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SHORT COMMUNICATION

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Preliminary approach to elucidate the role of pigment as a binding site for drugs and chemicals in anagen hair: differential uptake of ^{3}H-haloperidol by pigment-producing compared to non-pigment-producing cell lines

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Abstract A striking difference was observed for cellular-bound drug in HaCaT and Sk-Mel-1 cells for a fixed drug exposure time of 72 h and varying ^{3}H-haloperidol concentrations in the culture media. Drug uptake was dependent on drug concentration and linearly correlated for both the non-pigment- and the pigment-producing cells which however was different in magnitude. In an additional investigation the time course of drug uptake during ^{3}H-haloperidol exposure (400 pmol/ml; 28 days) revealed increasing drug concentrations in the Sk-Mel-1 population, whereas drug concentrations in the keratinocytes reached a plateau within a short time period. In contrast to the HaCaT cells no tendency to saturation was observed for the pigment-producing cell line. At the end of the experiments ^{3}H-haloperidol concentrations in Sk-Mel-1 were found to be approximately tenfold higher than in HaCaT.

Keywords Cell culture · Drug uptake · Melanin · Hair · HaCaT · Sk-Mel-1

Introduction

The mechanisms of drug incorporation into hair have not been clarified at all. There is some evidence that melanin influences the results for particular substances in hair analysis. Moreover, the melanin-related drug adsorption might be modified by the frequency of drug consumption. According to the biochemical concept of drug incorporation during hair fibre formation, it has been suggested that particular drug substances are bound to melanin during melanogenesis as well as onto the surface of mature melanin granules in addition to other binding sites such as intermediate filaments (IF, IFAPs) and the cell membrane complex [8, 9].

Even in 1995 Nakahara and coworkers [5] pointed out that among other physico-chemical properties, the melanin affinity of a particular substance strongly influences its incorporation into hair.

In animals it has been shown for various drug substances that the concentrations are different in pigmented and non-pigmented hair fibres [2, 3, 10, 11, 12, 14]. Striking differences in drug concentrations were found for codeine and ofloxacin in the white, reddish-brown and black hair fibres of the same individual using tricoloured guinea pigs as an animal model. Additionally these studies revealed that the relevance of pigmentation on the analytical results depended on the amount of drug applied and on the time of exposure [10, 11].

Recently the findings for codeine in guinea pigs were confirmed for human hair by Kronstrand et al. [4]. Rothe et al. [13] already observed in grey haired patients under treatment of antidepressants that the concentrations of the target substances were higher in pigmented than in non-pigmented hair fibres.

In vitro investigations on human cell cultures may offer a solution to answer open questions in hair analysis because drug uptake in the growing hair follicle cannot be studied at the cellular level in vivo.

The aim of the present study was to mimic chronic drug consumption and to investigate the cellular uptake of haloperidol, a lipophilic drug with high melanin affinity, in HaCaT and Sk-Mel-1 cells. These cell lines are maintaining the expression of keratin and melanin synthesis and may therefore serve as a model for long term drug exposure of the cell populations present in the anagen hair root.

Materials and methods

Cell lines and materials

The spontaneously immortalised cell-line HaCaT (passages 35–45) was kindly provided by Prof. N. Fusenig (Deutsches Krebs-

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forschungszentrum, Heidelberg, Germany) and cultured at 5% CO_2/37 °C in 1 × MEM medium containing 10% fetal calf serum (FCS). For subcultures, cells were disaggregated with 0.1% trypsin/0.05% EDTA (1 : 1) solution and split out on a ratio of 1:10 every 10 days.

Sk-Mel-1 cells were obtained from the DSMZ (Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany) and cultured in 1× MEM medium containing 10% FCS and 1× non-essential amino acid mix.

[^{3}H(G)]-Haloperidol (specific activity 4,440,000 GBq/mmol) was obtained from NEN (Bad Homburg, Germany). The liquid scintillation cocktail (rotizint) was from Roth (Karlsruhe, Germany). All other chemicals were of anaytical grade and were purchased from Sigma (München, Germany), the cell media and medium supplements were from Gibco-BRL (Eggenstein, Germany).

Experimental design

The dependence of the drug uptake on drug concentration by the HaCaT and Sk-Mel-1 cells was investigated for a given exposure time of 72 h. The cell lines were cultivated separately in 250-ml flasks (Greiner, Frickenhausen, Germany) in the presence of ^{3}H-haloperidol concentrations of 200, 400, 600 and 800 pmol/ml culture medium. The concentration coefficient kc_{UP} was calculated according to: kc_{UP} = concentration in 10^6 cells/ml culture medium.

