Catecholamine Analogs as Potential Antitumor Agents

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Abstract □ The structural requirements for murine P-388 leukemia activity in the dopamine series were investigated. Eight of the 31 analogs evaluated possessed reproducible antitumor activity. The *ortho*-aromatic hydroxyl groups were required for activity, but the aminoethyl side chain was not crucial since this group could be replaced by methyl or aminomethyl groups with the retention of activity. The lack of activity of *O*-alkylated and monosubstituted analogs suggests that *o*-quinone formation may be important for activity, a possibility supported by the observed P-388 activity of 5-hydroxydopamine compared with the inactivity of the 6-hydroxy isomer.

Keyphrases □ Pyrocatecholamine—analogs, antitumor activity, structure–activity relationship □ Antitumor activity—pyrocatecholamine analogs, structure–activity relationship □ Structure–activity relationship—pyrocatecholamine analogs, antitumor activity

In a previous study of the antitumor properties of psychotropic drugs (1), dopamine (I) was found to possess reproducible activity against P-388 and L-1210 leukemia in mice. This compound had been shown earlier to be active against the sarcoma-180 tumor model (2), while conflicting reports exist on I activity against B16 melanoma (1, 3). Certain other catecholamine neurotransmitters and their precursors were reported to have cancer-related properties (2, 4–8). Even catechol (9) and simple *o*-aminophenols (10) were reported to be antitumor active. The objective of this study was the establishment of the structural features critical to *in vivo* catecholamine P-388 leukemia antitumor activity.

EXPERIMENTAL[1]

Antitumor activity was determined as percent T/C values[2], with T/C ≥125% defined as statistically significant (Table I). Dose–response studies were carried out for each compound according to published NCI protocols (11). Six mice per dose were treated intraperitoneally with 10^6 P-388 leukemia cells on Day 0. Control (untreated) mice usually died about Day 11. Mice receiving drug were treated on Days 1–9 with intraperitoneal doses of the compound under investigation. Physiological saline (0.9%) was the vehicle.

Duplicate tests were carried out on each compound. When the duplicate test results did not agree (one active and one inactive test), a third dose–response experiment was scheduled. The mice (average weight 20 g) were weighed on Day 5, and the weight difference between the treated and control mice (T − C) was taken as an indication of the dose toxicity. Weight losses greater than 4 g were considered excessive.

RESULTS

Dopamine hydrochloride activity against P-388 leukemia was reconfirmed (Table I). This compound was active in five of five experiments. L-Dopa (II), the physiological precursor to dopamine, was inactive. The initial part of the study focused on β-phenethylamine analogs (III–XIV) of dopamine (I). *N*-Methyldopamine (III) retained activity. Either mono- or dialkylation of the phenolic hydroxyl groups (IV and V) converted dopamine into inactive compounds. Removal of the hydroxyl group from position 3 (tyramine, VI) also abolished activity. Neither the monosubstituted analogs (VI–IX) nor unsubstituted β-phenethylamine (X) was active.

