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Glutathione S-Transferase in Normal Human Anagen Hair Follicles

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Glutathione S-transferase (GST) has been quantified and characterized in healthy human anagen hair follicles obtained from 36 men and 36 women (26 ± 7 years of age). GST activity was determined using 1-chloro-2,4-dinitrobenzene as a substrate, and the values in men were: 0.5 ± 0.2 mU/follicle, significantly different from women (0.36 ± 0.2 mU/follicle); 196 ± 98 mU/mg protein and 309 ± 158 mU/mg DNA without significant differences from women. Enzyme activity showed a high degree of inter-individual variability (23.5-fold when expressed per follicle, 18.29-fold expressed per mg of protein and 22.75-fold per mg of DNA) in the whole population and this variability was higher in women. Ion-exchange chromatography by KCl and enzyme immunoassay suggest that the GST present in hair follicles corresponds with the acidic form. The percentage of anagen hairs in each subject showed a positive correlation with the following parameters: GST/hair, GST/DNA and DNA/hair. It is concluded that GST may contribute to the maintenance of the hair growth cycle.

Key words: Glutathione-S-Transferase, Hair follicles.

Human epithelial hair-follicle cells have been used to evaluate biochemical parameters and the metabolism of carcinogens in order to assess individual susceptibility to several xenobiotic compounds with carcinogenic properties (21, 22). This is especially relevant in epithelial tumours since 80 % of all human tumours are epithelial in origin. Hair follicles have also been used as biopsy material to detect a number of genetic diseases such as:

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Fabry's disease (7), the Lesch-Nyhan syndrome (5) and Glucose-6-phosphate dehydrogenase deficiency (19). Many degenerative processes appear as a consequence of the activation of pre-electrophilic compounds through oxidative pathways as, for instance, in the activation of benzo(a)pyrene by the aryl hydrocarbon hydroxylase system (3). Fortunately detoxifying systems are also present in our tissues, which are able to neutralize a number of electrophilic compounds, thus facilitating their elimination.

One of these systems is the glutathione S-transferase (GST, EC 2.5.1.18), which facilitates the conjugation of glutathione (GSH) with the electrophilic center of a large variety of hydrophobic molecules (8, 10). Hence its protective function is of utmost importance. Multiple forms of this enzyme have been reported in a number of rat and human tissues (9, 16). According to their electrical, structural and immunologic properties, human GST isoenzymes can be grouped into at least three distinct classes (15), each one showing specific characteristic affinities for different electrophilic compounds: the α class (basic) presents high activity against organic peroxides, the μ class (neutral) neutralizes epoxides and the π class (acidic) is active against lipid hydroperoxides and some carcinogens (13, 15). Because the net balance between activating and detoxifying systems determines the real risk of suffering the consequences of electrophilic compounds, a lack of expression of some GST forms may diminish the detoxication ability of living cells.

The acidic transferases are the predominant forms in most extrahepatic tissues, including skin (6) but very little is known about the presence and characteristics of GST in human hair follicles. In our opinion using this specimen as a source of information concerning the variability in the individual expression of this detoxifying system can be worthwhile. The main scope of the present study was to quantitate GST acitivty in the human hair follicles in the anagen phase. A preliminary attempt was also made to characterize the different isoforms that may be present in this specimen.

Materials and Methods

Samples.— Hairs were collected from 72 healthy volunteers (36 men and 36 women), whose age ranged from 19 to 45 years (mean value 26±7 for all the subjects). None of them reported disturbances of hair growth. After plucking, hair follicles were examined with a stereoscopic microscope and the percentage of anagen hairs was calculated.

The keratinized distal portion of the hairs were discarded and the proximal hair roots coming from twenty anagen follicles were homogenated in a glass-glass Potter homogenizer, cooled with crushed ice, with 3 ml 0.1 M sodium phosphate buffer (pH 6.25). The soluble tissue extract (STE) was obtained by centrifugation at 100,000 x *g* for one hour. When samples were not used immediately they were stored at –80 °C.

