxido dismutasa (SOD), catalasa (CAT), glutatión S-transferasa (GST), glutatión peroxidasa (GPx), glutatión reductasa (GR) y la concentración de GSH se determinaron por espectrofotometría; la [TAs] se midió por espectrometría de absorción atómica. Los datos sugieren un incremento en la actividad de PNP (mediana= 0.034 U mg proteína^{-1}) con la presencia de TAs (mediana= 1.16 g L^{-1}). Para explicar la posible asociación de la actividad de las enzimas antioxidantes y la concentración de GSH, así como [TAs], con la actividad de PNP en la leche materna, se construyeron modelos lineales generalizados. En el modelo ajustado, la actividad de GPx y GR presentó una asociación estadística (p<0.01) con la actividad de PNP. Los resultados pueden sugerir que la actividad de PNP aumenta con la presencia de TAs como parte del mecanismo de desintoxicación en la leche materna.

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Palabras clave: *Arsénico. Estrés oxidativo. Leche maternal. Modelo lineal generalizado. Purina nucleósido fosforilasa.*

Abbreviations

As: Arsenic.
[TAs]: Total arsenic concentration.
As^{III}: Arsenite.
As^{V}: Arsenate.
CAT: Catalase.
GLM: Generalized linear model.
GPx: Glutathione peroxidase.
GR: Glutathione-disulfide reductase.
GSH: Glutathione.
GST: Glutathione S-transferase.
k: number of parameters in the model.
$\mu g\ L^{-1}$: Microgram per liter.
PNP: Purine nucleoside phosphorylase.
SH: Thiol group.
SOD: Superoxide dismutase.
U mg^{-1} prot: Units per milligram of protein.

Introduction

Breast milk has a unique composition, which makes it the ideal nutrient source for the growth and development of neonates. Milk synthesis is perhaps the most energetically costly component of parental investment for mothers. Our understanding of evolutionary and ecological variation in human milk synthesis is still limited[1]. Studies suggest that human milk can suppress oxidative damage in newborn infants more effectively than infant formula and that human milk contains a unique defense mechanism that is impossible to replace[2]. The antioxidant capacity of bioactive components, such as vitamins A, C and E, β-carotene, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), in breast milk are well known[2]. However, our understanding of how the interaction of these antioxidants *in vivo* contributes to detoxification of xenobiotics, particularly liposoluble compounds that can be mobilized to breast milk, is incomplete.

Arsenic (As) is a metalloid known to be toxic for humans and other organisms. As is classified as carcinogen (category A) by the US Environmental Protection Agency[3]. Its toxicity depends on its chemical form; inorganic As seems to be more toxic than methylated organic As, but recent reports show a different perspective[4-6]. Inorganic As includes arsenite (As^{III}) and arsenate (As^{V}) and can be methylated to form monomethylarsonic acid (MMA) or dimethylated to dimethylarsinic acid (DMA)[4,6] . In many studies, As exposure has been associated with increased risk to different types of cancer, including lung, skin, bladder and liver cancer[7]. Children with exposure to As *in utero* or during early life show chronic respiratory distress symptoms[8,9]. Humans can be exposed to As in several ways; drinking water is the most common source of exposure. Although, in some areas of the world, exposure levels can reach up to 200 μg As L^{-1}, reported total As concentration ([TAs]) in breast milk from exposed women was 2.3 $\mu g\ L^{-1}$ [10]. There is no consensus on safety limits for As in food and water; thresholds reported in the literature as safe limits for [TAs] in breast milk and drinking water range from 1 $\mu g\ L^{-1}$ to 25 $\mu g\ L^{-1}$[3,11,12].

Purine nucleoside phosphorylase (PNP) is a soluble enzyme localized in the cytosol of some cells and contains a thiol (SH) group[13]. PNP is an ubiquitous enzyme which plays an important role in the purine salvage pathway[14]. In rat liver and calf spleen, PNP reduces As^{V} to As^{III} [13,15]. The formation of As^{III} requires PNP and a SH group[16]. Various types of reactive oxygen species (ROS) are generated during As metabolism[17], and oxidative stress has been linked to the development of As-related diseases, including cancer[18]. The activity of glutathione S-transferase (GST), which participates in phase I and II of the detoxification process, seems to be involved in the As detoxification mechanism by conjugating it with glutathione (GSH)[19]. Some studies reported decreased GSH levels and GPx activity after As exposure in rat liver[6]. In addition, glutathione (GSH) and PNP seem to play a role in arsenolysis; it is suggested that As binds to certain proteins or is conjugated with GSH and related enzymes[16,20].

