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NITROSOUREAS

Modes of action and perspectives in the use of hormone receptor affine carrier molecules

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Abstract

Mechanisms of DNA adduct formation by antineoplastic 2-chloroethyl-N-nitrosoureas (CNUs) and of DNA damage induced by these compounds are discussed. CNUs are alkylating agents that form DNA-DNA cross-links as well as 2-chloroethylated and 2-hydroxyethylated adducts, the N-7-position of guanine being the predominantly alkylated site. A close correlation exists between the potential of a given compound to induce DNA-DNA cross-links and its antineoplastic effectiveness. However, levels of DNA-DNA cross-linking in bone marrow and extent of myelosuppression as measured in rodents are also closely correlated. The design of new cross-linking analogues capable of directing the antineoplastically relevant activity predominantly to the target tumour appears therefore to be of great promise. Cross-linking agents have been attached to a variety of steroid hormone carrier molecules and the conjugates have been tested in structure-activity studies using hormone-receptor containing animal tumours. These studies have revealed that some hormone-linked antineoplastic agents are highly effective in receptor positive experimental tumours and are superior to mixtures of unlinked alkylating agents with hormones. Indications for a relative enrichment of DNA damaging effects in the tumour tissue and for reduced myelotoxicity have been obtained with specific hormone conjugates.

Key words: Alkylating, cross-linking nitrosoureas, estradiol-linked, dihydrotestosterone-linked, CNC-amino acids, steroid esters, mammary, prostatic cancer.

N-(2-chloroethyl)-N-nitrosoureas (CNUs) are highly active anticancer agents with a broad antitumour spectrum in experimental models (1). Representatives of the first-generation in this class, such as N,N′-bis-(2-chloroethyl)-N-nitrosourea (BCNU), N-(2-chloroethyl)-N′-cyclohexyl-N-nitrosourea (CCNU), N-(2-chloroethyl)-N′-(4-methylcyclohexyl)-N-nitrosourea (MeCCNU) (Fig. 1) were introduced into the clinic in the early 60s in rapid succession because of their activity against Hodgkin and non-Hodgkin lymphomas, brain tumours, gastrointestinal tumours and some other neoplasms (2). Their clinical application, however, is limited because they show delayed and cumulative toxic side effects, bone-marrow suppression being prevalent and dose-limiting.

In the last 20 years, much effort was put forth by many groups to understand the molecular mechanisms in their actions and to obtain new congeners with higher effectiveness and/or lower toxicity (3–7). Many mechanistically important findings applying also to other agents have been obtained with this group of compounds. Among the CNUs of the second generation, 2-(N-(2-chloroethyl)-N-nitrosoureido)-D-deoxyglucopyranose (Chlorozotocin), N-(2-chloroethyl)-N′-(2-hydroxyethyl)-N-nitrosourea (HECNU), and N′-(N-(2-chloroethyl)-N-nitrosocarbamoyl)-glycinamide (CNC-glycinamide) are all characterized by their good solubility in water (Fig. 1). Chlorozotocin is a water soluble CNU analogue tested clinically (3), because it appeared to be less myelotoxic in animal experiments. It was, however, also less active in experimental tumour systems than CNUs of first generation (4–6). In clinical studies Chlorozotocin did not show reduced myelotoxicity (7).

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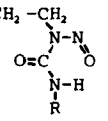
CNU:



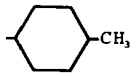
CNU derivative	-R
N,N'-Bis(2-chloroethyl)-N-nitrosourea (BCNU)	$-CH_2-CH_2-Cl$
N-(2-Chloroethyl)-N'-cyclohexyl-N-nitrosourea (CCNU)	
N-(2-Chloroethyl)-N'-(4-methylcyclohexylnitrosourea (methyl-CCNU)	
2-[N-(2-Chloroethyl)-N-nitrosoureido]-D-deoxyglucopyranose (chlorozotocin)	-D-2-deoxyglucopyranose
N-(2-Chloroethyl)-N'-(2-hydroxyethyl)-N-nitrosourea (HECNU)	$-CH_2-CH_2-OH$
N-[N'-(2-Chloroethyl)-N'-nitrosocarbamoyl]-glycinamide (CNC-glycinamide)	$-CH_2-CONH_2$

Fig. 1. Structures of some N-(2-chloroethyl)-N-nitrosoureas (CNUs).

Part of our own work has been devoted to the synthesis of water soluble analogues which, unlike chlorozotocin, should retain, as far as possible, their lipophilic properties. Lipophilicity is considered to be essential to the antineoplastic effectiveness of such compounds, especially against intracerebral tumours. By exchanging a chlorine atom in BCNU in the chloroethyl group at N'-position against a hydroxyl group, a new congener, HECNU, was obtained. HECNU is about 30 times more water soluble than BCNU, but still possesses a high lipophilicity, as exemplified by a log P (octanol/water) of 0.3 (8). The biological activity and some results from studies on the mechanism of the action of HECNU will be described below.

Mechanism of action

The molecular mechanisms by which CNUs exert their antitumour and toxic effects are rather complex. They are monofunctional as well as bifunctional alkylating agents, DNA alkylation and cross-linking being considered an important factor in their activity. Pathways of CNU-decomposition to highly reactive alkylating and carbamoylating species that have been proposed by several groups are summarized in Fig. 2. Chloroethyldiazohydroxide and hydroxyethyldiazohydroxide or the equivalent bifunctional electrophiles and chloroethylisocyanate are considered the main ultimate reactive agents. The monofunctional alkylating intermediate hydroxyethyldiazohydroxide alkylates RNA and DNA, forming monoadducts, whereas the bifunctional alkylating intermediate chloroethyldiazohydroxide alkylates RNA and DNA, forming monoadducts and DNA–DNA interstrand or DNA-protein cross-links. The isocyanate carbamoylates the functional groups of peptides or proteins (Fig. 2) (9).