Table I—P-388 Lymphocytic Leukemia Antitumor Activity

Compound	Dose Range, mg/kg/day	Optimum Dose, mg/kg/day	T/C, %	T − C, g
I	600–37.5	600	168	−1.7
	1200–150	600	153	−1.6
II	600–100	400	102	−0.9
	600–100	600	109	−0.5
III	600–100	400	156	−0.5
	600–100	600	126	−2.8
IV	600–100	200	110	−2.5
	600–100	100	108	−0.7
V	600–100	600	114	+0.1
	600–100	400	107	−1.5
VI	600–100	200	102	−0.7
	600–100	200	101	−0.1
VII	600–50	50	106	−1.7
	600–50	50	101	−2.2
VIII	400–50	50	105	+0.9
	400–50	50	100	−1.9
IX	400–50	100	105	−3.0
	400–50	50	104	+0.1
X	600–50	50	104	−1.8
	600–50	50	104	−0.9
XI	200–1.56	1.56	106	0.0
	200–1.56	1.56	94	+0.4
XII	600–50	50	111	−1.2
	600–50	100	106	−0.6
XIII	50–6.25	12.5	101	−0.9
	50–6.25	25	100	−0.7
XIV	600–50	200	131	−3.7
	600–50	400	129	−2.7
XV	50–6.25	50	111	−3.1
	50–6.25	25	101	−3.9
XVI	400–50	400	168	−3.6
	600–50	400	153	−4.1
XVII	200–50	100	110	+1.5
	200–50	50	105	−1.2
XVIII	700–350	700	158	−2.9
	700–350	600	144	−2.6
XIX	600–100	100	116	+0.3
	600–100	100	104	−0.3
XX	600–100	200	109	−0.6
	600–100	200	105	−1.2
XXI	100–6.25	6.25	116	−0.7
	100–6.25	6.25	110	−0.8
XXII	600–50	100	109	−0.1
	600–50	50	103	+0.4
XXIII	50–3.12	6.25	113	0.0
	50–3.12	3.12	112	−0.5
XXIV	400–25	200	164	−2.7
	400–25	200	133	−2.2
XXV	600–50	200	131	−1.4
	600–50	100	129	+0.4
XXVI	600–50	50	111	−1.8
	600–50	50	104	−3.5
XXVII	600–50	100	107	0.0
	600–50	100	105	0.0
XXVIII	600–50	400	115	−1.3
	600–50	400	111	−3.8
XXIX	600–50	100	118	−0.9
	600–50	50	103	+0.5
XXX	600–50	400	132	−2.2
	600–50	600	126	−1.4
XXXI	600–50	200	118	−0.7
	600–50	600	111	−0.8

[1] The compounds studied were obtained from the Developmental Therapeutics Program of the National Cancer Institute (NCI) or from commercial sources (Aldrich and Sigma). The P-388 lymphocytic leukemia testing was carried out at Hazleton Laboratories, Vienna, Va., under NCI Contract N01-CM5-7007.

[2] Percent T/C = (treated survival/control survival) × 100.

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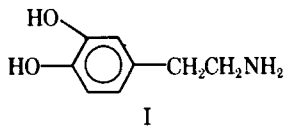
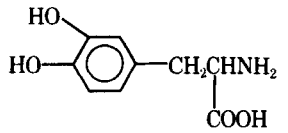


I



II

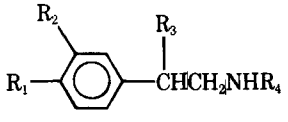
Among the four α-hydroxy compounds tested (XI–XIV), only isoproterenol (XIV) was reproducibly active. *dl*-Norepinephrine (XI) was inactive; *l*-epinephrine was P-388 inactive in earlier NCI testing. When a third hydroxyl group was added to dopamine in position 6 (XV), a P-388-inactive material resulted. When the extra hydroxyl group was added to position 5, however, the resulting 3,4,5-trihydroxy-β-phenethylamine (XVI) was as active as I.

The availability of benzylamine analogs of dopamine (XVIII–XXII) allowed evaluation of the importance of the aminoethyl side chain. Compound XVIII possessed good, reproducible P-388 activity. However, alkylation of one (XIX) or both (XX) hydroxyl groups again produced inactive materials. Halogens (XXI) could not be substituted for hydroxyl groups with retention of activity.

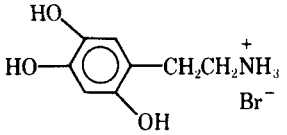
Since the dopamine aminoethyl group did not appear critical for activity, 4-substituted catechol (pyrocatechol) analogs with simple side chains (XXIII–XXX) were evaluated. Replacing the aminoethyl group in I with either a methyl (XXIV) or an aldehyde (XXV) function provided active compounds, but none of the four carboxyl-containing analogs (XXVI–XXIX) was active. Although catechol (XXIII) was inactive, *o*-aminophenol (XXX) possessed modest, but reproducible, activity. Gallic acid (XXXI), the carboxyl counterpart of active 5-hydroxydopamine (XVI), was inactive. The high optimal doses observed for most active compounds in this series were indicative of their relative nontoxicity.

