Synthesis and Antineoplastic Activity of CNC-Cysteamine and Related Compounds*, **

Wei-ci Tang***, Johannes Schmid, Heinz-Herbert Fiebig, and Gerhard Eisenbrand

1 Institute of Toxicology and Chemotherapy, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany
2 Department of Internal Medicine, University of Freiburg, D-7800 Freiburg, Federal Republic of Germany
3 Department of Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-6750 Kaiserslautern, Federal Republic of Germany

Summary. N′-[N-(2-Chloroethyl)-N-nitroso]carbamoyl cysteamine (CNC-cysteamine) and several related compounds have been synthesized and tested against L 1210 leukemia in mice. Reaction of N-(2-chloroethyl)-N-nitrosocarbamoyl azide (CNC-azole) with cysteamine yielded CNC-cysteamine and bis(CNC)cystamine. Reaction of CNC-azole with cysteamine in the presence of triethylamine gave bis(CNC)cystamine. Unexpectedly, formation of CNC-cysteamine carbazolazide as a minor reaction product was also observed. N-(2-Chloroethyl)carbamoyl cysteamine 2-chloroethylcarbamate was formed when 2-chloroethyl isocyanate was reacted with cysteamine. Nitrosation of this cysteamine N,S-dicarbamoyl derivative led to formation of a mixture of two dinitroso isomers.

Preliminary testing of the newly synthesized CNC-derivatives against L 1210 leukemia in mice revealed that CNC-cysteamine, its disulfide bis(CNC)cystamine and CNC-cysteamine carbazolazide were highly active against L 1210 leukemia.

Key words: CNC-cysteamine – L 1210 leukemia – Antineoplastic activity – nitrosourea.

Introduction

Nitrosoureas belong to the most potent experimental anticancer agents known (Montgomery 1981; Eisenbrand et al. 1984). Some compounds are used clinically to treat malignant tumors, e.g., N,N′-bis(2-chloroethyl)-N-nitroso-N′-cyclohexyl-N-nitrosourea (BCNU), N-(2-chloroethyl)-N′-cyclohexyl-N-nitrosourea (CCNU), N-(2-chloroethyl)-N′-(trans-4-methylcyclohexyl)-N-nitrosourea (MeCCNU), N-(2-chloroethyl)-N′-(2,6-dioxo-3-piperidyl)-N-nitrosourea (PCNU) (Montgomery 1981) and N-(2-chloroethyl)-N′-(4-amino-2-methylpyrimidine-5-yl)methyl-N-nitrosourea (ACNU) (Ogawa 1981). The clinical use of nitrosoureas is however rather limited because of the delayed and cumulative toxic side effects they exhibit especially to the bone marrow (Carter 1981). In view of their high anticancer potential in a wide spectrum of experimental tumors, many attempts have been made to develop analogues with lower toxicity. Examples are N′-[N-(2-chloroethyl)-N-nitroso]carbamoyl amino acid derivatives (Tang and Eisenbrand 1981; Ehresmann et al. 1984), the nitrosourea nucleosides (Prusoff et al. 1983), steroid linkes nitrosoureas (Lam et al. 1979; Zeller et al. 1984), fluoro analogues of 2-chloroethyl nitrosoureas (Johnston et al. 1984), nitrosoureas with sugar moieties (Akaike et al. 1982) including chlorozotocin (Talley et al. 1981) and compounds which are both highly water soluble and lipophilic, such as N-(2-chloroethyl)-N′-(2-hydroxyethyl)-N-nitrosourea (HECNU) (Eisenbrand 1984).

In this paper we report the synthesis and the preliminary results of testing on L 1210 leukemia of a series of nitrosoureas containing thiol, disulfide or thiolcarbamate groups, derived from cysteamine.

Chemistry

N′-[N-(2-Chloroethyl)-N-nitroso]carbamoyl cysteamine (CNC-cysteamine, 3) was obtained by reaction of cysteamine with CNC-azole (Eisenbrand 1978). The corresponding disulfide, N′,N″-bis[N-(2-chloroethyl)-N-nitroso]carbamoyl cysteamine (bis(CNC)cysteamine, 5) was isolated as a by-product in 15% yield. It evidently forms by reaction of CNC-az-
ide with cystamine, generated by concomitant oxidation of cysteamine, or forms directly by concomitant oxidation of CNC-cysteamine itself (Scheme 1).