According to Buckley (10) formation of a gem-diol or diolate tetrahedral intermediate, as first proposed by Snyder & Stock (11) and by Lown & Chauhan (12), appears the most probable pathway, since the collapse of this intermediate accounts for all products derived from CNUs (Fig. 3).

CNUs are known to produce DNA–DNA cross-links and good correlations have been observed between killing of various cell lines and the production of interstrand cross-links (13). Interstrand cross-link formation by CNUs is a two-step process involving rapid alkylation through the ultimate electrophile, 2-chloroethyldiazohydroxide or the like, followed by a much slower second alkylation through nucleophilic displacement of the chlorine at the beta carbon by an appropriate site of a DNA base (14). Hydroxyethylation represents by far the greatest proportion of DNA alkylation in vitro. The process of

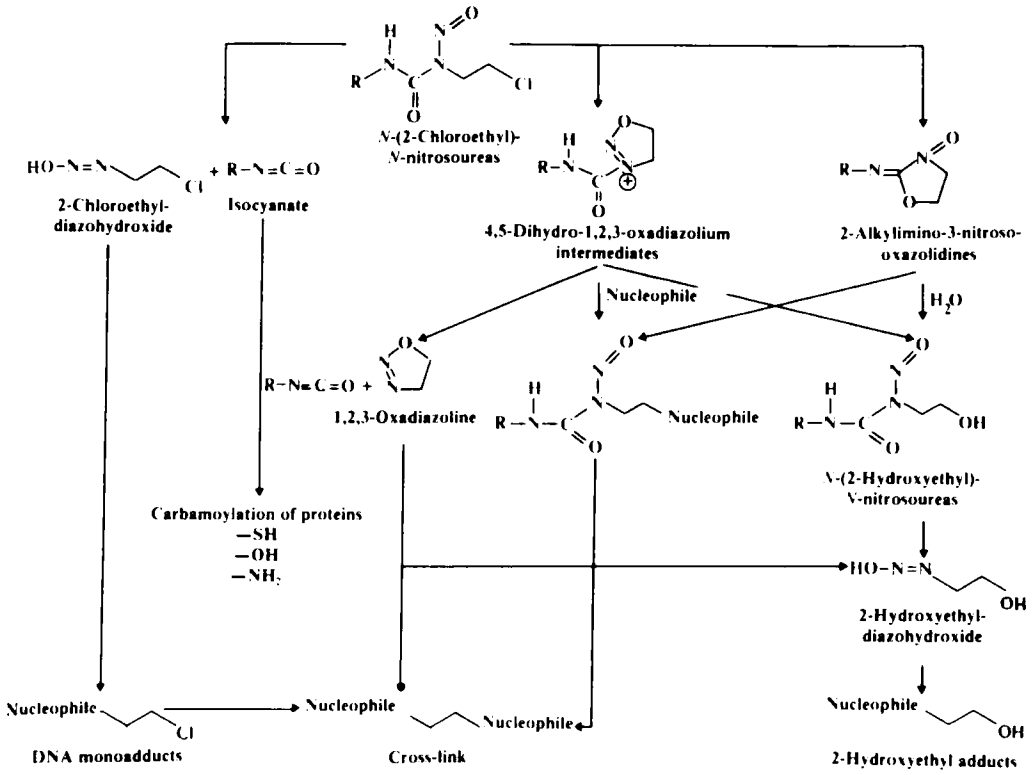


Fig. 2. Possible reactions of derivatives of N-(2-chloroethyl)-N-nitrosoureas in vivo (according to (12)).

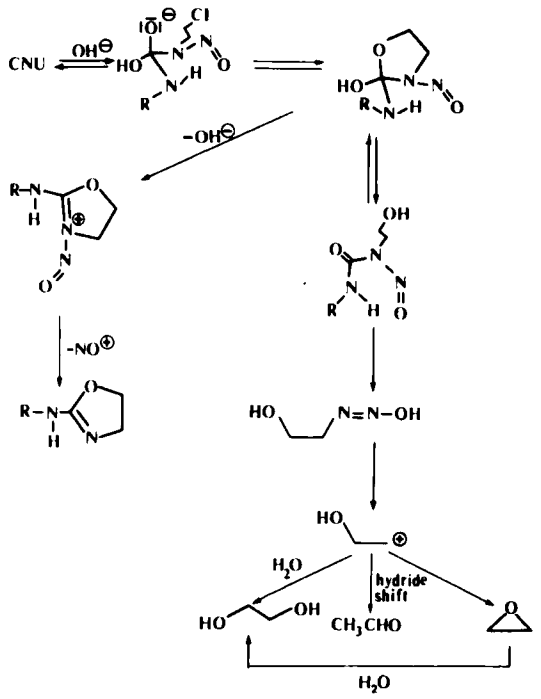


Fig. 3. Possible pathway for the decomposition of N-(2-chloroethyl)-N-nitrosoureas (according to (11), modified).

generating hydroxyethylating electrophiles from 2-haloethylnitrosoureas is impressively effective: even β-fluorine, an atom normally bound firmly to carbon, can easily be replaced by a hydroxyl group. Hydroxyethyl derivatives are not formed hydrolytically from haloethyl derivatives of DNA bases (14). Instead, intermediate formation of 3-nitroso oxazolidine derivatives appears to be responsible, as has been shown by Lown & Chauhan (12), who synthesized these compounds and elucidated their reactivity towards nucleophiles (Fig. 3).