In a previous study, GST activity was determined in breast milk as part of the detoxification process against [TAs] and some other trace elements[21]. In that study, the mathematical model explained only 40% of the potential As detoxification mechanism[21]. Therefore, we hypothesized that PNP and GSH may play an important role in the arsenolysis in breast milk. The aims of this study were a) to measure PNP activity in breast milk; b) to estimate the possible association of PNP activity (in groups with low PNP activity, <0.003 U mg^{-1} protein, and high PNP activity, >0.003 U mg^{-1} protein) with SOD, CAT, GST, GPx, and glutathione reductase (GR) activities, as well as with GSH and TAs concentrations, and c) to analyze the potential role of PNP in As detoxification in breast milk.

Methods

Sampling

Sampling was performed between July and December 2011; 108 breast milk samples were collected for a study of xenobiotics in breast milk in Mexican women[22]. Concentration of trace elements, among them TAs, were measured in all breast milk samples. [TAs] in breast milk samples from 26 women were above the analysis detection limit (DL, 0.02 $\mu g\ L^{-1}$), used here as a cut-off point. From the rest of the samples (82) with [TAs] below 0.02 $\mu g\ L^{-1}$, another 26 samples were randomly drawn. A total of 52 samples were taken to measure and compare the activities of PNP, SOD, CAT, GST, GPx and GR, as well as GSH concentration.

Informed consent was obtained from all the donor women. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by an *ad-hoc* review panel of the Capítulo Baja California Sur de la Academia Nacional Mexicana de Bioética, A.C.

Total arsenic concentration

Breast milk samples were transferred into Teflon vessels and digested with 70% nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) in a microwave oven (Mars 5x, CEM, Matthew, NC, USA). [TAs] was quantified using a hydride system (HG 3000, GBC, Australia) coupled to an atomic absorption spectrophotometer (XplorAA, GBC, Braeside Australia)[23,24]. The minimum DL for [TAs] was 0.02 μg L^{-1}. Analyses were performed in duplicate and included appropriate blanks; calibration standards and certified material (GBW10017) of milk were included in each run, with ≥ 90% recovery.

Purine nucleoside phosphorylase (PNP, EC. 2.4.2.1) activity

PNP activity was quantified in breast milk by using a colorimetric assay as described by Chu et al. [25]. Briefly, each sample was incubated with potassium phosphate buffer (22 mM, pH 7.5) containing xanthine oxidase (XO, 167 U), horseradish peroxidase (2000 U), 4-aminoantipyrine (160 mM), potassium ferrocyanide (120 μM), 3,5 dichloro-2- hydroxybenzenesulfonic acid (8 mM), and inosine (12 mM). Production of N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzoquinone-monoimine was followed at 520 nm in a spectrophotometer (Beckman Coulter DU 800, Fullerton, CA, USA) and the change in absorbance was recorded at 165 nm every 5 s for 180 s. Results are expressed as units (U) mg^{-1} protein. One unit of PNP activity is defined as the amount of enzyme necessary to deplete 1 μM of inosine per minute at 25°C[26]. The assay DL for PNP activity was 0.003 U mg^{-1} protein.

Antioxidant enzyme activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity

SOD activity was measured following the method described by Suzuki (2000). In a cuvette, working solution (sodium carbonate 50 mM; xanthine 0.1 mM; nitro blue tetrazolium (NBT) 0.025 mM; ethylenediaminetetraacetic acid (EDTA) 0.1 mM), XO (1 U mL^{-1} in 2 M ammonium sulfate), and the breast milk sample were mixed. The change in absorbance was registered every 30 s during 5 min at 560 nm. Data were expressed in units of SOD mg^{-1} of protein[27]. One unit of SOD activity is defined as the amount of enzyme required to inhibit 50% of the maximum reaction of the superoxide radical (O_2•-) with NBT.

