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(54) Title: PROGLUMIDE AND PHARMACEUTICAL COMPOSITIONS CONTAINING IT FOR USE IN THE TREATMENT OF NEOPLASTIC AFFECTIONS

(57) Abstract

A new therapeutic use of D,L-4-benzamido-N,N-dipropyl-glutamic acid (proglumide) in the treatment of neoplastic affections.

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Proglumide and pharmaceutical compositions containing it for use in the treatment of neoplastic affections.

The subject of the present invention is a new therapeutic use of D,L-4-benzamido-N,N-dipropyl-glutamic acid (proglumide) and its pharmaceutically-acceptable salts, for use in the treatment of neoplastic affections in which there is a pathological cell increase indirectly affected by gastrin, cholecystokinin (CCK), other bioactive peptides or by related mechanisms which are not yet well clarified.

The applicants have found that the said drug, already widely used in the treatment of gastric ulcers (see for example Merck Index No. 7680, 10th edition), in fact has an unexpected but extremely interesting therapeutic activity, which is that of significantly inhibiting the cell increase of tumour cells resulting from chemically induced pancreatic, colic or gastric cancer or of destroying such cells.

The invention is based on the discovery that the polypeptide hormone gastrin has a trophic effect on the digestive epithelium, just as the hormone CCK has a trophic action on pancreatic acini. Thus the chronic administration of gastrin to rats (or of pentagastrin which is the biologically active part of the physiological hormone) causes hyperplasia of the fundic and colic mucous membranes. Recently (Winsett et al - Surgical Forum, 33, 384 (1982)) it has been shown that gastrin stimulates the growth, in mice, of transplantable colic tumours obtained by chemical induction.

It has also been shown that the chronic treatment of rats with cerulein (a synthetic analog of CCK) causes hyperplasia of the pancreatic acinus cells (Solomon et.

al. - An. J. Physiol 235, E714-719 (1978)).

On the basis of these theoretical premises, there has been a desire to experiment to see whether proglumide might behave as an antagonist to the growth of induced gastro-intestinal tumours and to evaluate whether this action could also affect the survival time of the animals.

This antitumoral activity of proglumide, which is the subject of the present invention, will thus be illustrated by a series of pharmacological experiments in vitro and in vivo arranged to show both the qualitative and quantitative aspects of the antitumoral activity and the mechanism by which this activity is manifested.

Experiment 1

Action of Proglumide on the rate of pentagastrin-induced growth of normal and tumoral colic cells.

Male mice having a weight of 20-25g were innoculated subcutaneously in the interscapular region with a suspension of 8×10^4 colic-adenocarcinoma tumour cells taken from a mouse.

Four groups of 12 animals were used, that is: a group of control animals, a group of animals treated with 200 mg/kg i.p. doses of proglumide 3 times a day, a group of animals treated with 200 µg/kg of pentagastrin every eight hours and a group of animals treated with proglumide and pentagastrin in the manner indicated above.

After 20 days the animals were killed and the fundic mucous membranes and tumours were removed, weighed and extracted to determine the DNA. The results obtained are given in Table 1.

TABLE 1

Antagonistic activity of Proglumide towards the growth of colic tumours induced by pentagastrin

GROUPS	No Animals	Dose	Weight of the mucous membrane (mg)	student's t	fundic DNA (mg)	student's t	weight of the colic tumour (mg)	student's t	tumour DNA (mg)	student's t
A): Control	12	-	15.9 ± 1.44	-	0.21 ± 0.02	-	500 + 12.6	-	2.90 + 0.23	-
B): Pentagastrin (PEG)	10(°)	200 mcg/kg-3 per day	40.9 ± 1.26	12.78 (***)	0.49 ± 0.03	9.46 (***)	800 ± 12.8	16.6 (***)	4.56 ± 0.46	3.37 (**)
C): Proglumide (PR)	12	200 mcg/kg-3 per day	13.3 ± 1.58	1.21	0.12 ± 0.02	3.17 (**)	470 ± 11.5	1.75	2.54 ± 0.23	1.10
D): PEG + PR	12	200 mcg/KG PEG + 200 mg/kg PR	16.0 ± 1.27	vsA: 0.05 vsB: 13.7 (***)	0.21 ± 0.05	vsA:0 vsB: 4.43 (***)	519 ± 11.8	vsA: 1.11 vsB: 16.1 (***)	2.80 ± 0.24	vsA: 0.32 vsB: 3.57 (**)

(°) 2 animals died during the treatment

(**) : P < 0.01
(***) : P < 0.001

The data given in the table show that PEG causes both significant hyperplasia of the fundic mucous membrane and a significant increase in the weight of the mucous membrane tumour and of the DNA content. However proglumide, at the doses used, is capable of inhibiting significantly both of the trophic actions of pentagastrin .