The biological consequences of hydroxyethylation have been investigated by a comparative study on the antileukemic activities of CNUs and their respective N-(hydroxyalkyl)-N-nitroso isomers (15). As can be seen in Table 1, there is a clear difference between N-(2-chloroethyl)-N-nitrosourea analogues and their 2-hydroxyethyl counterparts: the former, with the intrinsic ability to cross-link, are much more active. It can be concluded that, if at all, 2-hydroxyethylation contributes only to a small extent to antileukemic activity, as shown here for rat leukemia L 5222. On the other hand, N-(2-hydroxyethyl)-N-nitrosourea analogues have been found to be strongly mutagenic and carcinogenic (16, 17), suggesting that 2-hydroxyethylation probably is a lesion more relevant for malignant transformation than for antitumour efficacy.

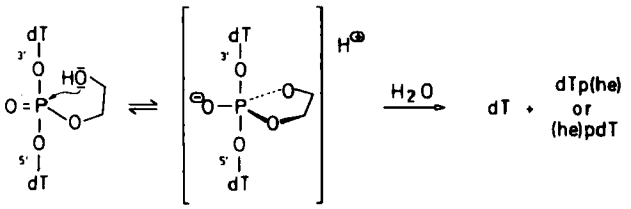


Fig. 4. Proposed mechanism of (2-hydroxyethyl)-phosphotriester decomposition.

Furthermore, the DNA phosphate groups also can be alkylated by alkylating carcinogens (18, 19). Lown & McLaughlin (20) distinguished 2 rates of DNA strand cleavage in vitro induced by CNUs. A relatively fast single strand scission at high pH that is caused by hydrolysis of the alkali labile phosphotriesters and a second one which is a much slower process generated by apurinic sites. Studies on the stability of 7-alkylated guanosines have been reported (21). A quantitative study on the rates of hydrolysis of di(2'-deoxythymidine)-phosphotriesters, generated by alkylation with methylnitrosourea (MNU) or 2-hydroxyethylnitrosourea, showed that the methylphosphotriester was stable for more than 3 days at pH 7.4 whereas the corresponding 2-hydroxyethyl analogue was much more unstable with a half-live of 60 min at pH 7.4 (22).

A strong instability of the 2-hydroxyethylphosphotriesters can be reconciled with intermediate formation of a dioxaphospholane ring that decomposes into phosphodiesters, releasing (2'-deoxythymidine)-5'-(2-hydroxyethyl)-phosphate ((he)pdT), and 2'-deoxythymidine (dT) as shown in Fig. 4 (22). Obviously, introduction of 2-hydroxyethyl phosphotriester groups strongly decreases the stability of the sugarphosphate back bone of DNA, eventually resulting in DNA single strand breaks (23).

In addition to alkylation, carbamoylation by isocyanates also induces a multiplicity of biomolecular effects. It has been shown, for instance, to inhibit the ligation of DNA strand breaks induced by x-rays (24), to inhibit repair of alkylated DNA (25) and to inhibit excision repair of UV-irradiated DNA (26). Moreover, reduction of glutathione levels in rodent liver (27) inhibition of glutathione-reductase in erythrocytes (28, 29) and other effects due to carbamoylation of proteins (30) have been described.

Inhibition of glutathion (GSH) reductase is a well known side effect of strongly carbamoylating CNUs, such as BCNU. In the lung this has been found to be a risk factor in inducing pulmonary fibrosis seen in about 30% of patients treated with this drug, most probably by inactivating a very important defense mechanism against oxidative stress (31).

A comparison of GSH reductase inhibitory effects in lung and brain of rats shows that the strongly carbamoylating agent BCNU causes a strong inhibition of GSH reductase in these organs whereas practically no effect is

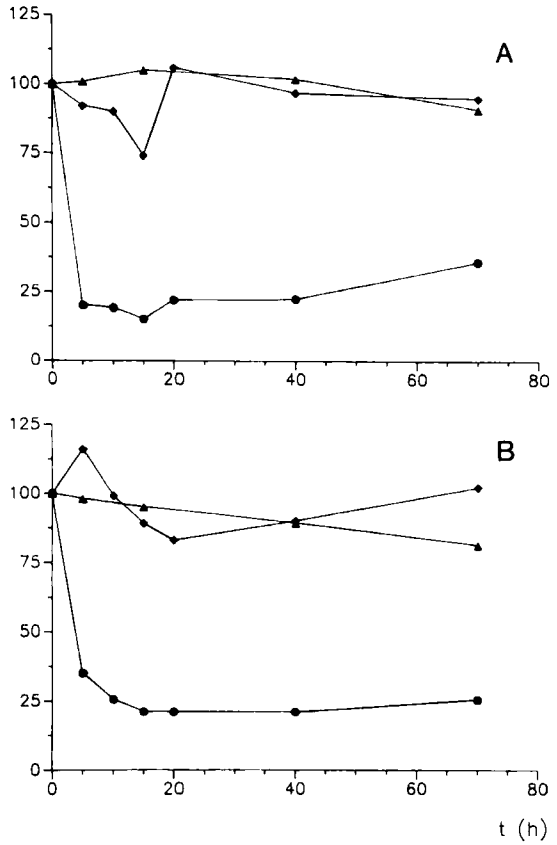
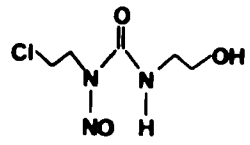
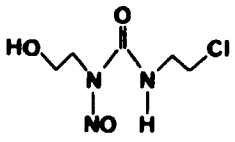
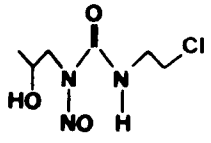
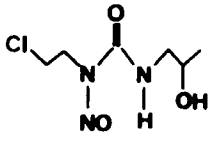


Fig. 5. Inhibition of glutathione-reductase by HECNU (◆—◆), CNC-glycinamide (CNC-GA (▲—▲)) and BCNU (●—●) in lung (A) and brain (B).