The time dependence on the cellular uptake of ^{3}H-haloperidol (400 pmol/ml culture medium) was followed for 28 days. Long term drug exposure was achieved by ^{3}H-haloperidol liberation from osmotic pumps (Charles River, Sulzfeld, Germany). At given time intervals (3, 6, 9, 15, 21 and 28 days) cell portions were removed and washed 4 times with cold PBS-buffer. Media and washing solutions were collected and stored at –20 °C until analysed at the end of the experiment. Cell counting was performed by haemocytometry under the microscope and the cell viability was estimated by trypan blue exclusion by at least two independent observers. All experiments were run in duplicate, and measurements were done in triplicate. Controls such as formalin-treated cells in culture were included. ^{3}H-haloperidol uptake was quantified by liquid scintillation spectrometry using the Tricarb 1600 TR analyser (Canberra Packard, Dreieich, Germany). Cell-bound ^{3}H-haloperidol was calculated from calibration curves which were linear. The mean of the assay runs (range ≤ 10%) was used for data analysis. Uptake values and total melanin were normalised to 1×10^6 cells.

The total melanin content of the Sk-Mel-1 cells was determined by spectrophotometry (UV 240-spectrophotometer, Shimadzu, Duisburg, Germany) according to Ozeki et al. [7] at the end of the 28-day experiments. Calibration was performed using Sepia melanin and synthetic eumelanin (Sigma, München, Germany).

Results

Radioactivity in formalin-treated cells was close to the background. When Sk-Mel-1 and HaCaT cells were incubated with ^{3}H-haloperidol 92 ± 4% of the radioactivity added was found in the culture media plus washing solutions. The relatively small amount taken up by the cells implies that for simulating concentration ranges present in serum the use of radiolabelled substrates in cell culture models is indispensable.

A striking difference was observed for cellular bound drug in HaCaT and Sk-Mel-1 cells at the chosen drug exposure time (72 h) and varying ^{3}H-haloperidol concentrations in the culture media (Fig. 1). Concentration-dependent and linearly correlated drug uptake was observed for both the non-pigment-producing cells and pigment-producing cells. However, at a given concentration and exposure time the capacity to bind the drug was always higher for the pigment-producing cells than for the keratinocytes. Concentration coefficients for drug uptake were kc_{UP} = 0.006 for the non-pigmented keratinocytes HaCaT and kc_{UP} = 0.025 for the pigmented cell-line Sk-Mel-1.

The time course of drug uptake showed that prolonged drug exposure revealed increasing drug concentrations in the Sk-Mel-1 population, whereas drug concentrations in the keratinocytes reached a plateau within a short time period (Fig. 2). In contrast to the HaCaT cells, the pigment-producing cell line showed no tendency to saturation under the experimental conditions. The time-dependent coefficient for Sk-Mel-1 was kt_{UP} = 0.915. At the end of the experiments ^{3}H-haloperidol concentrations in Sk-Mel-1 were found to be approximately tenfold higher than in HaCaT.

The melanin content was 25 µg/10^6 Sk-Mel-1-cells for all experiments.

Discussion

The present study showed that non-pigment-producing and pigment-producing cells in culture were both able to take up ^{3}H-haloperidol from the medium. Pigment-pro-

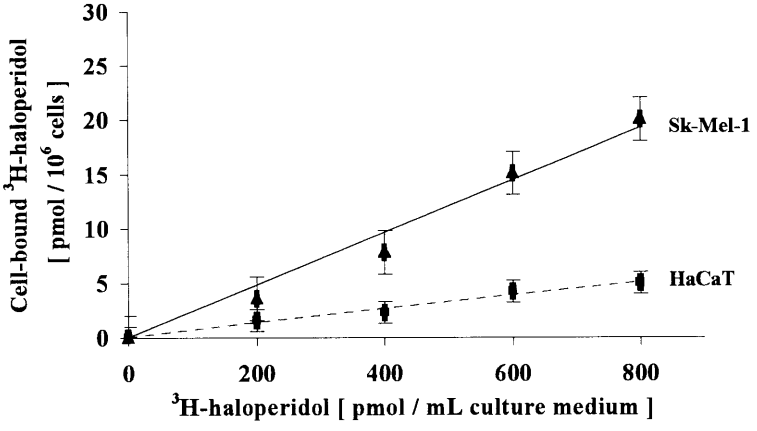

Fig. 1 Concentration-dependent and linearly correlated uptake of ^{3}H-haloperidol by HaCaT (r = 0.994) and Sk-Mel-1 (r = 0.995) after 72 h. Melanin content of Sk-Mel-1 25 µg/10^6 cells