Glutathione S-transferase (GST) assay.— GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma) as substrate, according to the method described by HABIG *et al.* (9). One hundred µl of 25 mM GSH were added to 1.8 ml of STE. After incubation for 5 min at 25 °C, the reaction was started by the addition of 100 µl of 25 mM CDNB dissolved in ethanol and monitored spectrophotometrically by the increase in absorbance at 340 nm and 25 °C in a thermostatted Perkin Elmer Lambda-2 spectrophotometer. Automatic correction for the non-enzymatic reaction was made in the absence of STE. Enzyme activity was expressed in mU (one Unit = 1 µmol of CDNB conjugated in one minute).

Protein was determined by the method of LOWRY *et al.* (14) using bovine serum albumin (Merck) as standard. The assay of DNA was performed in the homogenate according to the method described by KAPUSCINSKI and SKOCZYLAS (11) using calf thymus DNA (Sigma) as standard. Briefly, 100 µl of homogenate was solubilized with 50 µl of a 1 mg/ml solution of pronase (Sigma) and diluted with 850 µl of water. After incubation for 30 min at 37 °C, 1.4 ml of 10 mM Tris, 100 mM NaCl and 10 mM EDTA buffer (pH 7) were added and the mixture was transferred to a quartz cuvette. Finally, 600 µl of a 0.28 µM fluorescent reactive DAPI (4',6-diamidino-2-phenylindole, Sigma) was added, and the DNA-DAPI complex was measured at an excitation wavelength of 360 nm and an emission wavelength of 458 nm in a Perkin Elmer LS 50 luminescence spectrometer.

Ion-exchange chromatography.— One hundred hair follicles were homogenated and centrifuged as described above in 3 ml of 10 mM Tris-HCl, 1 mM GSH, pH 7.8 buffer. STE was added into a 1 x 10 cm glass-column, filled with DEAE-Sephacel (Pharmacia) and equilibrated with the homogenizing buffer. Fractions (0.7 ml) were collected from the moment of introduction of the sample into the column, and after the addition of a continuous gradient of KCl (0-300 mM) with the same Tris-HCl buffer. GST activity was measured in each fraction, as described above, in a COBAS MIRA (Roche) Autoanalyzer with the appropriate volume reductions.

Enzyme immunoassay.— Primary sheep anti-human GST π polyclonal antibody was used to start an ELISA assay, carried out in Falcon micro-titer plates (Becton Dickinson Co. Oxnard, California) (1) using goat biotinylated secondary anti-sheep antibody and ExtrAvidin-Peroxidase complex (Sigma). The peroxidase reaction was performed with an o-phenylene-diamine (Roche) (0.1 %) H_2O_2 (0.01 %) solution in 0.1 M citrate buffer, pH 4.5. A Whittaker Microplate Reader 2001 (Anthos Labtec Instruments, Salzburg, Austria) was used at 550 nm. The immunoasay was performed in crude STE and in chromato-eluted samples.

Statistical analysis.— Normality test, comparison between mean values and regression analysis were performed by the MICROSTAT Program (Ecosoft, Inc., Indianapolis, USA).

Results

Table I shows the values obtained for GST activity, protein and DNA content, comparing men and women. The percentage of anagen hairs was significantly lower in women than in men. In addition, GST activity per hair and protein and

Table I. *Biochemical parameters and degrees of variability (D.V.) for GST activity, protein and DNA content in freshly plucked hair roots.*
For differences between men and women p values *< 0.01; **< 0.05; ***< 0.001. N.S. not significant.

	Men	D.V.	Women	D.V.
GST (mU/follicle)	0.5 ± 0.2*	12.6	0.36 ± 0.2	21.1
Protein (µg/follicle)	2.79 ± 1.22**	7.5	2.23 ± 0.93	8.3
DNA (µg/follicle)	1.66 ± 0.31***	2.0	1.40 ± 0.33	2.8
GST (mU/mg prot.)	196.18 ± 98.14 N.S.	13.1	172.79 ± 81	15.0
GST (mU/mg DNA)	309.21 ± 158.29 N.S.	9.7	257.19 ± 129.3	18.9
Anagen hairs (%)	73.9 ± 21.8**	–	65.6 ± 19.1	–

DNA content per hair were also significantly lower in women. If GST activity was expressed by protein or DNA, the results were similar in both sexes and no significant differences have been appreciated.