Table 1

Antileukemic activities of N-(2-chloroethyl)-N'-hydroxyalkyl-N-nitrosoureas and their isomeres N-hydroxyalkyl-N'-(2-chloroethyl)-N-nitrosoureas against mouse leukemia L1210 and rat leukemia L 5222

	L1210	L5222
	+++	++
	–	+
	–	+
	+++	++

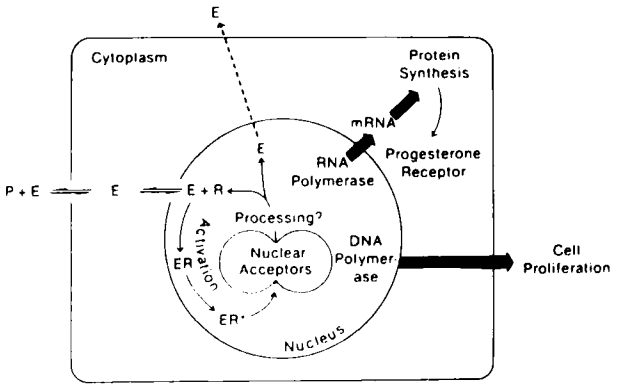


Fig. 6. Model of oestrogen action. Plasma oestrogen (E) diffuses directly into the nucleus, where it binds with nuclear oestrogen receptor (R), and initiates protein synthesis and cell proliferation (according to (38)).

Table 2

Relative binding affinities of estradiol (E_2) derivatives to oestrogen receptors of calf uterus cytosol

Substance	RBA-value (%)
3-CNC-L-alanyl-E_2	4.7
17-CNC-L-alanyl-E_2	0.8
3,17-bis-(CNC-L-alanyl)-E_2	0.05
3-CNC-L-alanyl-L-alanyl-E_2	10.0
17-CNC-L-alanyl-L-alanyl-E_2	2.8
6α-CNC-L-alanyl-E_2	0.28
E_2	100

seen after HECNU application, although the latter is penetrating much easier the blood/brain barrier (Fig. 5). Therefore, inhibition of GSH reductase appears to be a factor probably of relevance in determining differential toxicity of these agents, especially after repeated administration.

Furthermore, reaction products of various isocyanates with glutathione have been studied for chemical and biological activities (32). In general, reaction with glutathione represents an important detoxification pathway for isocyanates generated in vivo by nitrosourea decomposition.

However, 2-chloroethylisocyanate, the decomposition product of BCNU, reacts with glutathione by forming 2-chloroethyl-S-carbamoyl glutathione. This GSH adduct is exceptional because it reacts with DNA-bases as an alkylating agent, transferring a 2-aminoethyl group to N^7 of guanosine (32). This lesion can either lead to depurination, creating an apurinic site in DNA, or might even more easily lead to imidazole ring opening (21). As could be expected, 2-chloroethyl-S-carbamoyl glutathione was found to be a strong mutagen in the Ame's test and a potent inducer of DNA strand breaks in a human lymphoid cell line. In summary, whereas for all other CNUs GSH-carbamoylated adducts were either not genotoxic or were not formed at all. BCNU forms a strongly genotoxic intermediate by reaction with GSH. This might well explain why BCNU has been found to be much more toxic and more carcinogenic than other CNUs in comparative long-term studies under repeated application (33).

Steroid linked N-(2-chloroethyl)-N-nitrosocarbamoyl (CNC)-amino acids

Many human tumours have been found to contain hormone receptors, e.g. mammary carcinoma, prostatic tumours, various gastrointestinal tumours and others (34–37). The rationale of synthesizing hormone-linked antineoplastic agents is to benefit from the selective binding of hormones to their receptors in receptor-containing tissues, thereby directing a cytotoxic agent more selectively to target cells. A model for oestrogen action on the subcellular level and its translocation to nuclear acceptor sites after binding to a nuclear receptor protein is demonstrated in Fig. 6 (38).

An important parameter for the potential value of a given steroid-linked derivative is its affinity to the corresponding hormone receptor (39). Relative binding affinities (RBA-values) were measured in receptor preparations from calf uterus cytosol according to established methods (40). Biological activity was tested on receptor-positive tumour models, such as MNU-induced mammary carcinoma in rats, MXT mammary carcinoma in mouse and Noble Nb-R prostatic carcinoma in rats. Some results are discussed here.

Among the estradiol (E_2)-linked CNC-amino acids, a marked influence of the position of the ester bond on the RBA-value can be seen (Table 2). The 6-α-ester, for example, has a very low affinity, although the OH-groups considered relevant for receptor interaction are left free, but this is in accordance with a rather low RBA-value for the parent 6-α-hydroxyestradiol itself (6.3%). On the other hand, relatively high apparent RBA-values of the 3-esters were found to result from cleavage of the phenolic ester bond under the incubation conditions, liberating E_2 (41). This competes for the binding site, causing an apparently higher RBA-value. Dipeptide esters invariably exhibit higher RBA-values than the amino acid esters.