Experiment 2

Antagonism of proglumide to the rate of incorporation of tritiated thymidine in colic tumour cells, stimulated and unstimulated by pentagastrin.

The groups of animals and the treatments were identical to those given in the preceding experiment. On the seventh day, two hours after the final treatment, the animals were killed and the tumours which had developed were explanted.

Small pieces of tissue were incubated at 37° for 30 minutes in a Dulbecco-modified Eagle-type culture medium together with 2 μCi of (3-H)-thymidine. The reaction was interrupted by the addition of 0.4N perchloric acid containing carrier thymidine. The samples were homogenised with 2 ml of 0.2N perchloric acid and RNA and proteins were removed by conventional techniques.

The DNA content of the samples was determined by the colorimetric method with diphenylamine (Biochem. J. 62, 315-323 (1956)).

The incorporation of the (3-H) thymidine in the DNA was determined by counting coupled aliquots of filtrate in a liquid scintillator under conditions suitable for counting the tritium.

The results were expressed as disintegrations per minute per microgram of DNA.

The results obtained are given in Table No. 2 and are expressed as a percentage variation with respect to the controls of the dpm of tritiated thymidine per mcg of DNA.

TABLE 2

Influence of Proglumide and Pentagastrine on the incorporation of tritiated thymidine in the synthesis of DNA in cultures of tumour cells in vitro.

GROUPS	DOSE	% VARIATION WITH RESPECT TO THE CONTROL GROUP (mean ± S.E.)	STUDENT'S t (P)
A: Controls		100.0±4.9	
B: Pentagastrin (PEG)	200 mcg/kg (3 per day)	285.0±20.3	vsA:8.859 (<0.001)
C: Proglumide (PR)	200 mg/kg (3 per day)	88.2±5.2	vsA:1.65
D: (PEG) + (PR)	200 mcg PEG (3 per day) + 200 mg PR (3 per day)	112.0±8.4	vsA:1.24 vsB:7.88 (<0.001)

From the data given in the table it is seen that pentagastrin accelerates the replication of DNA in colic tumour cells; in fact, at the doses used, the incorporation of tritiated thymidine, is found to be increased by about three times compared to the controls, an increase which, on a statistical basis, is highly significant.

Proglumide alone reduces the incorporation but not significantly, while the combined treatment with pentagastrin and proglumide means that the antagonistic action of the latter prevents the pentagastrin from reaching the target tissue and there is thus a clear reduction (statistically significant) in the incorporation of tritiated thymidine in the DNA compared with the group of animals treated solely with pentagastrin.

Experiment 3

In order to check the possibility that proglumide can influence the survival time of animals having a transplanted colic adenocarcinoma, the following experiment was carried out.

Male mice having a weight of 20-25g were innoculated subcutaneously, in the interscapular region, with a suspension of $1x10^5$ colic-adenocarcinoma tumour cells taken from a mouse. Four groups of 12 animals were used, that is: a group of control animals and three groups of animals treated with 100, 200 and 400 mg/kg i.p. doses of proglumide respectively, three times a day.

This treatment was continued up to the 35th day, the day on which the final control animal died. The

straight line of regression for the survival time of the various groups, starting from the first day on which a death occurred was calculated by the least squares method. It was thus possible to calculate an ED50, that is, the single daily dose of the drug capable of increasing the survival time of the animals by 50%.

The results obtained are given in Table No. 3.

TABLE 3

Effect of Proglumide on the survival time of mice innoculated with colic adenocarcinoma.

TREATMENT	DAYS ON WHICH DEATHS (INDICATED IN PARENTHESES OCCURRED) starting from time 0.	(%) DEATH TOTAL	CALCULATED STRAIGHT LINE OF REGRESSION (1) AND COEFFICIENT OF CORRELATION (r)	DAYS OF LIFE PER ANIMAL (AVERAGE)	ED50
Control group	17(1)-20(1)-22(2)-24(1)-26(2)-29(1)-30(1)-32(2)-35(1)	100	n° animals= 22.35 - 0.647 (r = 0.995)	25.3	-
Proglumide 100 mg/kp i.p.	20(1)-24(1)-27(2)-29(1)-30(1)-33(1)-34(2)	75	n° animals = 22.47 -0.545 (r = 0.98)	30.1	342.9 (r = 0.99)
Proglumide 200 mg/kg i.p.	22(1)-23(1)-25(1)-28(1)-30(2)-33(1)-35(1)	66.6	n° animals = 23.06 -0.55 (r = 0.99)	31.5	
Proglumide 400 mg/kg i.p.	23(1)-28(1)-31(1)-33(1)	33.3	n° animals = 18.82 -32 (r = 0.98)	40.4	

(1) The straight lines of regression were calculated from the day at which the first death occurred.

