Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8–12, pp. 681–683. 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Equilibrium kinetics of the new experimental anti-tumour compound SK&F 104864-A in aqueous solution*

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Abstract: The equilibrium kinetics of lactone ring hydrolysis in the new experimental anti-tumour compound SK&F 104864-A, (*S*)-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride, have been studied. Only one product is formed, SK&F 105992. A stability-indicating HPLC method has been optimized to perform the analysis. The pH is the main factor influencing equilibrium; at pH ≥10 the lactone ring is quantitatively opened while at pH values ≤4 the lactone form is exclusively present. Other parameters, such as buffer ions and ionic strength, do not influence equilibrium. Complexation with dimethyl-β-cyclodextrin stabilizes the lactone form. Other cyclodextrins do not show this stabilization.

Keywords: *Anti-tumour agents; SK&F 104864-A; degradation; equilibrium kinetics; stabilization.*

Introduction

(*S*)-9-Dimethylaminomethyl-10-hydroxy-camptothecin hydrochloride (SK&F 104864-A, NSC 609669, Fig. 1), a semisynthetic analogue of camptothecin, shows considerable cytotoxic and antitumour activity in animal test systems [1]. The compound has been selected by the EORTC for further clinical testing.

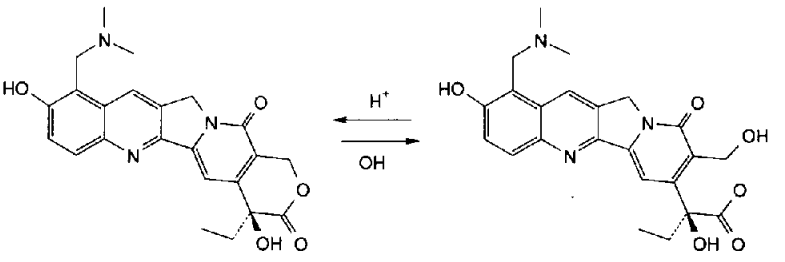
SK&F 104864-A is known to undergo a pH-dependent, reversible hydrolytic dissociation of its lactone function (Fig. 1) [1]. Knowledge of this hydrolytic process is important for the evaluation of clinical studies since the open form (SK&F 105992, Fig. 1) does not exhibit any topoisomerase I inhibitive activity.

In present study the kinetics of the hydrolytic process are studied in order to optimize the pharmaceutical formulation and to predict *in vivo* activity of the compound.

Experimental

Chemicals

SK&F 104864-A was obtained from Smith Kline & Beecham Laboratories (King of



SK&F 104864-A SK&F 105992

Figure 1
Hydrolysis of SK&F 104864-A to SK&F 105992.

*Presented at the Second International Symposium on "Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

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Prussia, PA, USA). The cyclodextrins were from Nihon Shohukin Co. Ltd (Tokyo, Japan) and were used as received. All other chemicals were of analytical grade and de-ionized water was filtered through a Milli-Q Water Purification System (Millipore, Bedford, MA, USA) before use.

HPLC

The HPLC system comprised a Model M6000 pump and a U6K injector from Waters Associates (Milford, MA, USA) and a Fluorescence Detector F1000 from Merck Hitachi (Tokyo, Japan). The column (100 × 4 mm) was packed with 5-μm Hypersil ODS; the solvent comprised methanol–water–triethylamine (50:49.7:0.3, v/v) with 1 mM sodium dioctylsulphosuccinate and 23 mM ammonium phosphate buffer (pH 6.0) and was adjusted to pH 6.0 with phosphoric acid. The flow rate was 1.0 ml min^{-1} and the fluorescence detector system operated at 377 nm (excitation) and 505 nm (emission). The HPLC system is a modification of a system described earlier [1].

The HPLC system provides an excellent separation between the parent compound SK&F 104864-A and its open form (SK&F 105992) (Fig. 2) and is, thus, stability indicating.

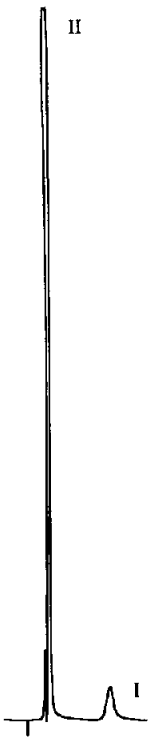

Figure 2
Chromatogram of a hydrolysed solution of SK&F 104864-A (**I**) at pH 8.0 at equilibrium. **II** = SK&F 105992. Detection by fluorimetry.