Table 3 shows the extent of DNA–DNA cross-links induced by CNC-ala-E_2-17-ester in the bone marrow and in mammary tumours of the rat (42), in comparison with cross-links induced in the same organs after administration of an equimolar mixture of CNC-L-alanine and E_2. Cross-links form at a much higher rate after application of the ester, as compared to the equimolar mixture. In comparison to CNC-ala, the CNC-ala-E_2-17-ester induces about 3 times more cross-links in the bone marrow but about 8 times more in the receptor-containing tumour tissue.

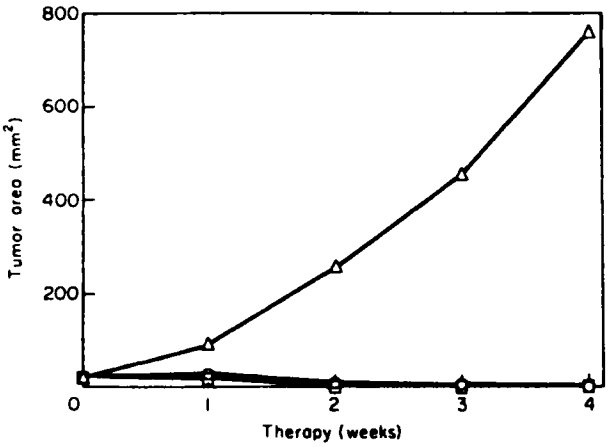


Fig. 7. Chemotherapy of Noble Nb-R prostate carcinoma of the rate with 19-nortestosterone-linked CNC-amino acids. O—O 17-CNC-glyc-19-nortestosterone, □—□ 17-CNC-L-alanyl19-nortestosteron, △—△ control.

The biological activity of the E_2-linked CNC-amino acid esters in MNU-induced rat mammary carcinoma is summarized in Table 4 (43, 44).

As can be seen in Table 4 remarkable differences in biological activity became apparent with regard to the site of binding to the carrier. The 17-position of E_2 is superior to the other positions tested for linking CNC-L-alanine. The 17-ester not only shows highest antitumour effectiveness, but also lowest toxicity. Optimum antitumour effectiveness is already reached at 75 μmol/kg. CNC-L-alanine alone and the unlinked mixture of CNC-L-alanine and E_2 are definitely inferior. The considerable tumour-inhibitory effect of the unlinked mixture at 75 μmol/kg appears to be a combined effect of both components. E_2 is known to have tumour-inhibitory activity on hormone-dependent mammary carcinoma at pharmacological doses. However, toxicity of the unlinked mixture was considerably higher at the 75 μmol/kg dose level, causing 90% mortality at week 10. Linking of CNC-L-alanine at 3-position of E_2 yields a compound that exerts a moderate antineoplastic effectiveness but is highly toxic even at the lowest dose. The corresponding E_2-3,17-diester is definitely less active, showing a similarly high and unacceptable toxicity. The analogue 6-α-ester displays no significant antineoplastic effect but is highly toxic. The lack of antitumour effect of the latter 2 compounds correlates with their lower RBA-values. Ovariectomy had a remarkable tumour-inhibitory effect at week 4, that, however, to a great extent was lost at week 7 (43).

Hormonal side effects and antitumour activities appear not to be correlated. In the Dorfman uterotrophic activity test, the 3- and 17-ester as well as the 3,17-diester were of similar activity, although their antitumour effects were quite different (43).

In treatment of hormone-independent tumours the 17-ester lost its therapeutic advantage and did not differ from CNC-L-alanine (43). This confirms the importance of receptor contents for therapy by this agent.

To further verify the potential validity of the hormone carrier concept CNC-amino acides of androgens were also synthesized. Affinities to androgen, progesterone and oestrogen receptors were determined (Table 5). Antineoplastic activity of selected compounds was tested in a prostatic tumour model as well as in MNU-induced mammary carcinoma of the rat, that also has been shown to contain androgen receptors.

Table 3

DNA–DNA cross-links (rad-equiv.) in mammary-carcinoma and bone marrow 16 h after intraperitoneal administration of 75 μmol/kg CNC-L-ala-E_2-17-ester, CNC-L-alanine and of a mixture from equimolar CNC-L-alanine and estradiol

Treatment	DNA–DNA-cross-links (rad.-equiv.)	
	Mamma-ca.*	Bone-marrow
CNC-L-ala	7	6
CNC-L-ala + E_2	14	4
17-CNC-L-ala-E_2	54	19

* Pooled tumour tissue.

Table 4

Antineoplastic activity of CNC-ala esters of E_2, in comparison with unlinked single agents and ovariectomy. Compounds were given on days 1, 8, 22, 29 after randomization in equimolar dosage, i.p., dissolved in DMSO

Substance	Dose (μmol/kg)	% T/C* week		% mortality week		
		4	7	4	7	10
Control		100	100	0	15	50
Ovariectomy		12	69	0	10	30
CNC-ala	45	62	63	0	20	50
	67	38	39	20	30	70
	101	32	50	70	70	90
E_2 + CNC-ala	54 each	25	32	0	10	20
	75 each	42	24	0	70	90
CNC-ala-E_2-3-ester	75	48	44	10	50	90
	105	21	35	30	50	90
	147	15	23	40	70	100
CNC-ala-E_2-17-ester	38	19	51	0	30	70
	54	9	12	0	20	30
	75	18	10	0	30	30
	105	19	26	0	10	70
	147	25	34	0	50	90
	206	10	19	20	80	90
6α-CNC-alanyloxy OH-E_2-6-ester	38	72	107	0	60	90
	75	96	94	30	80	90
3,17-bis(CNC-alanyl)E_2	54	38	97	0	20	90
	75	28	48	0	20	80
	105	16	29	0	50	100

* Mean tumour volume of treated rats in % of untreated control.

