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Received November 14, 1975.
Accepted for publication January 26, 1976.
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Differing Antitumor Activities of the Hydrochloride and Methiodide Salts of 1-Ethyl-3-(3′-dimethylaminopropyl)-carbodiimide

Keyphrases □ 1-Ethyl-3-(3′-dimethylaminopropyl)carbodiimide—hydrochloride and methiodide salts, antitumor activity compared, mice □ Antitumor agents, potential—1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide, hydrochloride and methiodide salts compared, mice

To the Editor:

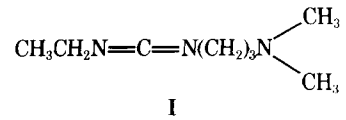
Cellular surface modifications effected by the reaction of mouse neuroblastoma C1300 and carcinoma TA3 cells with 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride (II) have been demonstrated *in vitro* to result in an increased susceptibility of these cells to immune lysis by soluble antibodies (1). This carbodiimide derivative also has been shown to exert an *in vivo* antitumor activity. On the basis of the following lines of evidence, this activity appears to result from an enhanced host immune reactivity against drug-modified tumor cells:

1. Chemotherapeutic effectiveness against neuroblastoma C1300 solid tumors increases with increasing host levels of cytotoxic serum antibodies (1).

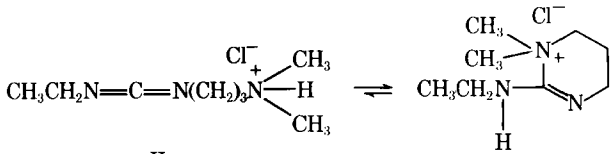
2. Drug inhibition of TA3 ascites carcinoma growth is not observed in hosts that have received an immunosuppressive 700-rad whole body dose of cobalt-60 radiation (2).

In addition to having chemotherapeutic potential, the *in vivo* action of this carbodiimide derivative may be useful for tumor diagnosis using the scintillation camera, since the enhancement of host immune reactions in neoplastic tissue could create a favorable condition for the selective uptake of radioisotopically labeled compounds (3).

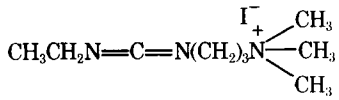
On the basis of NMR and IR evidence, the water-soluble hydrochloride salt[1] (II) of the carbodiimide base 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (I) has been found to exist in aqueous solution at physiological pH as an equilibrium mixture of two isomeric species: 7.4% open chain carbodiimide hydrochloride (II*a*) and 92.6% 2-ethylamino-3,3-dimethyl-3,4,5,6-tetrahydropyrimidine chloride (II*b*) (4, 5). Because of the structural complexity introduced by isomerism of II, we synthesized and tested the chemotherapeutic activity of the water-soluble carbodiimide salt 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide methiodide (III), which is formed by reaction of I with methyl iodide (4).



I



II*a* II*b*



III

NMR and IR studies showed that III exists exclusively as the open chain carbodiimide at physiological pH (5). From a comparison of the biological effects of II and III, we sought to gain evidence as to whether the structural features of II—apart from its chemically reactive carbodiimino functionality—are important factors in the antitumor action exerted by this compound.

In vitro cytotoxicity assays were carried out to assess the relative abilities of II and III to increase the complement-dependent (6) antibody-mediated lysis of neuroblastoma C1300 target cells. The antiserum used was drawn from A/HeJ mice[2] bearing 21-day-old neuroblastoma C1300 tumors. The antiserum was added to test cultures so that the final dilution was 1:640 (v/v). Rabbit serum was absorbed with C1300 cells by the method of Boyse *et al.* (7) to remove naturally occurring toxic substances (8); the absorbed rabbit serum was then placed in test cultures at a final dilution of 1:40 (v/v) as a source of the complement.

Lysis of C1300 target cells was determined from the percentage release of the intracellular chromium-51 label during an 8-hr incubation at 37°. The method used to label C1300 target cells with chromium-51, the preparation of the antiserum and complement, and details of the cytotoxicity assay procedure were described previously (1). Compounds II and III were added to test cultures at an identical concentration of 0.16 m*M*. On the basis of drug tolerance studies, this concentration of carbodiimide was determined to be the maximum that was not toxic to C1300 cells over the 8-hr incubation used in all cytotoxicity assays.

Results of *in vitro* cytotoxicity assays are summarized in Table I. Neuroblastoma C1300 target cell lysis by A/HeJ immune serum increased significantly when II was added to test cultures (from 13.4 to 27.8%). Addition of the open chain carbodiimide III, however, did not lead to increased target cell immune lysis.

[1] Ott Chemical Co., Muskegon, Mich.

[2] Jackson Laboratory, Bar Harbor, Me.

