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New antitumoral cyclopeptides

Short Communication

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Summary. Chlamydocin is a powerful *in vitro* antitumoral agent, quickly inactivated *in vivo*. A series of cyclic tetrapeptides related to chlamydocin or HC toxin and bearing a bioactive alkylating group on an ε-amino-lysyl function have been examined for their antitumoral activity on L1210 and P388 murine leukemia cell lines. One analog was found to be potent at inhibiting L1210 cell proliferation and had a higher therapeutic index than the reference compound bis-β-chloroethylnitrosourea on the *in vivo* P388-induced leukemia model.

Keywords: Amino acids – Peptides – Cyclopeptides – Chlamydocin – HC toxin – Alkylating agent – Antitumoral agent

Introduction

Chlamydocin has been shown by Closse and Huguenin (1974) to be a cyclic tetrapeptide [cyclo(Aib-Phe-DPro-Aoe) where Aoe means (2S, 9S)2-amino-8-oxo-9,10-epoxydecanoic acid] exhibiting powerful *in vitro* antitumor activity. Stahelin and Trippmacher (1974) have determined its cytostatic activity on the inhibition of proliferation of mastocytoma P-815 cells (mice) : an $ED_{50} = 0.36$ ng/ml, 10 times higher than for other well-known antimitogenic agents (e.g. vincristine, colchicine or amethopterin) has been obtained.

Other cyclic tetrapeptides containing the same Aoe residue show several biological activities. These activities are largely overlaped and depend on the peptide sequence. For example, HC-toxin: cyclo(Ala-DAla-Aoe-DPro), as pointed out by Walton et al. (1985), is more phytotoxic host-specific and less cytostatic than chlamydocin.

Unfortunately, this remarkable cytotoxic activity fades during *in vivo* test. As outlined by Stahelin and Trippmacher (1974), chlamydocin increases only very slightly the life span (ILS < 15%) of mice inoculated with tumor P-815 cells

even at high doses (160 mg/Kg). This discrepancy has been explained by the rapid inactivation of this compound in the blood stream. The keto epoxidic group of Aoe is essential for compound activity as demonstrated after its reduction or hydrolysis, Closse and Huguenin (1974). As it is assummed that this group acts as an alkylating agent, its replacement by other similar but more stable chemical functions would be benefit.

Basing on this assumption, we have prepared a series of cyclic peptides belonging either to the *chlamydocin* or to the *HCtoxin* family, containing a lysine in the place of Aoe. This provided a free amino function on Nε for the anchoring of the well known alkylating β-chloroethyl-nitrosourea [-CON(NO)C_2H_4Cl], respectively giving cyclopeptides **1** and **2** or of the N,N-di-(β-chloroethyl)-4-aminobenzoyl group [-p$COC_6H_4N(C_2H_4Cl)_2$], respectively leading to cyclopeptides **3** and **4**.

Material and methods

Peptide synthesis

The synthesis of these new analogs will be described in detail elsewhere. The cyclisation step which is always the bottleneck in the synthesis of small cyclic peptides was carried out with satisfactory yields (50% to70%), using diethylphosphorocyanidate as the activating agent of the Pro-carboxylic group of the two linear precursors.

Biological activity

For *in vitro* experiments, the inhibitory concentration (IC_{50}) was determined after 48 h incubation of L1210 cells at 37°C according to Alley et al. (1990). *In vivo* experiments were carried out on hybrid female mice $B_6D_2F_1$. The drugs were administred Ip as a single injection on day 1. The antitumor activity was estimated according to Geran et al. (1972).

Results

Cyclopeptide **1** exhibits (Table 1) a tenfold higher activity than BCNU and more than twentyfold higher than the HC toxin analog **2**. Nitrogen mustard cyclopeptide **3** is slightly active whereas **4** is inactive. Compound **1** was therefore investigated further in an *in vivo* P-338 leukemia model. The results summarized in Table 2 demonstrate a high activity of compound **1** (considerably higher than that of 5-fluoro-uracil used as a positive control). At similar molarity (128 and

Table 1. Inhibitory potency of cyclopeptides **1–3** on L1210 cell line *in vitro*

Compound	IC_{50} (μM) (± standard error)
1	0.84 ± 0.10
2	26.4 ± 5.2
3	43.2 ± 8.2
BCNU*	9.2 ± 1.0

* Bis-β-chloroethyl nitrosourea

Table 2. In vivo antileukemic activity of **1** in mice injected with P338 cells

Compound *	Dose mg/Kg	(μMol/kg)	Δ Weight *g* D (1–4)	T/C %	Survivals at D60
1	5	8	+1.3	128	0/8
1	10	16	+0.9	142	0/8
1	20	32	+0.8	150	0/8
1	40	64	+1.3	195	0/8
1	60	96	+0.4	229	1/8
1	80	128	0	>600	6/8
BCNU	30	143	−0,6	500	4/8
5-FU	80	610	0	164	0/8
Control	—	—	+1.4	100	0/20

* Abbreviations: *BCNU* Bis-β-chloroethylnitrosourea; *5-FU* 5-fluoro-uracil.

143 μM, respectively) compound **1** and BCNU have comparable activity with a T/C ratio of 600 and 500, respectively. No sign of toxicity is detectable for compound **1** under these conditions, since no loss of weight is seen and the number of survivals at day 60 is even higher (6 out of 8) for **1** than for BCNU.

Discussion

From the *in vitro* experiments, it can be concluded that the peptide sequence of the nitrosourea analogs **1** and **2** plays an important role in target recognition, since the biological response is higher (about 20 times) for the chlamydocin compared to the HC toxin analog, as this was the case for the natural parent compounds, Walton et al. (1985).

The main result of this study was obtained in combining the favorable structure of a cyclotetrapeptide derived from chlamydocin with a classical nitrosourea alkylating substituent, giving rise to a potent antiproliferative agent showing important activity *in vivo*.

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