Glutathione S-transferase (GST, EC 2.5.1.18) activity

GST activity was determined by measuring the change in absorbance caused by the synthesis of thioether glutathione dinitrobenzene complex as a product of the reaction between GSH and 1-chloro-2,4-dinitrobenzene (CDNB)[28]. Working solution (0.1 M phosphate buffer, 10 mM GSH, 60 mM EDTA), CDNB (10 mM) and sample were mixed in a cuvette. Change in absorbance was measured every 30 s during 6 min at 340 nm. Enzyme activity was expressed in units mg^{-1} of protein. One unit of GST activity is defined as the amount of enzyme that catalyzes the production of 1 μmol of CDNB per min.

Glutathione peroxidase (GPx, EC 1.11.1.9) activity

To determine GPx activity, the continuous decrease in reduced nicotinamide adenine dinucleotide phosphate (NADPH) concentration was measured while GSH levels were maintained following the method of Flohé and Günzler (1984). Phosphate buffer solution (100 mM), EDTA (50 mM), sodium azide (20 mM), GR (15 U mL^{-1}), NADPH (1.5 mM), GSH (250 mM), sample and H_2O_2 (10 mM) were mixed in a cuvette and the change in absorbance at 340 nm was recorded every 3 s during 40 s. Enzyme activity was expressed in milliunits of GPx mg^{-1} of protein. One unit of GPx activity is defined as the amount of enzyme necessary to oxidize 1 μmol of NADPH per min[29].

Glutathione-disulfide reductase (GR, EC 1.8.1.7) activity

Catalytic activity of GR was measured following the decrease in absorbance during NADPH oxidation[30,31]. All solutions were maintained in an ice-cold bath except phosphate buffer (500 mM, pH 7.2). In a cuvette, phosphate buffer, EDTA (50 mM), NADPH (2 mM), sample and oxidized glutathione (GSSG; 10 mM) were mixed. Change in absorbance was recorded at 340 nm for 60 s. Enzyme activity was expressed in milliunits of GR mg^{-1} of protein. One unit of GR is defined as the amount of enzyme needed to reduce 1 μmol of GSSG to GSH per min.

Catalase (CAT, EC 1.11.1.6) activity

The methodology proposed by Aebi (1984), as described by Vázquez-Medina et al. (2006) was followed in order to analyze CAT activity in breast milk sam-

ples. The decay in the concentration of H_2O_2 (20 mM in phosphate buffer, 0.1 M) was followed in a spectrophotometer; the change in absorbance per minute at 240 nm was recorded. CAT activity is expressed in units of mg^{-1} protein. One unit of CAT activity is defined as the amount of enzyme required to reduce 1 μmol of H_2O_2 per minute[32,33].

Glutathione concentration

Samples were homogenized 1:10 w/v in ice-cold 5% sulfosalicylic acid (previously degassed by 10 min bubbling with nitrogen gas), bubbled with nitrogen gas for 10 s, and centrifuged at 17,005 × *g* at 4°C for 5 min. Supernatants were immediately used to measure total glutathione levels (GSH-Eq = GSH + 2GSSG). GSH-Eq were determined by following the rate of reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) at 412 nm in the presence of GR in 125 mM potassium phosphate buffer supplemented with 6 mM EDTA. Briefly, samples, 0.21 mM NADPH, 600 μM DTNB, and GR (0.5 U mL^{-1}) were mixed in a cuvette. Absorbance was read every minute at 412 nm. GSH-Eq concentration was calculated using a standard curve (GSH, 0–8 μM) [34, 35]. The results were expressed in nmol of equivalents of glutathione (GSH-Eq) per milligram of protein.

Total soluble protein content

To standardize the data, the amount of total soluble proteins in each sample was determined using the method described by Bradford (1976), using a commercial kit (Bio-Rad Laboratories, Hercules, CA, USA). The samples were diluted 1:100 with phosphate buffer (50 mM, pH 7.5, 1 mM EDTA). Absorbance was read at 590 nm in a semiautomatic analyzer (Microlab 300, Vital Scientific, Netherlands). The results are expressed in mg of protein mL^{-1} [27,36].

Statistical analyses

Descriptive statistics were calculated, including medians, 10th and 90th percentiles. The value DL/2 was used for statistical analyses in those cases where PNP and [TAs] values were below the DL analysis[37]. Activities of PNP, SOD, GST, GPx, GR, CAT and GSH concentration, as well as [TAs], were not normally distributed (Kolmogorov-Smirnov $p<0.05$). Therefore, non-parametric (Mann-Whitney) tests were applied. PNP activity was set as a categorical variable and data were grouped accordingly (low PNP activity, < 0.003 U mg^{-1} protein, n=30; high PNP activity, > 0.003 U mg^{-1} protein, n=22; with values of 0 and 1, respectively).