Table I—Effect of Carbodiimide Derivatives on Neuroblastoma C1300 Immune Lysis

Serum and Dilution (v/v)	Complement-Dependent C1300 Target Cell Lysis[a], %		
	Serum	Serum + II (0.16 m*M*)	Serum + III (0.16 m*M*)
Immune A/HeJ[b] (1:640) + rabbit complement (1:40)	13.4 ± 2.1 (6)[c]	27.8 ± 2.5 (6)	13.2 ± 0.9 (2)

[a] The percentage of complement-dependent antibody-mediated C1300 target cell lysis was determined from the release of intracellular chromium-51 label during an 8-hr incubation at 37° (1). The percent lysis is expressed relative to a "background" release of chromium-51 label measured in control cultures. Contents of control cultures were the following: (*a*) C1300 target cells alone; (*b*) target cells and II (0.16 m*M*); (*c*) target cells and III (0.16 m*M*); (*d*) target cells and rabbit complement; (*e*) target cells, rabbit complement, and II (0.16 m*M*); (*f*) target cells, rabbit complement, and III (0.16 m*M*); (*g*) target cells and immune serum; (*h*) target cells, immune serum, and II (0.16 m*M*); and (*i*) target cells, immune serum, and III (0.16 m*M*). The average percent release of chromium-51 label did not differ significantly among these cultures and was 25.3 ± 0.7 (*SE*). Additional controls provided the following information: (*a*) a 1:640 dilution of serum from nonimmunized A/HeJ mice was not cytotoxic to C1300 target cells; and (b) antiserum from A/HeJ mice immunized against C1300 cells was not cytotoxic to unrelated target cells, specifically A/HeJ spleen cells. [b] Immune serum was drawn from syngeneic A/HeJ mice bearing 21-day-old C1300 solid tumors. [c] Mean ±*SE* (number of determinations).

Previous *in vivo* chemotherapy trials demonstrated that II exerts an antitumor activity against neuroblastoma C1300 tumors only during advanced stages of growth (3 weeks posttransplantation), when the host level of circulating cytotoxic antibody is high (1). This pattern of drug response is in distinct contrast to that observed with antimetabolites [*e.g.*, 6-thioguanine (1)], which are most effective against young, rapidly growing tumors. We, therefore, tested the relative chemotherapeutic effectiveness of II and III by administering these compounds to A/HeJ mice bearing 21-day-old neuroblastoma C1300 tumors. The procedures used for the preparation of tumors and injected drugs were described previously (1).

Compounds II and III were dissolved in 0.9% NaCl solution and injected intraperitoneally in a volume of 0.2 ml; control mice received 0.2 ml of 0.9% NaCl solution. The dosage of II and III used in chemotherapy trials, 50 mg/kg, was the maximum tolerated level for A/HeJ mice. The chemotherapeutic effect of II and III was measured as a *T/C* ratio, defined as the average tumor weight of drug-injected mice divided by the average tumor weight of 0.9% NaCl solution-injected mice at 7 days postinjection. Calculation of a T/C ratio was based on tumor weights from 10 pairs of mice; each drug-injected mouse was matched with a control 0.9% NaCl solution-injected mouse bearing a C1300 tumor of identical size and shape at the time of injection.

In vivo chemotherapy trials demonstrated that a 50-mg/kg ip dose of II exerted a significant inhibitory effect on the subsequent growth of 21-day-old C1300 tumors, with the T/C ratio at 7 days postinjection being 0.51. Based on the Student *t* test, the difference in the average weight of tumors from drug-injected and 0.9% NaCl-injected control mice was significant at $p < 0.001$. Compound III did not exert a statistically significant chemotherapeutic effect. Following administration of a 50-mg/kg ip dose of III to mice bearing 21-day-old C1300 tumors, the T/C ratio at 7 days postinjection was 0.94.

Results presented here thus indicate that the open chain carbodiimide III fails to enhance tumor immune lysis *in vitro* or to exert an antitumor effect *in vivo*, in distinct contrast to the activity demonstrated for the carbodiimide derivative (II). This observation indicates that the chemotherapeutic effect of II is not associated solely with its chemically reactive carbodiimino functional group but depends as well on other aspects of molecular structure.

In this regard, one principal difference between II and III is the permanent charge of the quaternary amine of III in contrast to the partially uncharged state of the tertiary amine of II*a* [pKa ~ 10.75 (5)]. As a consequence, II may partition into and chemically modify the tumor cell surface to a greater extent than III. The difference in charge character of II and III may also influence their metabolism, relative tissue distributions, and plasma clearance rates in a manner that favors a greater *in vivo* chemotherapeutic effect by II.

A second major structural difference between II and III is the existence of II predominantly as the reduced pyrimidine species (II*b*). The potential role of this structure in the antitumor activity of II remains obscure, but information could possibly be gained through the synthesis and biological testing *in vitro* and *in vivo* of new carbodiimide compounds that isomerize to form heterocyclic structures.

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Received July 28, 1975.
Accepted for publication January 9, 1976.
Supported by U.S. Atomic Energy Commission Contract W-7405-eng-48 with the Lawrence Berkeley Laboratory.
The technical assistance of Alice Beckmann is gratefully acknowledged.

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