

Deoxythymidine pools of human skin and guinea pig organs

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The activity of antiviral nucleoside analogues like acyclovir is influenced by a number of cellular factors, one being the deoxythymidine (dThd) concentration. We therefore analysed the dThd concentration in human plasma and skin and in organs of guinea pig, the common experimental animal. High-performance liquid chromatography showed low amounts of dThd in human skin, 0.20–1.15 nmol/g, whereas guinea pig skin and spleen had 20–30 nmol/g and the concentration in guinea pig plasma was 10-times higher than in human plasma. These animals are therefore in this respect less suitable as accurate models for antiviral nucleoside activity in humans.

Deoxythymidine HPLC Tissue concentration

1. INTRODUCTION

The primary site of herpes simplex virus infection in man is the skin. One therefore needs to know about the presence here of naturally occurring molecules which compete with antiviral drugs. A common experimental animal model for the assay of antiviral drug efficiency is the guinea pig.

The activation of a number of modern antiviral substances is dependent on viral or cellular deoxythymidine kinases (dTKs); examples of these compounds are acyclovir, bromovinyldeoxyuridine and iododeoxyuridine. Addition of the natural substrate of dTK, deoxythymidine (dThd), decreases the activity of these substances [1,2]. It has been shown that the addition of dThd to herpes simplex virus-infected cell cultures reverses the action of dTK-activated antiviral compounds [1,3]. Some cell cultures endogenously exhibit high levels of intracellular dThd [1].

To obtain a basis for the interpretation of animal and clinical data, we have studied the intracellular levels of naturally occurring dThd in selected tissues and in plasma of human and guinea pig origin.

2. MATERIALS AND METHODS

2.1. Samples

Male 6-week-old Dunkin-Hartley guinea pigs were from J.A. Sahlin, Malmö, Sweden. The animals were killed using pentothal, dissected and the organs frozen. Human skin was from patients at the Department of Plastic Surgery, Karolinska Hospital, Stockholm, and immediately frozen at -70°C . The patients were admitted to the hospital for the removal of benign birthmarks. The skin was extensively washed with 70% alcohol prior to removal. The washing procedure did not affect the dThd content (not shown). Heparinized blood was from healthy humans and the chilled plasma immediately separated by centrifugation.

2.2. Reagents

dThd was from Sigma (St. Louis, MO); methanol of analytical grade from Riedel-de Haen, Hannover; acetonitrile of chromatographic grade from Merck, Darmstadt.

2.3. Chromatography

High-performance liquid chromatography (HPLC) was performed on a liquid chromato-

graph (Waters, Milford, MA). Analytical 5 or 10 μm Rad-PAK C_{18} columns (8 mm \varnothing \times 100 mm, Waters) were used under conditions previously described [1]; 10 mM ammonium acetate in water, pH 4.7:acetonitrile (95.0:5.0); flow rate 2.5 ml/min, ambient temperature; detector sensitivity 0.01.

2.4. Sample preparations

Ice-cold 1 M perchloric acid (PCA) was added to tissue samples, which were subsequently disintegrated by Ultraturrax (Janke and Kunkel, FRG). After centrifugation the supernatant was neutralized with ice-cold 4 M KOH with 0.4 M KH_2PO_4 . Cell debris and precipitates were removed by an additional centrifugation and the supernatant was loaded on a Sep-Pak C_{18} cartridge [4].

Plasma samples were loaded directly on Sep-Pak C_{18} cartridges (Waters).

3. RESULTS

The dThd content was analysed from the skin of 6 patients undergoing plastic surgery (table 1). Only low amounts of dThd were found in human skin.

The dThd concentration varied between different organs of the guinea pigs (table 2). The highest concentrations were found in the skin and spleen (20.4–30.5 nmol dThd/g wet wt). In the intestine the concentration of dThd was 8.93 nmol/g and levels around 3 nmol/g or lower were found in the other organs studied. A higher dThd concentration was found in guinea pig plasma than in human plasma: 1.12 μM vs 0.09 μM (not shown).

Table 1
dThd concentration of human skin

Sample	[dThd] (nmol/g wet wt)
1	<0.20
2	1.09
3	1.15
4	<0.20
5	0.48
6	<0.20

Table 2

dThd concentration of organs of three guinea pigs

Organ	[dThd] (nmol/g wet wt)	(range)
Skin	20.4	(16.0 – 27.6)
Spleen	30.5	(24.2 – 36.8)
Intestine	8.93	(8.40– 9.70)
Kidney	1.50	(0.95– 1.99)
Brain	1.98	(1.09– 2.66)
Liver	0.68	(0.67– 0.70)
Heart	2.42	(1.77– 3.31)
Lung	3.66	(2.54– 5.55)
Eye	2.24	(1.97– 2.50)
Pancreas	2.62	
Adrenal	4.64	(3.68– 6.94)

4. DISCUSSION

High contents of dThd were found in guinea pig skin. The amounts ranged from 16.0 to 27.6 nmol/g (average 20.4 nmol/g). The dThd concentration for guinea pig skin seems to be of the order of 20 μM . Addition of 10 μM dThd to herpes simplex-infected human embryonic lung fibroblasts increases the 50% virus inhibiting concentration of acyclovir by a factor of approx. 20 [1]. The high amounts of dThd in guinea pig skin indicate that the guinea pig is less suitable as a model system for antiviral nucleosides. This is especially important for cutaneous herpes treated with antiviral substances with a high K_m value for dTK.

Conflicting reports have appeared about the plasma and serum concentrations of dThd in healthy humans. Reports utilizing HPLC methods have given dThd values of 0.43–0.60 μM in human plasma [5,6]. Others, using radioimmune or microbiological assays, have reported lower values [7–10]. Part of the difference may result from different procedures for collecting samples. dThd may be degraded by deoxynucleoside phosphorylase [11] or phosphorylated by deoxythymidine kinase [12], present in plasma. Further, the HPLC methods were previously utilized close to the sensitivity limit. In this paper we utilized a new Sep-Pak procedure for the purification and concentration of the samples, thereby improv-

ing the sensitivity of the assay by a factor of 10. However, the dThd concentration of $0.09 \mu\text{M}$ found in human plasma is still close to the sensitivity limit of the improved assay and should be interpreted with caution. The dThd concentration found in guinea pig plasma was 10-times higher than in plasma of humans. Our results are in accordance with those of Nottebrock and Then [8] who report that rodents have a higher serum dThd concentration than other species. These findings further point to the unsuitability of guinea pigs as test animals.

Currently we are investigating dThd contents of other human organs. Knowledge of the various dThd concentrations is of importance when treating herpes virus, since infections in different organs may require different doses of the antiviral drug to overcome competition from endogenous nucleosides.

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