Multivariate analyses were performed using generalized linear models (GLM) considering a *Gamma* distribution *error* to explain the PNP activity (response variable) measured in the breast milk using a *log* canonical link function[38,39]. The *Gamma* distribution is helpful as an alternative to the *Gaussian* distribution *error* for continuous data[21,39,40]. The explanatory variables considered in building the models were SOD, GST, GPx, GR, CAT activities, GSH concentration and [TAs]. The simplification and selection of the minimal adequate model was performed starting with the maximal model using all the variables of interest (*k=7; k=number of parameters*). To choose the minimal adequate model, the backwards procedure was applied, evaluating all the alternative models by testing the contribution of each variable in turn ($p \leq 0.05$) and the change of the residual deviance in each step[21,41,42]. The distribution of residuals of the minimal adequate fitted model was evaluated as a diagnostic method and for model validation[39].

Results

Data grouped by purine nucleoside phosphorylase (PNP) activity level

In 30 of the 52 (58%) analyzed breast milk samples, PNP activity levels were <0.003 U mg^{-1} protein. Data for SOD, CAT, GST, GPx and GR activities, GSH concentration and [TAs], grouped according to PNP activity, are summarized in Table I. SOD and GPx activities were lower (85.7% and 80.0%, respectively $p<0.01$) in the group with PNP activity >0.003 U mg^{-1} protein than in the group with PNP activity <0.003 U mg^{-1} protein. GST, GR and CAT activities were 50.0%, 50.0% and 44.3% lower, respectively, in the group with PNP activity >0.003 U mg^{-1} protein than in the group with PNP activity <0.003 U mg^{-1} protein ($p>0.05$). GSH levels were 2.1% higher in the PNP activity >0.003 U mg^{-1} protein than in the PNP activity <0.003 U mg^{-1} protein group ($p>0.05$). [TAs] was 11500% higher in the group with PNP activity >0.003 U mg^{-1} protein than in the group with PNP activity <0.003 U mg^{-1} protein; however, this difference was not statistically significant ($p=0.32$).

Association between PNP activity and the covariables

The GLM contributes to explain the variability of PNP activity in breast milk in association with [TAs], the antioxidant enzyme activities, and GSH concentration. The analysis started with the maximal model with *k=7* (*k*= number of parameters), PNP activity (intercept), activities of SOD, GST, GPx, GR and CAT, GSH concentration and [TAs] (β=-3.302 *Std. Error* = 0.4703, $p<0.01$, *scale* 1.767, residual deviance = 114.560), where 6 of the 7 parameters were not significant. Simplification of the initial model was achieved by using

Table I
Median purine nucleoside phosphorylase activity (PNP, U mg^{-1} protein), the enzymatic antioxidant defense system, and total arsenic concentration in breast milk of women (n=52) inhabiting Baja California Sur, Mexico

Variable	*PNP activity <0.003 (n=30)*	*P10*	*P90*	** PNP activity >0.003 (n=22)*	*P10*	*P90*	*Median difference %*	***p*
PNP	nd	nd	nd	0.034	0.007	0.101	-	-
SOD	590.83	37.65	1560.56	84.26	11.39	1163.37	-85.74	0.01
GSH	22.68	8.29	49.63	23.17	8.29	53.83	2.13	0.71
GST	0.004	0.0	0.032	0.002	0.0	0.029	-50.00	0.20
GPx	0.1	0.02	1.8	0.02	0.0	0.69	-80.00	0.01
GR	0.02	0.0	0.14	0.01	0.0	0.06	-50.00	0.08
CAT	1.05	0.14	38.72	0.58	0.02	34.01	-44.29	0.22
[TAs]	0.01	0.01	6.27	1.16	0.01	10.49	11500	0.32

Data are shown as median. P10-P90, Percentile 10th and 90th;*compared group; **statistical significance by Mann-Whitney Test; PNP, purine nucleoside phosphorylase activity U mg^{-1} protein; nd, non-detectable; SOD, superoxide dismutase activity, U mg^{-1} protein; GSH, glutathione concentration, nmol mg^{-1} protein; GST, glutathione S-transferase activity, U mg^{-1} protein; GPx, glutathione peroxidase activity, mU mg^{-1} protein; GR, glutathione reductase activity, mU mg^{-1} protein; CAT, catalase activity, U mg^{-1} protein; [TAs], total arsenic concentration, μg L^{-1}.