From an examination of the table it is seen that the treatment with proglumide significantly increases, and in a dose dependent manner, the survival time of animals innoculated with colic adenocarcinoma. This effect is probably related to the antagonistic action of proglumide towards endogenous gastrin which, as previously explained, has a stimulating effect on the growth of gastrointestinal tumour cells. An ED50 value of about 340 mg/kg was also calculated, this being the dose which enables the survival time of the animals to be doubled.

Experiment 4

Inhibiting action of proglumide on the rate of growth of pancreatic adenocarcinoma induced by CCK-8

Male hamsters were innoculated in the cheek pouch with a suspension of $1x10^5$ pancreatic adenocarcinoma tumour cells. After five days from the innoculation the animals were divided at random into four groups of 10 animals each, that is a control group, a group of animals treated with 250 mg/kg i.p. of proglumide three times a day, and a fourth group treated with proglumide and CCK-8 in the manner described above.

After 15 days of this treatment the animals were killed and the normal pancreas and the pancreatic tumours introduced into the cheek pouches were removed and weighed. The DNA was extracted and measured by conventional techniques.

The results obtained are given in Table 4 where they are expressed as average values $\pm$ S.E.

The data given in the Table show that the hormone cholecystokinin (of which CCK-8 is the biologically

active component), which has a trophic action on normal pancreatic cells, also stimulates the growth of pancreatic adenocarcinoma. Proglumide, which is a specific antagonist to CCK, antagonises both these actions of CCK-8 to a highly significant extent.

The experimental data given above seem fully to support the theory that the use of proglumide may be particularly favourable in the treatment of neoplasia in general and, more particularly, of that which is certainly sustained by endogenous bioactive polypeptides (such as gastrin and CCK), such as gastrointestinal and pancreatic neoplasia.

This treatment may in fact be extremely advantageous in that the rate of proliferation of these tumour cells has been shown to be stimulated and sustained by polypeptides, such as gastrin and cholecystokinin, for which proglumide is a specific antagonist in target cells, that is in gastric cells, colic cells and in the pancreatic acinus. Thus by antagonising these hormones in a selective manner, proglumide may act advantageously in blocking the indiscriminate development of these neoplastic forms or even causing their regression. This antagonism has been shown by various techniques, both in vivo and in vitro, by the study of the incorporation of tritiated thymidine in the DNA, which incorporation occurs in the S phase of the cell cycle, a phase on which the polypeptide hormones seem to exercise their trophic action.

It has also been shown that proglumide has an effective action even on the endogenous component of these hormones as shown by the increase in the survival time of animals in which colic adenocarcinoma had been

implanted and which were treated with proglumide. Pharmaceutical forms including proglumide for use in the treatment of neoplastic affections are of conventional type. They may include pharmaceutically-acceptable inactive ingredients, such as binders, excipients, dispersants, preservatives, humectants and dyes possibly in association with drugs having an antimitotic action.

TABLE 4

Inhibiting action of Proglumide on the rate of growth of normal and tumoral pancreatic cells induced by CCK-8.

TREATMENT	N° Animals	DOSE	WEIGHT OF THE PANCREAS (mg)	student's t	PANCREATIC DNA (mg)	student's t	WEIGHT OF THE PANCREATIC CARCINOMA (mg)	student's t	TUMORAL DNA	student's t
A: Control	10	-	380 ± 29.7	-	0.8 ± 0.10	-	113 ± 7.66	-	0.4 ± 0.05	-
B: CCK-8	8(°)	10mcg/kg (3 per day)	600 ± 54.1	3.76(**)	1.5 ± 0.11	4.72(***)	180 ± 15.2	4.17(***)	0.7 ± 0.13	2.79(*)
C: Proglumide (PR)	10	250mg/kg (3 per day)	330 ± 40.7	0.99	0.9 ± 0.13	0.61	100 ± 8.6	1.15	0.4 ± 0.06	0
D: PR + CCK-8	10	10mcg/kg CCK-8 + 250mg/kg PR	370 ± 38.0	vsA:0.2 vsB: 3.58(**)	1.0 ± 0.09	vsA:1.56 vsB: 3.56(**)	108 ± 8.8	vsA:0.45 vsB: 4.3(***)	0.4 ± 0.09	vsA:0 vsB: 2.33(*)

(°) 2 animals died during the treatment

(*) : P < 0.05

(**) : P < 0.01

(***) : P < 0.001

CLAIMS

1. Proglumide and its salts which are pharmaceutically-acceptable for its use in the treatment of tumoral affections in which bioactive polypeptides or similar mechanisms are involved.