\begin{align*}
\text{HS-CH}_2\text{-CH}_2\text{-NH}_2 + \text{Cl-CH}_2\text{-CH}_2\text{NCO-N}_3 \rightarrow \text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} \\
\text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} \rightarrow \text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \\
\text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} \rightarrow \text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \\
\text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} \rightarrow \text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \\
\text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} \rightarrow \text{HS-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO}
\end{align*}

Scheme 1

The reaction of cystamine with CNC-azide led to 5 as the major product. Formation of N’-[N-(2-chloroethyl)-N-nitroso]carbamoyl cystamine carboxylazide (CNC-cystamine carboxylazide, 6) was observed under these reaction conditions as a side reaction, yielding about 10% of 6. This side reaction was not observed in the absence of triethylamine (Scheme 2).

\begin{align*}
\text{S-CH}_2\text{-CH}_2\text{-NH}_2 + \text{Cl-CH}_2\text{-CH}_2\text{NCO-N}_3 \rightarrow \text{S-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \\
\text{S-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \rightarrow \text{S-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \\
\text{S-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \rightarrow \text{S-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \\
\text{S-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO} \rightarrow \text{S-CH}_2\text{-CH}_2\text{-NH-CO-N-CH}_2\text{-CH}_2\text{-Cl} + \text{NO}
\end{align*}

Scheme 2

The synthesis of 5 has already been described (Imbach et al. 1981). The authors reported that 5 was obtained by the reaction of cystamine with N-Hydroxysuccinimide N-(2-chloroethyl)-N-nitroso carbamate (Martinez et al. 1982). By nitrosation of bis(2-chloroethylcarbamoyl)cystamine, mixtures of two isomers, containing 35% of compound 5 (Imbach et al. 1981) or of three isomers, with only 1% of compound 5 (Fahri et al. 1984) have been obtained.

The previously undescribed compound 6 contains a bifunctionally alkylating nitrosourea, connected via a disulfide bridge to a carbamoyl azide. The usefulness of 6 in attaching the nitrosourea group covalently to appropriate carrier molecules, such as peptides, proteins or hormones is under study at present.

2-Chloroethyl isocyanate (7) carbamoylates not only the amino group, but also the thiol group of cysteamine, yielding N-(2-chloroethyl)carbamoyl cysteamine 2-chloroethylcarbamate (8). The nitrosation of 8 with dinitrogen tetroxide in an organic solvent or by means of NaNO₃ in anhydrous formic acid produces a mixture of two dinitroso isomers: CNC-cysteamine N-(2-chloroethyl)-N-nitosocarbamate (9) and N’-[N-(2-chloroethyl)carbamoyl]-N’-nitrosocysteamine N-(2-chloroethyl)-N-nitosocarbamate (10). Moreover, 3 can also be carbamoylated by 2-chloroethyl isocyanate to form CNC-cysteamine 2-chloroethylcarbamate (11). Nitrosation of 11 under the same conditions mentioned above did not give the expected compound 9 alone, but a mixture containing 9 and 10. The composition of nitrosation products was established by elemental analysis and by ¹H NMR spectral data. The ¹H NMR spectrum of the nitrosation mixture showed a complete lack of the signal of the NH-proton of the carbamate moiety, whereas the ¹H NMR spectrum of 11 showed a triplet of this proton at δ = 5.82 ppm (Fig. 1).

In the ¹H NMR spectra, all compounds containing a CNC-group showed the characteristic pattern of two triplets at δ = 3.5 ppm (Cl-CH₂) and at δ = 4.1–4.2 ppm (CH₂-N-NO) (Johnston et al. 1966). The peaks of N-CH₂ protons of the cysteamine moiety were located between the above mentioned triplets. The peaks of 8-CH₂ protons appeared upfield. The free SH group of 3 showed up as a triplet (D₂O exchangeable) at δ = 1.49 ppm (Fig. 1).

In the IR spectra (KBr), peaks of the following groups were prominent: NH(3315–3335 cm⁻¹); amide I (1660–1708 cm⁻¹); amide II (1530–1535 cm⁻¹); N-N=O (1490 cm⁻¹) and C-C₁ (1440 cm⁻¹). For com-

Fig. 1. The ¹H NMR spectra of CNC-cysteamine and some related compounds, from top down: CNC-cysteamine(3), bis(CNC)cysteamine(5), CNC-cysteamine carboxylazide(6), CNC-cysteamine 2-chloroethyl-carbamate(11), and mixture of CNC-cysteamine N-(2-chloroethyl)-N-nitosocarbamate and N’-[N-(2-chloroethyl)carbamoyl]-N’-nitrosocysteamine N-(2-chloroethyl)-N-nitosocarbamate(9/10)