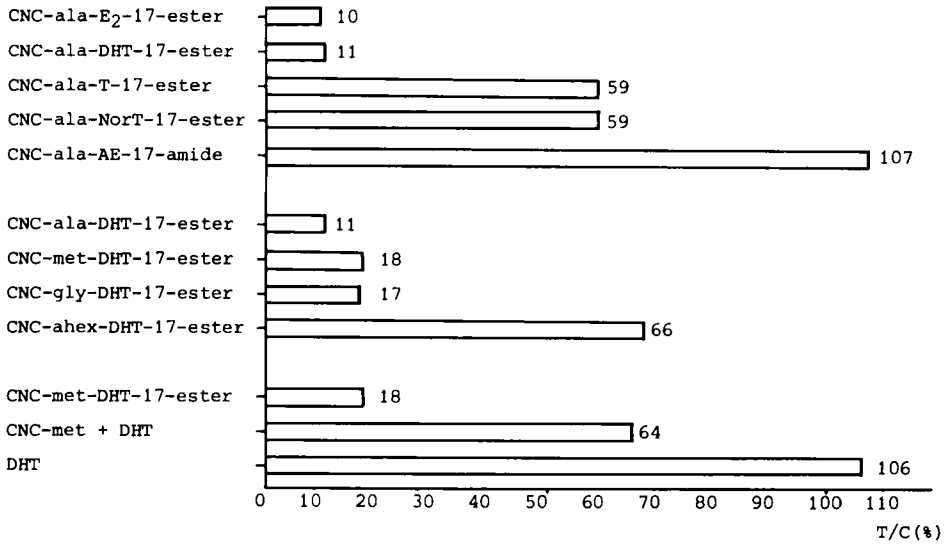


Fig. 8. Structure activity relationship of steroid-linked CNC-amino acid esters in therapy of MNU-induced mammary carcinoma in rats. Treatment: esters at optimal dose levels on days 1, 8, 22, 29 after randomization, i.p.; evaluation: 6 weeks after beginning of therapy.

Table 5

Receptor binding affinities of androgen-linked CNC-amino acid esters to receptor of calf uterus cytosol

Substances		Relative binding affinity to receptor (% RBA)		
CNC-amino acid	Steroidhormone	Oestrogen-	Progesterone-	Androgen-
CNC-glycine	Testosterone	<0.01	0.6	1.0
CNC-sarcosine	Testosterone	<0.01	0.2	1.7
CNC-L-alanine	Testosterone	<0.01	0.3	1.9
CNC-L-methionine	Testosterone	<0.01	0.4	2.4
CNC-glycine	Dihydrotestosterone	<0.01	0.7	4.3
CNC-sarcosine	Dihydrotestosterone	<0.01	0.2	3.5
CNC-L-alanine	Dihydrotestosterone	<0.01	0.12	5.0
CNC-L-methionine	Dihydrotestosterone	<0.01	0.4	4.5
CNC-glycine	19-nortestosterone	<0.01	1.8	4.4
CNC-sarcosine	19-nortestosterone	<0.01	1.7	4.7
CNC-L-alanine	19-nortestosterone	<0.01	0.8	7.5
CNC-L-methionine	19-nortestosterone	<0.01	2.6	6.1

As can be seen derivatives of 19-nortestosterone appear to display higher binding affinities to both receptors, as compared to those of testosterone or dihydrotestosterone. There is no detectable affinity to the oestrogen receptor (45).

Results of therapy of the Noble Nb-R prostatic carcinoma of the rat are shown in Fig. 7 (46).

The CNC-alanine and CNC-glycine esters of 19-nortestosterone were given i.p. in DMSO at a dose of 50 mg/kg on days 1, 7 and 21. Both substances display very high tumour-inhibitory effectiveness, tumours being no longer detectable after termination of therapy. Weights of prostates and of seminal vesicles were not significantly reduced indicating low androgenic side effects. At equimolar dosage, CNC-alanine was much more toxic than the 19-nortestosterone ester. Animals died before termination of the experiment from strong systemic toxicity with a preference for nephrotoxicity. These results show that binding to the carrier hormone brings about a very remarkable decrease in toxicity of this cytostatic agent.

Structure activity relationships of the steroid-linked CNC-amino acid esters in treatment of MNU-induced mammary carcinoma in rats are summarized in Fig. 8. It is noticeable that at optimal dose level the dihydrotestosterone (DHT)-17-ester of CNC-alanine showed a tumour-inhibitory activity comparable to that of the corresponding E_2-17-ester, whereas the corresponding testosterone-(T)-17-ester and the 19-nortestosterone (NorT)-17-ester

only had marginal activity. The 3-hydroxy-17-amino-1,3,5(10)estratrien-(AE)-17-amide of CNC-L-alanine (CNC-ala-AE-17-amide) did not show any effect. Among the DHT-17-esters of various CNC-amino-acids there was no remarkable difference in activity. Esters of CNC-ω-aminohexanoic acid (ahex), however, had only insignificant effectiveness. The unlinked mixture of CNC-L-methionine and DHT was much less active than the corresponding ester. DHT alone was totally ineffective.

Studies on bone marrow toxicity of CNC-ala and the 17-esters of E_2 and DHT were carried out by measuring effects on pluripotent bone marrow stem cells (CFU-s) in mice. Substances were given to NMRI female mice by i.p. injection (days 1, 8, 22, 29 at a dose of 34 mg/kg). The results show that CNC-alanine is practically not influencing CFU-s, whereas application of E_2 results in some transient depletion at day 8 with recovery at week 4. In contrast, 17-CNC-L-alanyl-E_2 is cumulatively toxic, showing a strongly damaging effect to CFU-s after 4 applications. In contrast to the E_2-ester, the corresponding DHT analogue behaves quite differently: initially, a slight reduction to about 30% after the first application was seen, but thereafter a rapid recovery back to pretreatment values was observed, although treatment was continued up to the full dose (4×35 mg/kg).