the backward procedure and evaluating the alternative models by testing the contribution of each variable in turn ($p<0.05$) as well as the change of the deviance at each step[43]. The minimal adequate model, shown in Table II, included $k=2$, PNP activity (intercept), GR and GPx activities (β=-3.621, *Std. Error*= 0.235, $p<0.01$, *scale* 1.963, residual deviance =129.262). This model adjusted PNP activity from the measured value of 0.0015 U mg^{-1} protein to the fitted values of 0.0196 U mg^{-1} protein. The equation for the minimal-fitted model was generated in terms of the explanatory variables with significant contribution to PNP activity (Table III). The residuals of the minimal selected model showed homoscedasticity in the distribution, suggesting constant variance as expected for a fitted model (Fig. 1).

Discussion

To our knowledge, this is the first report of PNP activity in human breast milk. A specific search for similar publications was performed on January 4th, 2015 in Ovid (all available databases), Direct Science and Springer Link using the following keywords: milk or breast milk and purine nucleoside phosphorylase. The search yielded zero publications.

The PNP activity in human breast milk samples (mean= 0.034 U mg^{-1} protein, this study) was lower than that in red blood cells (mean=18.21 U mg^{-1} protein) and higher than the activity in plasma that was below detection limits[26]. Therefore, the activity of PNP, as for most enzymes, appears to be tissue specific.

Table II
Coefficients fitted by the generalized liner model (GLM) (k=2) with Gamma error distribution for purine nucleoside phosphorylase activity (PNP) activity (U mg^{-1} protein) in breast milk of women (n=52) inhabiting Baja California Sur, Mexico

Model	*Parameter*	*B*	*Std. Error*	*95% Wald Confidence Interval*		*Hypothesis Test*		
				Lower	*Upper*	*Wald Chi-Square*	*df*	*Sig.*
PNP	Intercept	-3.621	0.235	-4.080	-3.161	238.007	1	<0.01
	GR	-1.150	0.115	-1.376	-0.924	99.357	1	<0.01
	GPx	-0.270	0.046	-0.360	-0.180	34.628	1	<0.01
	(Scale)	1.963	0.318	1.428	2.697			

Std. Error: Standard error.
df: Degrees of freedom.
Sig: Significance.
PNP: Purine nucleoside phosphorylase activity, U mg^{-1} protein.
GPx: Glutathione peroxidase activity, mU mg^{-1} protein.
GR: Glutathione reductase, activity mU mg^{-1} protein.

Table III
*Fitted models for purine nucleoside phosphorylase (PNP), glutathione peroxidase (GPx), and glutathione reductase (GR) activity in breast milk of women (*n=52*) inhabiting Baja California Sur, Mexico.*

Variable	*Model*	*Median PNP measured*	*Median PNP fitted model*
PNP	$PNP = e^{-3.621+1.150(GR)+0.270(GPX)}$	0.0015	0.0196

PNP: Purine nucleoside phosphorylase activity, U mg^{-1} protein.
GPx: Glutathione peroxidase activity, mU mg^{-1} protein.
GR: Glutathione reductase activity, mU mg^{-1} protein.

[TAs] in breast milk appeared to be associated with PNP activity (Table I). Most of the As excreted in breast milk is in the trivalent inorganic form[44]. It is possible that PNP contributes to the transformation of As^{V} to As^{III} in breast milk, as was previously suggested to occur in rat liver and calf spleen[13,15]. Two pathways have been proposed for the metabolism of As in humans. 1) The classical pathway suggests that once As^{V} enters the cells via a phosphate transporter, it undergoes sequential reduction and oxidative methylation; in human liver PNP reduces As^{V} to As^{III} [18]. Another pathway suggests that As either binds to certain proteins or conjugates with GSH and related enzymes, such as GST, GPx and GR, with subsequent methylation resulting in two end products, MMA and DMA. Methylated As is usually viewed as less toxic than inorganic As[5,6] and, thus, the methylation of inorganic As was considered to be a detoxification process. However, recent studies have provided evidence that human cells are more sensitive to the cytotoxic effects of MMA than of As^{III} [6,20]. This could be the reason for most of the excreted As in breast milk being in the trivalent form[44].