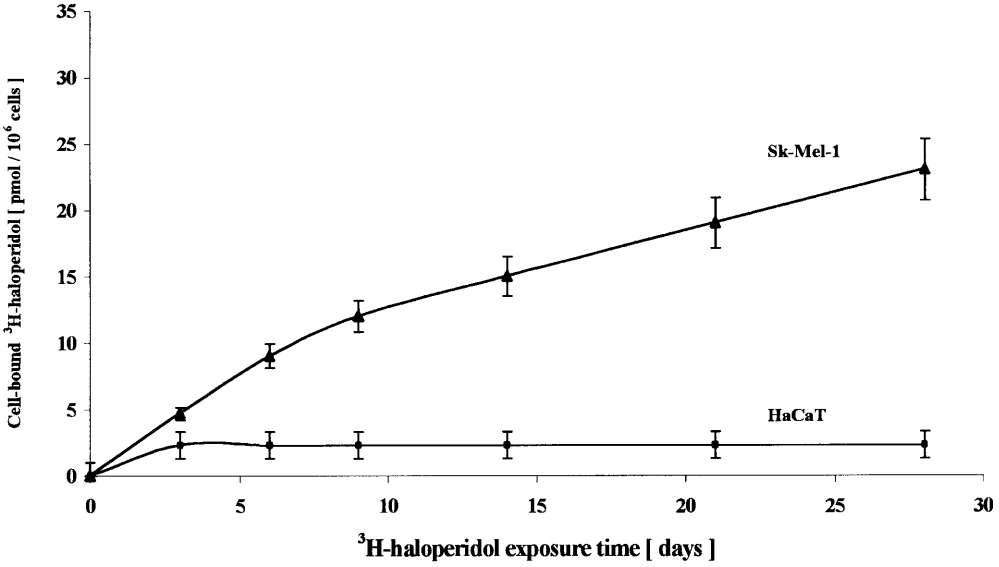

Fig. 2 Time course for uptake of ^{3}H-haloperidol for HaCaT and Sk-Mel-1 (r = 0.996) at constant drug concentration (400 pmol/ml culture medium). Melanin content of Sk-Mel-1 25 µg/10^6 cells

ducing cells (melanocytes) and non-pigment producing cells (keratinocytes) were chosen as a model for the cell populations present in the hair root. Although primary cell cultures were regarded as superior, the established cell lines HaCaT and Sk-Mel-1 were used in the preliminary investigations, thus allowing unlimited access by passaging and cryopreservation as well as excellent reproducibility and consistence. The HaCaT cell line is widely used in dermatological research. It is derived from normal human abdominal skin and described to exhibit a differentiation profile comparable to normal human keratinocytes [1]. The Sk-Mel-1 cell line is derived from a human malignant melanoma and maintained melanin expression [6].

The drug binding was more pronounced in the melanin-producing cell population in general, which was found to have a high capacity to accumulate and concentrate ^{3}H-haloperidol. Additionally an increase of the striking difference for cell-bound ^{3}H-haloperidol was observed after long-term drug exposure. It has been suggested that this is partly due to the presence of acidic organelles, which may entrap ^{3}H-haloperidol, which is a weak base but is predominantly associated with the cell pigments [8]. Subcellular constituents other than melanin may contribute only to a minor extent to differences in drug affinity between different cell types as exemplified in our study. The present results from cell cultures underlined the observations of Uematsu and co-workers [15], who suggested a possible linkage of haloperidol excretion into hair with hair pigmentation as early as 1990.

The experimental results from cell cultures are in full agreement with those of the animal studies which demonstrated that the hair fibre concentrations for substances with melanin affinity are influenced by pigmentation and even confirmed the relevance of pigmentation as a function of drug concentration and drug exposure time at a given melanin content [10, 11]. Therefore non-pigment and pigment-producing cell lines may serve as an appropriate model for basic experiments as well as for some elucidation of the mechanisms of drug uptake into the different cell populations of the hair root and localisation of the drug binding, although the differences in the proliferation rate in cell culture and in vivo should be noted.

An interesting and important finding was the limited capacity of the keratinocytes for ^{3}H-haloperidol binding. This finding confirmed the observations in animals for the low drug content of white, non-pigmented hair fibres [2, 3, 10, 11, 14]. The experiments clearly demonstrated that the duration of drug exposure does not influence the drug uptake in these cell types in contrast to pigment-producing cell types (Fig. 2).

In the hair follicle pigment-producing cells, the melanocytes, are located around the apex of the papilla. In contrast to the matrix cells, the keratinocytes, these cells are differentiated, are in a very close contact with the blood supply from the dermal papilla and do not migrate [8]. During long-term drug consumption the pigment-producing melanocytes are permanently exposed in the hair follicle. For drug substances with a high melanin affinity a permanent concentration gradient will result because the particular drug is entrapped by the melanins and during melanin formation as demonstrated by the results of the in vitro experiments (Fig. 2). The cell products of the melanocytes, the melanin granules, are phagocytised by the presumptive cortical cells passing by on their way upwards to the keratinisation zone. Therefore, drug-transfer by the melanin granules is likely to occur.

Taken together it can be suggested from the results of our previous animal studies and the present cell culture experiments that for substances with high a melanin affinity there are at least four factors that determine the relevance of pigmentation. The decisive parameters of a complex mathematical function for the influence of pigmentation in hair analysis are thought to be the type of pigment (eumelanin > pheomelanin), the amount of melanin in the hair sample as well as drug concentration and time of drug

exposure during hair growth. As a conclusion, although preliminary in nature, the results obtained for ^{3}H-haloperidol by non-pigment and pigment-producing cell lines underlined the influence of pigmentation for the scenario of heavy chronic drug consumption in hair analysis.

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