2. Proglumide and its salts which are pharmaceutically-acceptable for its use in the treatment of gastro-intestinal and pancreatic tumoral affections sustained by bioactive polypeptides such as gastrin and cholecystokinin.

3. Proglumide and its pharmaceutically-acceptable salts according to Claims 1 or 2 when administered in association with antimitotic drugs.

4. Pharmaceutical composition for use in tumour therapy according to Claims 1 or 2 including proglumide or a pharmaceutically-acceptable salt thereof as the active agent.

5. Pharmaceutical composition according to Claim 4 further including a drug having an antimitotic action.

6. Pharmaceutical composition according to Claims 4 or 5, further including pharmaceutically-acceptable, inactive ingredients selected from the group consisting of excipients, binders, dispersants, preservatives, humectants, colouring agents and mixtures thereof.

7. The use of proglumide and/or its pharmaceutically-acceptable salts for the preparation of a drug for the therapeutic treatment of tumoral affections.

8. A method of treatment of tumoral affections in humans comprising administering an effective amount of proglumide or a pharmaceutical-acceptable salt thereof to a human suffering from said affections.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 85/00673

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 31/165

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K 31/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	The Merck Index, tenth edition, Merck & Co., Inc., 1983, Rahway, New York, (US), see page 1120, monograph 7680 "Proglumide" (cited in the application)	1-4,6-7
Y	Surgical Forum, volume 33, 1982 Owen E. Winsett et al.:"Gastrin stimulates growth of colon cancer", pages 384-386, see the whole document (cited in the application)	1-4,6-7
Y	Gastroenterology, volume 80, no. 5, 1981 B. Rae-Venter et al.:"Gastrin receptors in human colon carcinoma", page 1256, see the whole abstract	1-4,6-7
Y	Surgical Forum, volume 35, 1984 Proceedings for the 40th annual sessions of the forum on fundamental surgical problems 70th clinical congress, American College of Surgeons San Francisco, October 1984 P. Singh et al.:"Gastrin receptors in a mouse colon cancer cell line responsive to trophic effects of gastrin",	./.

* Special categories of cited documents. [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20th May 1986	17 JUIN 1986
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	pages 205-206, see the whole document	1-4,6-7
Y	Proc. Natl. Acad. Sci. USA, volume 78, no. 10, October 1981, (US) W.F. Hahne et al.:"Proglumide and benzotript:Members of a different class of cholecystokinin receptor antagonists", pages 6304-6308, see page 6304, left-hand column; page 6307, right-hand column, last seven lines of the discussion	1-4,6-7
X,P	Ann. Surg., volume 202, no. 3, September 1985, R. Daniel Beauchamp et al.:"Proglumide, a gastrin receptor antagonist, inhibits growth of colon cancer and enhances survival in mice", pages 303-309, see the whole document	1-4,6-7
Y	J. Clin Gastroenterol, volume 5, no. 1, February 1983 C.B.H.W. Lamers et al.:"The effect of a gastrin-receptor antagonist on gastric acid secretion and serum gastrin in the Zollinger-Ellison syndrome", pages 21-25, see page 21, introduction; page 23, right-hand column, last five lines of the discussion	1-4,6-7
Y	Rev. Med. Limoges, volume 5, no. 3, 1974, R. Claude et al.:"Intérêt du milide (*) dans le traitement des affections gastroduodénales (à propos de 30 cas)", pages 183-187, see page 185, right-hand column; page 186, table III	1-4,6-7

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Digestive Diseases and Sciences, volume 29, no. 8, August Supplement 1984 P. Singh et al.:"Mouse colon cancer and trophic effects of pentagastrin in relation to gastrin receptor levels in vivo", page 80S, see the whole document --	1-4,6-7
Y	Surgical Forum, volume 32, 1981 Courtney M. Townsend et al.:"Stimulation of pancreatic cancer growth by caerulein and secretin", pages 228-229, see the whole document --	1-4,6-7
		./.

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim numbers °°), because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

°°) - 6, as far as claim 6 refers to claim 4,
- 5, and partly 6 as far as claim 6 refers to claim 5 (PCT, art. 6);
- 8 (see PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.