ETHYL GLUCURONIDE CONCENTRATION IN HAIR IS NOT INFLUENCED BY PIGMENTATION

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Abstract — This work shows that the concentration of ethyl glucuronide (EtG) in hair, a marker for the evaluation of the alcohol consumption, is not influenced by the presence or absence of melanin. The results confirm that, unlike many other substances, the EtG determination in hair has not to take into account the hair colour for the correct interpretation of hair testing results.

Ethyl glucuronide (EtG) is a minor metabolite of ethanol, which is detectable in body fluids and tissues for an extended time period even after complete elimination of ethanol from the body (Schmitt et al., 1997; Wurst et al., 1999). Since its determination in hair was proposed as a tool for the detection of alcohol consumption, growing interest was observed for this marker and the many technical efforts performed to improve its sensitivity made the limit of detection decrease from about 2 ng/mg hair (Skopp et al., 2000) to values close to 2 pg/mg hair (Yegles et al., 2004). It was rapidly demonstrated that the presence of EtG allowed to distinguish hair of excessive and chronic alcohol consumers from hair of moderate drinkers and teetotallers where EtG was not detectable (Alt et al., 2000). Recently, cutoffs were proposed to distinguish teetotallers from social drinkers, and social drinkers from excessive and chronic alcohol consumers (Pragst and Yegles, 2006). Other studies demonstrated the existence of proportional relationships between the daily amount of alcohol intake and the EtG concentration in hair (Appenzeller et al., 2007; Politi et al., 2006), and that variations in EtG concentration in segmented hair provide an overview of the drinking history of individuals (Appenzeller et al., 2007).

Nevertheless, incorporation pathways of EtG in hair are not fully understood. In particular, the importance of melanin has not yet been documented. Probably because of the mean age of patients having alcohol abuse problems (Appenzeller et al., 2005a; Appenzeller et al., 2005b), many patients present partially unpigmented hair (grizzled subjects). Several studies showed that among patients diagnosed as alcoholic (according to ICD10), the percentage of grizzled subjects (white, grey, or dark streaked with grey hair) varied from 18 to 50% (Alt et al., 2000; Appenzeller et al., 2007; Janda et al., 2002; Skopp et al., 2000). Since melanin was demonstrated to influence the deposition of many drugs in hair (Rothe et al., 1997), the aim of the present letter was to clarify this critical point for EtG, in order to confirm the usefulness of the determination of EtG in hair for the evaluation of the alcohol consumption.

Hair samples were discarded specimens collected post mortem from 21 subjects with blood alcohol concentration ranging from 0 to 5 g/l. A selection of subjects with blood alcohol concentration of up to 5 g/l increased the chance that the hair specimens tested positive for EtG. This study was approved by the Luxembourg Ethics Committee. After washing hair samples with water and with acetone, pigmented and white hair shafts from each specimen were manually separated and pulverized in a ball mill (Retsch, Haan Germany). The relative part of white hair shafts ranged from 28 to 84% (w/w). EtG extraction from hair, GC-MS analysis and repeatability were fully described in previous work (Yegles et al., 2004). The limit of detection and limit of quantification were 2 and 4 pg/mg hair, respectively. For all specimens but one, analysis was performed in triplicate.

In the 21 specimens investigated in this study, the EtG concentration in hair ranged from 6 to 1239 pg/mg and one specimen had EtG concentration below the limit of quantification. No significant intra-individual difference in the EtG concentration between white and pigmented hair was observed (Fig. 1). The curve slope was 1.001 and the coefficient of correlation was 0.9939 (P < 0.0001). These
results demonstrate that the concentration of EtG in hair does not seem to be affected by its melanin content.

In different studies performed on human (Rothe et al., 1997) or animals (Green and Wilson, 1996; Pötsch et al., 1997), differences in drug concentration between white and pigmented hair were observed mainly for basic drugs (pKa from 8 to 10). As described by Pragst and Balikova (2006), the incorporation of drugs into hair from blood is facilitated for lipophilic molecules which can easily penetrate membranes. Furthermore, the relatively low pH in melanocytes and the affinity of melanin for basic drugs leads to their accumulation in pigmented hair. At the opposite, EtG is clearly hydrophilic and has a pKa of 3.21 (Krivankova et al., 2005). It has a rather negative charge at physiological pH, and would not be influenced by such interactions. Its concentration would hence not differ between pigmented and white hair, which is in agreement with the present experimental results. We demonstrated here that unlike many other substances, the EtG determination in hair has not to take into account the hair colour for the correct interpretation of hair testing results.

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