This finding shows that by attachment to an appropriate hormone carrier bone marrow toxicity of nitrosoureas can be suppressed without concomitant loss in antitumour efficacy. Other parameters of bone marrow function showed essentially the same.

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REFERENCES

1. Johnston TP, McCaleb GS, Opliger PS, Montgomery JA. The synthesis of potential anticancer agents. XXXVI. Nitrosoureas. II. Haloalkyl derivatives. J Med Chem 1966; 9: 892–911.
2. Carter SK, Schabel FM Jr, Brodes L, Johnston TP. 1.3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and other nitrosoureas in cancer treatment. Adv Cancer Res 1972; 16: 273–332.
3. Johnston TP, McCaleb GS, Montgomery JA. Synthesis of chlorozotocin, the 2-chloroethyl analog of the anticancer streptozotocin. J Med Chem 1975; 18: 104–6.
4. Zeller WJ, Eisenbrand G, Fiebig HH. Examination of four newly synthesized 2-chloroethylnitrosoureas in comparison with BCNU, CCNU, MeCCNU, chlorozotocin and hydroxyl-CNU in preterminal rat leukamia L 5222. J Cancer Res Clin Oncol 1979; 95: 43–9.
5. Fiebig HH, Eisenbrand G, Zeller WJ, Zentgraf R. Anticancer activity of new nitrosoureas against Walker carcinosarcoma 256 and DMBA-induced mammary cancer of the rat. Oncology 1980; 37: 177–80.
6. Spreafico I, Filippeschi S, Falautano P, et al. The development of novel nitrosoureas. In: Prestayko AW, Baker LH, Crooke ST, Carter SK, Schein PS, eds. Nitrosoureas, current status and new developments. New York: Acad. Press. 1981: 175–91.
7. Talley RW, Samson MK, Brownlee RW, Samhouri AM, Fraile AM, Baker LH. Phase II evaluation of chlorozotocin (NSC-178248) in advanced human cancer. Eur J Cancer 1981; 17: 337–43.
8. Eisenbrand G, Fiebig HH, Zeller WJ. Some new congeners of the anticancer agent 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU). Synthesis of bifunctional analogues and water soluble derivatives and preliminary evaluation of their chemotherapeutic potential. Z Krebsforsch 1976; 86: 279–86.
9. Eisenbrand G. Anticancer nitrosoureas: Investigations on antineoplastic, toxic and neoplastic activities. In: O'Neill IL, von Vorstel RC, Miller CT, Long J, Bartsch H. eds. N-Nitroso compounds: Occurrence, biological effects and relevance to human cancer (IARC Sci Publ No 57), Lyon: Int Agency for Res on Cancer 1984: 695–708.
10. Buckley W. Structure-activity relationship of N-(2-chloroethyl)-N-nitrosoureas. 1. Deuterium isotope effects in the hydrolysis of 1-(2-chloroethyl)-1-nitrosoureas: evidence for the rate-limiting step. J Org Chem 1987; 52: 484–8.
11. Snyder JK, Stock LM. Reactions of alkylnitrosoureas in aqueous solution. J Org Chem 1980; 45: 1990–9.
12. Lown JW, Chauhan SMS. Synthesis of specifically ^{15}N- and ^{13}C-labeled antitumor (2-haloethyl)nitrosoureas. The study of their conformations in solution by nitrogen-15 and carbon-13 nuclear magnetic resonance and evidence for stereoelectronic control in their aqueous decomposition. J Org Chem 1981; 46: 5309–21.
13. Sariban E, Kohn KW, Zlotogorski C, et al. DNA cross-linking responses of human malignant glioma cell strains to chloroethylnitrosoureas, cisplatin and diaziquone. Cancer Res 1987; 47: 3988–94.
14. Ludlum DB, Tong WP. Modification of DNA and RNA bases by the nitrosoureas. In: Serrou B, Schein PS, Imbach JL, eds. Nitrosoureas in cancer treatment. Amsterdam: Elsevier, Biomedical Press, 1981: 21–31.
15. Zeller WJ, Lijinsky W, Eisenbrand G. Antitumor activity of 1-nitroso-1-(2-chloroethyl)-3-(hydroxyalkyl)ureas and of 1-nitroso-1-(hydroxyalkyl)-3-(2-chloroethyl)ureas. J Cancer Res Clin Oncol 1985; 109: A46.
16. Lijinsky W, Reuber MD. Carcinogenicity of hydroxylated alkylnitrosoureas and of nitrosooxazolidones by mouse skin painting and by gavage in rats. Cancer Res 1983; 43: 214–21.
17. Lijinsky W, Singer GM, Kovatch RM. Similar carcinogenic effects in rats of 1-ethyl-1-nitroso-3-hydroxyethyl-urea and 1-hydroxyethyl-1-nitroso-3-ethylurea. Carcinogenesis 1985; 6: 641–3.
18. O'Conner PJ. Interaction of chemical carcinogens with macromoleculs. J Cancer Res Clin Oncol 1981; 99: 167–86.
19. Singer B. All oxygens in nucleic acids react with carcinogenic ethylating agents. Nature 1976; 264: 333–9.
20. Lown JW, McLaughlin LW. Nitrosourea-induced DNA single-strand breaks. Biochem Pharmacol 1979; 28: 1631–8.
21. Müller N, Eisenbrand G. The influence of N^7-substituents on the stability of N^7alkylated guanosines. Chem Biol Interactions 1985; 53: 173–81.
22. Conrad J, Müller N, Eisenbrand G. Studies on the stability of trialkylphosphates and di-(2'-deoxythymidine)phosphotriesters in alkaline and neutral solution. A model study for hydrolysis of phosphotriesters in DNA and on the influence of a β-hydroxyethylester group. Chem Biol Interactions 1986; 60: 57–65.