PNP utilizes SH groups to reduce As^{V} to As^{III}; an elevated demand for the SH groups in GSH, therefore decreasing GSH concentration, could contribute to explain the lower GPx and GR activities in the group with PNP activity >0.003 U mg^{-1} protein, as shown in the proposed model (Table II, this study). This would suggest that GPx and GR activities catalyze the NAD(P)H-dependent reduction of GSSH to GSH, which contributes to maintaining adequate levels of the GSH pool and, thus, the redox status in breast milk. Keeping the SH groups in the milk proteins in their reduced form contributes to reducing the potential toxic effects of As[20]. Another explanation in the reduction of SH groups can be for the formation of As^{III}, this process requires both PNP and SH group, the formation of hypoxanthine from inosine by PNP involving phosphorolysis or arsenolysis. The formation of As^{III} during or following arsenolysis, reaction catalyzed by PNP, could be the result of thiol-involved hydrolysis of ribose 1-arsenate. A thiol and enzyme-assisted hydrolysis of ribose 1-arsenate could also occur prior to release from the catalytic site. The hydrolysis of ribose 1-arsenate is believed to occur by hydrolytic attack on As^{V}, resulting in the O-As bond being lost[16].

The bivariate analysis suggested lower SOD activity (86%, *p<0.01*) in the group with PNP activity >0.003 U mg^{-1} protein than in the group with PNP activity <0.003 U mg^{-1} protein (Table I, this study). This could be related to As-mediated formation of superoxide radical ($O_2^{\bullet-}$) and SOD activity contributing to protect cells from ROS-induced oxidative injury[6,45]. The mechanism responsible of ROS production is still not clear, but the formation of intermediary arsine species has been proposed[6]. CAT activity does not appear to affect PNP activity in its role in As detoxification in breast milk. However, CAT activity has been reported to be tissue specific; in studies of the effect of As^{V}, CAT activity was found in rat kidney cortex but not in the medulla[46].

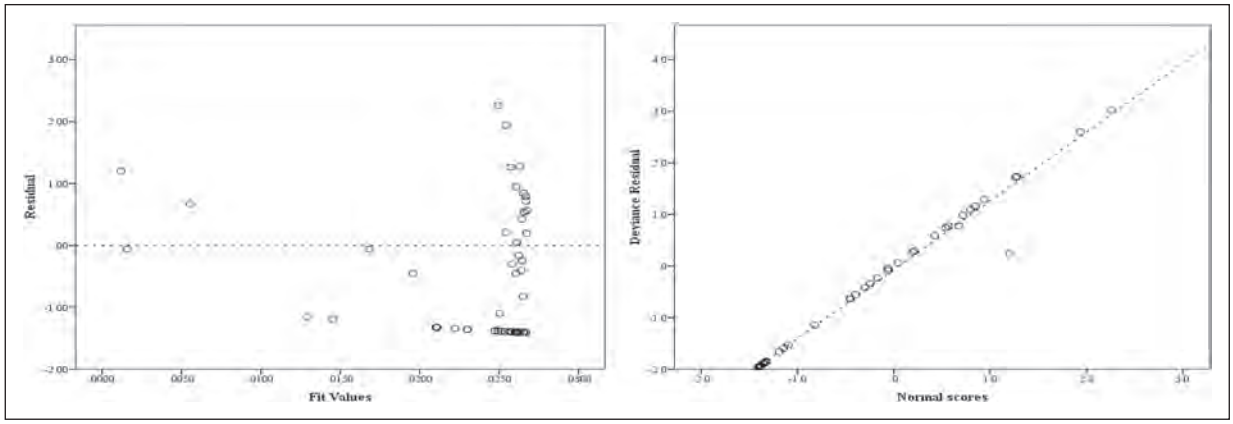

*Fig. 1.—Residual plots of the minimal adequate model for (*k=2*) purine nucleoside phosphorylase activity (U mg^{-1} protein) in breast milk of women (n=52) inhabiting Baja California Sur, Mexico.*

The main weakness in the present study is that only [TAs] was quantified; thus, the amount of the different As species (organic or inorganic; As^{V}, As^{III}, MMA or DMA) in breast milk samples is unknown. The results from this study suggest that PNP activity increases with [TAs], probably as part of an As detoxification mechanism in breast milk. Our proposed model explains 12% of PNP activity, the possible explanation is that we measured the [TAs] and not the concentrations of the specific forms of As^{V} that could contribute to PNP activity.

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