Eur. J. Clin. Chem. Clin. Biochem.
Vol. 31, 1993, pp. 41–45

© 1993 Walter de Gruyter & Co.
Berlin · New York

A HPLC-Based Chloramphenicol Acetyltransferase Assay for Assessing Hair Growth: Comparison of the Sensitivity of UV and Fluorescence Detection

By *D. J. Waldon, M. F. Kubicek, G. A. Johnson* and *A. E. Buhl*

Dermatology Department, The Upjohn Company, Kalamazoo, MI USA

(Received June 22/October 6, 1992)

Summary: In our attempt to measure hair growth by hair-specific markers, we used transgenic mice to express the chloramphenicol acetyltransferase gene under the control of an ultrahigh sulphur keratin gene promoter. To quantitate expression of the keratin gene, we required a chloramphenicol acetyltransferase assay which could measure enzyme activity in a single follicle and also could be used to assay a large number of samples without loss of sensitivity. We achieved this objective by utilizing a fluorescent substrate for chloramphenicol acetyltransferase. With HPLC-fluorescence detection, this substrate provides a sensitivity of less than 1×10^{-13} mol, which is 1000 times greater than that achievable with HPLC-UV detection in cultured follicles. Further, the assay was automated to facilitate the analysis of more than 100 samples/day. It should be possible to apply this fluorescent assay to a number of cell or tissue studies.

Introduction

Assessment of hair growth or the measurement of end points related to hair growth is difficult due to the very slow growth rate of hair. In an attempt to overcome this limitation, we utilized transgenic mice in which a gene for a hair-specific marker, an ultra high sulphur keratin, is inserted to control a chloramphenicol acetyltransferase marker gene. Measurement of chloramphenicol acetyltransferase activity in hair or vibrissae follicles can then be followed as a sensitive measure of hair growth. The use of chloramphenicol acetyltransferase as a reporter for genomic events is a well-established and widely used method for measuring transfected gene activity in mammalian cells and transgenic animals (1–3).

Due to the small amount of tissue available in single follicles, which would be optimal for assessing hair growth, a very sensitive assay is required. Previously, TLC-based radiometric and HPLC-UV detection methods have been used to detect chloramphenicol acetyltransferase activity in transgenic experiments, including hair growth studies. However, the analytic methods are not sufficiently sensitive to enable an accurate and quantitative assessment of chloramphenicol acetyltransferase activity in single follicles.

To achieve the necessary sensitivity in the chloramphenicol acetyltransferase assay for measuring enzyme activity in single follicles, we made use of a fluorescent chloramphenicol derivative as a substrate for the chloramphenicol acetyltransferase. When acetylated, the product is resolved by HPLC and the resulting fluorescence provides greater sensitivity than that previously reported using HPLC-UV detection methods. The analysis is also sufficiently sensitive for the easy detection of chloramphenicol acetyltransferase activity in single vibrassae follicles. This report describes the fluorescent assay and compares its sensitivity with that of an HPLC-UV detection-based assay.

Materials and Methods

The transgenic mice were offspring from a C57Bl × B6sjc crossed with a CF1. The pups were 3 days old and weighed between 1.8 and 2.4 grams. These mice express the chloram-