Kinetic experiments

The kinetic experiments were performed in the dark at 25°C at a buffer concentration of 0.01 M and ionic strength 0.3 M, unless stated otherwise. The reaction was initiated by spiking 100 μl of stock solution (1 mg ml^{-1}) in methanol into 5.0 ml of pre-adjusted buffer, leading to an initial SK&F 104864-A concentration of 20 μg ml^{-1}.

At appropriate time intervals, samples of 10 μl were injected into the HPLC system.

The effects of pH, temperature, ionic strength, buffer concentration, concentration of SK&F 104864-A and inclusion in cyclodextrins on the equilibrium have been investigated.

Results and Discussion

Equilibrium kinetics

The degradation of SK&F 104864-A can be described by a simple reversible reaction mechanism [2]

$$A \underset{k_1}{\overset{k_1}{\leftrightarrow}} B,$$

in which both the forward and reverse reaction steps are pseudo-first-order processes with k_1 and k_1 as their respective rate constants.

The equilibrium constant K_e can be expressed as

$$K_e = [B]_e/[A]_e = \{[A]_o - [A]_e\}/[A]_e = k_1/k_{-1}, \quad (1)$$

where $[A]_e$ and $[B]_e$ are the equilibrium concentrations of A and B, respectively, and $[B]_e = [A]_o - [A]_e$, provided that $[B]_o = 0$ at $t = 0$.

Integration and rearrangement of equation (1) leads to

$$-\ln([A]_t - [A]_e) = -\ln([A]_o - [A]_e) + (k_1 + k_{-1})\cdot t, \quad (2)$$

in which $[A]_o$ and $[A]_t$ represent the initial concentration of A and the concentration of A at time t, respectively. Measurement of [A] at various time intervals before equilibrium is reached enables graphical calculation of $(k_1 + k_{-1})$ and $[A]_o$ from the slope and the intercept

of equation (2). Measurement of $[A]_e$ enables calculation of K_e using the obtained value of $[A]_o$. Subsequently, the values for k_1 and k_{-1} can be calculated.

Influence of pH

The pH has a major influence on the equilibrium constant K_e (Table 1). Primarily, k_1 increases with increasing pH; the influence of pH on k_{-1} is much smaller. As a result, K_e increases with increasing pH.

Influence of temperature

At increased temperature both k_1 and k_{-1} increase. However, no significant changes in K_e occur, indicating that the equilibrium itself is unaffected by temperature.

Influence of concentration changes

Changes in the concentrations of buffer ions or changes in the ionic strength of the solution did not affect the equilibrium reaction. Similarly changes in the initial concentration of the parent compound SK&F 104864-A had no effect.

Table 1
Influence of pH on the equilibrium

pH	K_e	k_1 (s^{-1})	k_{-1} (s^{-1})
≥10	—	open form	—
9	20.2	2.02×10^{-3}	1.00×10^{-4}
8	8.4	2.78×10^{-4}	3.33×10^{-5}
7	2.8	9.32×10^{-5}	3.34×10^{-5}
6	0.4	1.55×10^{-5}	3.62×10^{-5}
5	0.1	6.0×10^{-6}	6.0×10^{-5}
≤4	—	lactone	—

Table 2
Influence of cyclodextrin on the equilibrium at pH 8.0 and 25°C

Cyclodextrin	K_c	k_1 (s^{-1})	k_{-1} (s^{-1})
—	17.5	3.62×10^{-4}	2.16×10^{-5}
α	11.6	2.59×10^{-4}	2.26×10^{-5}
β	10.5	2.44×10^{-4}	2.32×10^{-5}
γ	13.8	3.38×10^{-4}	2.45×10^{-5}
HP-β	10.2	2.08×10^{-4}	2.04×10^{-5}
HP-γ	12.4	2.76×10^{-4}	2.22×10^{-5}
DM-β	5.3	1.02×10^{-4}	1.92×10^{-5}

Influence of cyclodextrin complexation

Table 2 summarizes the influence of various cyclodextrins on the SK&F 104864-A equilibrium reaction. Only dimethyl-β-cyclodextrin (DM-β) stabilizes the lactone form, as shown by a significant decrease in k_1, while k_{-1} is unaffected. The conditions for inclusion of SK&F 104864-A appear to be best with dimethyl-β-cyclodextrin.

References

[1] EORTC Protocol No. ND 02891, Version No. 2, August (1989).
[2] A.N. Martin, J. Swarbrick and A. Cammarata, *Physical Pharmacy* (2nd edn), p. 365. Lea & Febiger, Philadelphia (1969).

[*Received for review 5 April 1990*]