DISCUSSION

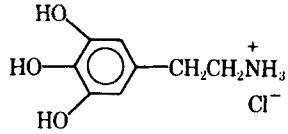
Eight of the 31 compounds evaluated possessed reproducible activity against P-388 leukemia. These compounds (I, III, XIV, XVI, XVIII, XXIV, XXV, and XXX) are *o*-dihydroxybenzene (catechol) derivatives



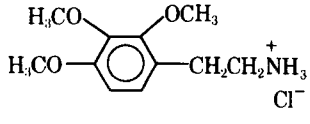
phenethylamine analogs	R_1	R_2	R_3	R_4	salt
I	OH	OH	H	H	HCl
III	OH	OH	H	CH_3	—
IV	OCH_3	OCH_3	H	H	—
V	OH	OCH_3	H	H	HCl
VI	OH	H	H	H	HCl
VII	OCH_3	H	H	H	—
VIII	NH_2	H	H	H	—
IX	NO_2	H	H	H	HCl
X	H	H	H	H	—
XI	OH	OH	OH	H	—
XII	OH	H	OH	H	HCl
XIII	H	OH	OH	H	HCl
XIV	OH	OH	OH	isopropyl	HCl



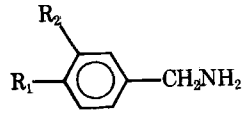
XV



XVI



XVII



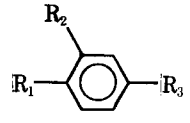
benzylamine analogs	R_1	R_2	salt
XVIII	OH	OH	HBr
XIX	OH	OCH_3	HCl
XX	OCH_3	OCH_3	—
XXI	Cl	Cl	—
XXII	H	H	—

and analogs. While the lead compound, dopamine, is a neurotransmitter, this physiological property may be related only coincidentally to P-388 activity since the analogous aminomethyl (XVIII) and methyl (XXIV) compounds also are active.

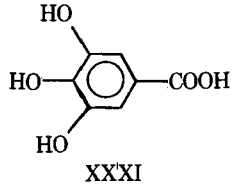
The fact that alkylation of one or both hydroxyl groups converts active compounds into inactive ones (I, IV, and V; XVIII, XIX, and XX) suggests that biological oxidation to *ortho*-quinones may be related to the observed antitumor activity. Studies of the oxidative pathways of brain catecholamines and the chemical reactivity of their oxidation products are consistent with this speculation. Tse *et al.* (12) showed that dopamine *o*-quinone was exceptionally reactive toward nucleophilic attack by cysteine or glutathione sulfhydryl groups. These reactions occurred 10^3 times faster than intramolecular cyclization by the aminoethyl side chain. Graham *et al.* (13, 14) studied the oxidation of dopamine, epinephrine, and norepinephrine and found dopamine to be the most easily autoxidized but the slowest to undergo intramolecular cyclization, thus giving it an opportunity to react with external nucleophiles.

Quinones as catechol oxidation products also have been suggested as biologically reactive intermediates (4, 8, 10, 15–20). Many of these studies were concerned with the elucidation of relationships existing between catecholamine oxidation products and malignant melanoma or neuroblastoma. While quinone–sulfhydryl interactions are studied most commonly, catecholamines also have been observed to cause DNA breaks (2), to inhibit prolactin secretion (4), and to be involved in the production of cytotoxic hydrogen peroxide and superoxide or hydroxyl radicals (14, 21, 22). A number of *o*-quinones tested by NCI were cytotoxic *in vitro* against KB cells (23). Several of these compounds possessed *in vivo* sarcoma 180 or Walker 256 activity. Furthermore, quinoid formation may possibly play a role in the P-388 activity of the polyhydroxypyridines (24).