Figure 1 depicts the differences between sexes for GST activity, protein and DNA content when expressed per hair. All these parameters followed a normal distribution pattern and the interindividual variability was different for each parameter (table I). Thus the greatest variability was observed for GST activity whereas the lowest was calculated for DNA. In all cases, this variability was higher in women than in men.

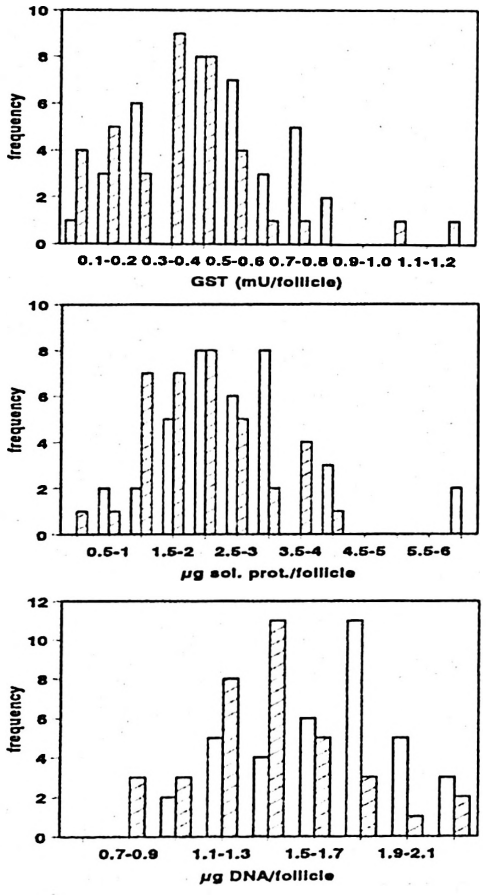

Fig. 1. *Comparison between sexes in freshly plucked hair follicles for GST activity, soluble protein and DNA content.*
Filled bars: men; dashed bars: women.

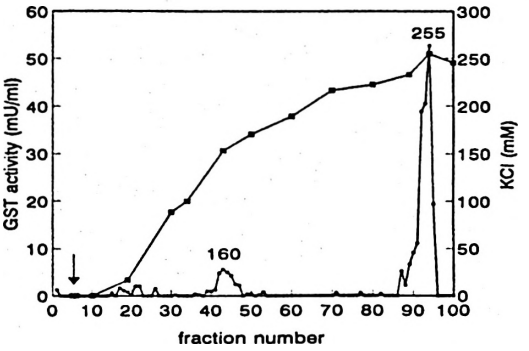

Fig. 2. *Elution profile of soluble GST obtained from 100 hair follicles. Ion Exchange Chromatography in DEAE-Sephacel.*
■: GST activity; □: KCl molarity. The arrow indicates the beginning of the continuous KCl gradient (0-300 mM). Numbers in eluted peaks indicate the molarity of KCl. Fractions of 0.7 ml.

Correlations between GST activity and other parameters, expressed "per hair" were as follows: protein ($r=0.39$, $p<0.001$), DNA ($r=0.29$, $p<0.05$) and percentage of anagen hairs (0.30, $p<0.01$). The percentage of anagen hairs also correlated positively with: DNA/hair ($r=0.28$; $p < 0.05$) and GST/DNA ($r=0.24$; $p < 0.05$). Differences in GST activity in relation to age were not significant.

Chromatographic analysis and immunoassay were performed in five subjects (2 women and 3 men). No GST activity was present in the eluted fractions before the addition of KCl gradient (fig 2). The greatest acitivity eluted at high concentrations of KCl, whereas a small activity (5-10 %) was eluted at low KCl concentrations.