23. Eisenbrand G, Müller N, Denkel E, Sterzel W. DNA adducts and DNA damage by antineoplastic and carcinogenic N-nitroso compounds. J Cancer Res Clin Oncol 1986; 112: 196–204.
24. Fornace AJ, Kohn KW, Kann HE. Inhibition of the ligase-step of excision repair by 2-chloroethyl isocyanat, a decomposition product of 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 1978; 38: 1064–9.
25. Heal JW, Fox PA, Schein PS. Effects of carbamoylation on the repair of nitrosourea-induced DNA alkylation damage in L 1210 cells. Cancer Res 1979; 39: 82–9.
26. Kann HE Jr, Schott MA, Petkas A. Effects of structure and chemical activity on the ability of nitrosoureas to inhibit DNA repair. Cancer Res 1980; 40: 50–5.
27. McConnell WR, Kari P, Hill DL. Reduction of glutathione levels in livers of mice treated with N,N'-bis(2-chloroethyl)-N-nitrosourea. Cancer Chemother Pharmacol 1979; 2: 221–3.
28. Frisher H, Ahmad T. Severe generalized glutathione reductase deficiency after antitumor chemotherapy with BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea). J Lab Clin Med 1977; 89: 1080–91.
29. Babson JR, Reed DJ. Inactivation of glutathione reductase by 2-chloroethylnitrosourea derived isocyanate. Biochem Biophys Res Commun 1978; 83: 754–62.
30. Reed DJ. Metabolism of nitrosoureas. In: Prestayko AW, Baker LH, Crooke ST, Carter SK, Schein PS, eds. Nitrosoureas, Current status and new developments. New York: Acad Press, 1981: 51–67.
31. Kehrer, JP Klein-Szanto AJP. Enhanced acute lung damage in mice following administration of 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 1985; 45: 5707–13.
32. Stahl W. Untersuchungen zur Reaktion von 2-Chlorethylnitrosoharnstoffen mit biologisch relevanten Thiolgruppen (Dissertation). Kaiserslautern: University of Kaiserslautern, 1987.
33. Habs M. Experimentelle Untersuchungen zur cancerogenen Wirkung zytostatischer Arzneimittel, Habilitationsschrift, Heidelberg: University of Heidelberg, 1980.
34. Knight WA, Livingston RB, Gregory EJ. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. Cancer Res 1977; 37: 4669.
35. Pichon MF, Pallud C, Brunet M, Milgron E. Relationship of progesterone receptors to prognosis in early breast cancer. Cancer Res 1980; 40: 3357.
36. Raynaud JP, Ojasso T. Tags for steroid receptors. Clin Neuropharmacol 1984; 7: 325.
37. Sica V, Nola E, Contieri E, et al. Estradiol and progesterone receptors in malignant gastrointestinal tumours. Cancer Res 1984; 44: 4670.
38. Mirecki DM, Jordan VC. Steroid hormone receptors and human breast cancer. Lab Medicine 1985; 16: 287–94.
39. Rochefort H, Garcia M. Interaction and actions of androgens on the estrogen receptor. In: Genazzani E, ed. Pharmacological modulation of steroid action, New York: Raven Press, 1980: 75–80.
40. Schneider MR. Entwicklung antineoplastischer Substanzen zur Therapie von Mamma- und Prostatacarcinom. Habilitationsschrift. Regensburg: Universität Regensburg, 1987.
41. Schreiber J. Darstellung steroidverknüpfter N-(2-chlorethyl)-N-nitroso-Harnstoffe und Untersuchung ihrer biologischen Wirkung (Dissertation). Heidelberg: Universität of Heidelberg, 1985.
42. Henne T, Berger MR, Schreiber J, Eisenbrand G, Zeller WJ, Floride JA. Levels of DNA–DNA interstrand cross-linking in various organs of SD-rats after treatment with estradiol-linked N-(2-chloroethyl)-N-nitrosocarbamoyl (CNC)-L-alanines. J Cancer Res Clin Oncol 1985; 109: A 41–Ther 18.
43. Berger M, Floride J, Schmähl D, Schreiber J, Eisenbrand G. Estrogen-linked 2-chloroethylnitrosoureas: Anticancer efficacy in MNU-induced rat mammary carcinoma, uterine activity in mice and receptor interactions, Eur J Cancer Clin Oncol 1986; 22: 1179–91.
44. Berger MR, Floride J, Schreiber J, Schmähl D, Eisenbrand G. Evaluation of new estrogen-linked 2-chloroethylnitrosoureas. J Cancer Res Clin Oncol 1984; 108: 148–53.
45. Eisenbrand G, Berger MR, Fischer J, Schneider MR, Tang W, Zeller WJ. Development of more selective anticancer nitrosoureas. Anti-Cancer Drug Design 1988; 2: 351–9.
46. Eisenbrand G, Berger M, Fischer J, Schneider M, Zeller W, Tang W. N-(2-chloroethyl)-N-nitrosocarbamoyl amino acid derivatives of steroid hormones. Cancer Treat Rev 1987; 14: 285–90.