The results indicate that within the series of compounds studied: (*a*) *o*-hydroxyl groups are required for activity; (*b*) *N*-methylation (III) of dopamine does not abolish activity but *O*-methylation (IV and V) does; (*c*) the aminoethyl side chain is not crucial for activity; (*d*) isoproterenol (XIV), but not epinephrine or norepinephrine, is active; (*e*) catechol is not active, but the 4-methyl analog (XXIV), a depigmenting agent which was used previously as a model compound in catechol oxidation (12) and



catechol analogs	R_1	R_2	R_3
XXIII	OH	OH	H
XXIV	OH	OH	CH_3
XXV	OH	OH	CHO
XXVI	OH	OH	COOH
XXVII	OH	OH	CH_2COOH
XXVIII	OH	OH	CH_2CH_2COOH
XXIX	OH	OH	CH=CHCOOH
XXX	OH	NH_2	H



XXXI

antimelanoma studies (25, 26), possesses activity; and (*f*) no carboxyl-containing catechol is active.

The data from the testing of the trihydroxyphenethylamines (XV and XVI) indicate that it may be important to restrict potential quinone formation possibilities to only an *o*-quinoid structure. The P-388-inactive neurotoxin, 6-hydroxydopamine (XV), when oxidized, can form either an *o*- or a *p*-quinone. When given this choice, quinones in general and XV in particular (13) form hydroxy-*p*-quinones, which weakly react with sulfhydryl groups (17). The other trihydroxy compound studied, 5-hydroxydopamine (XVI), can form only an *o*-quinone. It has good P-388 activity. Therefore, one would expect that 2-hydroxydopamine, the perdemethyl analog of XVII, should have P-388 activity. Studies are in progress to determine further structure–activity relationships among polyhydroxy derivatives of benzene and pyridine.

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Adsorption of Antineoplastic Drugs to Polyalkylcyanoacrylate Nanoparticles and Their Release in Calf Serum

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Received February 6, 1979, from the **Laboratoire de Pharmacie Galénique, Université Catholique de Louvain, B-1200 Brussels, Belgium*, and the ‡*School of Pharmacy, Federal Institute of Technology, Zürich, Switzerland.* Accepted for publication June 25, 1979.

Abstract □ Conditions are described for attaching anticancer drugs to polyalkylcyanoacrylate nanoparticles, a new drug delivery system for cells that exhibit endocytic uptake. They are ultrafine, metabolizable, and able to associate with various drugs in a nonspecific manner. Data are given for the *in vitro* degradation and for drug release from this new drug carrier system.

Keyphrases □ Polyalkylcyanoacrylate nanoparticles—adsorption and release of dactinomycin and methotrexate, liberation kinetics, controlled release □ Dactinomycin—controlled-release dosage forms, polyalkylcyanoacrylate nanoparticles, adsorption and release kinetics □ Methotrexate—controlled-release dosage forms, polyalkylcyanoacrylate nanoparticles, adsorption and release kinetics □ Dosage forms, controlled release—polyalkylcyanoacrylate nanoparticles, adsorption and release of dactinomycin and methotrexate

Entrapment of cytotoxic drugs inside endocytizable carriers such as liposomes improves drug specificity and reduces toxicity toward nondiseased cells (1, 2). Work in this field has resulted in the development of polyacrylamide nanocapsules (3, 4). Polyacrylamide nanocapsules also may be useful in promoting cellular uptake *via* endocytosis for compounds that do not gain access to lysosomes (5).

Due to their polymeric nature, these small capsules (diameter of ~200 nm) may be more stable than liposomes in biological fluids and during storage (6–9). Furthermore, they can entrap various molecules in a stable and reproducible way (3, 5). However, this new lysosomotropic carrier is unlikely to be digested by lysosomal enzymes, which may restrict its clinical use. With these considerations, biodegradable nanoparticles made by polymerization of various alkylcyanoacrylate monomers were developed recently (10, 11). Similar polymers are used in surgery as sutures and adhesive agents (12, 13).

This paper describes techniques for attaching two cytostatic drugs to these polyalkylcyanoacrylate particles. Data concerning the degradability of these nanoparticles and drug liberation from this new intracellular formulation (14) are presented also.

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