Enzyme immunoassay performed with these fractions and in the STE samples of the same donors gave positive reactivity to the anti-π antibody at 1/10,000 dilution.

Discussion

Very few data are available about GST activity in human hair follicles. In cultured human skin keratinocytes, BLACKER *et al.* (2) demonstrated the existence of only the π class of isoenzyme, with increasing activity during the differentiation process. PRUCHE *et al.* (17) evaluated the activity of GSH-related enzymes in hair follicles from a reduced group of subjects, concluding that GST activity diminished in aged (> 80 years) individuals.

The results reported here demonstrate that the differences observed between sexes, in GST activity per hair, were only due to the smaller amount of protein and DNA per hair in women, since these differences disappeared when enzyme activity was expressed as a function of protein or DNA content. Moreover, this activity correlated significantly, though not to a great extent, with protein and DNA content in each subject.

Although the major form of GST found in skin keratinocytes is the π class (2), small 8 amounts of the α class have also been reported in human dermis and epidermis (6). The present chromatographic and immunoassay analyses must be regarded as preliminary, although it would appear from these results that only a major acidic GST isoform is present in hair follicles. If basic GST forms were also present, they should have not been retained in the column and should have eluted before starting the KCl gradient. The minor fraction, which eluted at low KCl concentration, may correspond with a "near neutral" GST isoform, but this conclusion is doubtful since it reacted positively with the anti-π antibody. All these aspects need to be confirmed in the future by using more specific procedures and in a greater number of subjects.

KERMICHIETAL *et al.* (12) demonstrated a high detoxifying activity associated with the hair follicle, and a decrease in GSH content with ageing has been observed in many tissues and organs (17). The involvement of free radicals in the ageing process (18) may critically affect the life span of the tissues (4). Assuming the protective role of the GST system, those subjects having a low activity will be at greater risk to the deleterious effect of electrophilic substances. In this sense, it is important to emphasize the large inter-individual variations in enzyme activity (more than 20 fold in the whole population) presented here. In normal human urinary bladder a dispersion factor of 17 was previously found (13) whereas STRANGE *et al.* (20) reported a variability of six fold in human erythrocytes.

Whether the GST/GSH system is involved in the maintenance of the hair-growth cycle remains to be elucidated, but if the positive correlation between the percentage of anagen hairs, GST activity and DNA content is considered, it may be assumed that low expression of the GST/GSH system can contribute to the impairment of hair growth, especially when electrophilic substances are present in excess.

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A. M. PUERTO, M. R. NOGUÉS, I. CERVELLÓ, M. GIRALT, F. ORTÍN y J. MALLOL. *Glutation S-transferasa en folículos pilosos humanos anágenos sanos.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (2), 103-108, 1994.

Se determina la actividad glutation S-transferasa (GST) en folículos pilosos anágenos sanos de 36 varones y 36 mujeres (edad 26 ± 7 años), usando 1-cloro-2,4-dinitrobenceno como substrato. Los valores en varones fue-

ron: 0,5 ± 0,2 mU/folículo, significativamente diferente respecto a mujeres (0,36 ± 0,2 mU/folículo); 196 ± 98 mU/mg proteína y 309 ± 158 mU/mg DNA sin diferencias significativas respecto a mujeres. Dicha actividad muestra un elevado grado de variabilidad interindividual (23,5 veces expresada por folículo, 18,29 por mg de proteína y 22,75 por mg de DNA) en el conjunto de la población, siendo mayor entre las mujeres. Mediante cromatografía de intercambio iónico con ClK y enzimoinmunoensayo se lleva a cabo una caracterización preliminar del enzima. Los resultados sugieren que el isoenzima GST presente en folículo piloso humano corresponde a la forma ácida. El porcentaje de cabellos anágenos en cada individuo presenta una correlación positiva con los siguientes parámetros: GST/folículo, GST/-DNA y DNA/folículo. Se concluye que la GST puede jugar un papel importante en el ciclo del folículo piloso.

Palabras clave: Glutation S-transferasa, Folículo piloso.

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