COMBINED THERAPY AGAINST TUMORS COMPRISING SUBSTITUTED ACRYLOYL DISTAMYCIN DERIVATIVES AND RADIOTHERAPY

The present invention relates to the field of cancer treatment and provides an antitumor therapy comprising the combined use of a substituted acryloyl distamycin derivative, more particularly a α-bromo- or α-chloro-acryloyl-distamycin derivative, with radiotherapy.

The treatment of tumours with ionising radiation, also referred to as radiotherapy, is extensively used in cancer therapy as it provides destruction of tumour cells together with inhibition of tumour cell growth, presumably through DNA damage.

Some therapeutic compounds, which are known as being cytotoxic per se, hence susceptible of being used in the therapy of cancer, are also endowed with radiosensitisation activity as they are capable of inducing DNA radiation damage in response to ionizing radiation.

So far, the possibility of combining both cytotoxic agents, e.g. a given radiosensitiser and radiotherapy, with the expectation of getting a supra-additive antitumor effect in comparison to the single cytotoxics alone, is of utmost importance in cancer therapy.

Among the several compounds endowed with antitumor activity and also known as possessing radiosensitisation activity see, for instance, cisplatin, gemcitabine, navelbine, tomudex, nicotinamide, paclitaxel, docetaxel, simvastatin and topotecan.

In addition, the use of halogenated DNA ligands as possible radiosensitisers, also including some distamycin derivatives, were disclosed by R. Martin et al. in the international patent application WO 90/12321.

For a general reference to distamycin, an antibiotic substance with antiviral and antiprotozoal activity, as well as to the several derivatives thereof which are known as cytotoxic agents see, for instance, Nature 203: 1064 (1964); J. Med. Chem. 32: 774-778 (1989); and the international patent applications WO 90/11277, WO 98/21202, WO 99/50265, WO 99/50266 and WO 01/40181, all in the name of the applicant itself and herewith incorporated by reference.

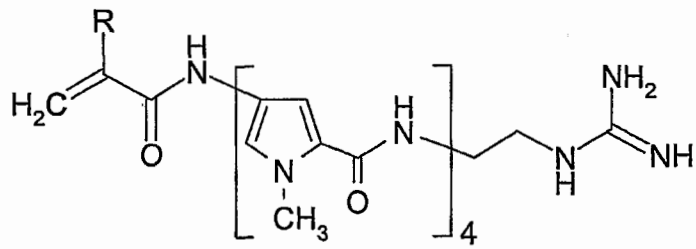
Among the several distamycin derivatives being disclosed so far, a class of α–bromo- or α-chloro-acryloyl-distamycins, as per the aforementioned international patent application WO 98/04524, were found to possess a significant antineoplastic activity.

We have now found that these same compounds are also unexpectedly endowed with a remarkable radiosensitisation activity which render their use, in combination with radiotherapy, particularly advantageous in cancer therapy.

It is therefore a first object of the present invention, the use of a α-bromo- or α–chloro-acryloyl-distamycin derivative in the preparation of a medicament having radiosensitisation activity.

In the present description, unless otherwise specified, with the term "radiosensitisation activity" it is intended the aforementioned capability of a compound, or medicament thereof, to act as a radiosensitiser. With the term "radiosensitiser", in its turn, we refer to a compound or medicament which is capable of increasing or otherwise improving tumor cells destruction in response to ionizing radiation.
Finally, the term "ionizing radiation" is the one conventionally adopted in the therapeutic field of cancer treatment and includes photons having enough energy for bonds ionization such as, for instance, α-, β- and γ-rays from radioactive nuclei as well as x-rays.

According to a preferred aspect of the invention, the α-bromo- or α–chloro-acryloyl-distamycin-derivative is a compound of formula (I) below



(I)

wherein R is a bromine or chlorine atom, more preferably bromine, or a pharmaceutically acceptable salt thereof.

Pharmaceutically acceptable salts of the compounds of formula (I) are the salts with pharmaceutically acceptable inorganic or organic acids such as, for instance, hydrochloric, hydrobromic, sulfuric, nitric, acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic, p-toluenesulfonic acid and the like; the hydrochloride salt being the preferred one.

Even more preferably, the acryloyl-distamycin derivative for use as radiosensitiser is the compound N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl]carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (internal code PNU 166196A).

The combined therapy of the invention is suitable for the treatment of various tumor forms such as, for instance, breast, ovary, lung, colon (including rectus), kidney, stomach, pancreas, liver, head and neck, esophagus, uterus (including body and cervix), vagina, melanoma and non malanoma skin cancer, as well as sarcomas.

From all of the above and unless otherwise specified, it is clear to the skilled person that the α–bromo- or α–chloro-acryloyl-distamycin derivative may be administered to mammals, including humans, through the usual routes, for example parenterally, e.g. by intravenous injection or infusion.

The dosage will depend from several factors, also including the selected schedule of administration which may comprise repeated doses, for instance once a day, once a week, twice a week, and the like, as the case may be.

As a non limiting example, suitable dosages may range from about 0.05 mg/m^2 to about 10 mg/m^2.

For any indication concerning suitable pharmaceutical forms for administering the acryloyl-distamycin derivatives in re, hence including any pharmaceutically acceptable excipient, see the aforementioned international patent application WO 98/04524.

A further aspect of the present invention is to provide a method of treating a mammal,

including humans, suffering from a neoplastic disease state, which method comprises administering to said mammal a α–bromo- or α–chloro-acryloyl-distamycin derivative and radiotherapy, in amounts and according to a schedule treatment effective to produce a synergistic antineoplastic effect.

By the term "synergistic" effect, as used herein, it is meant the inhibition of the growth tumor, preferably the complete regression of the tumor, by administering an effective amount of the above acryloyl distamycin derivative and the ionizing radiation to mammals, including humans. By the term "administered " or "administering", as used herein, it is meant parenteral (e.g. intravenous) administration.

As far as the schedule treatment is concerned, exposure to radiotherapy may either occur simultaneously whilst administering the medicament comprising the α-bromo- or α–chloro-acryloyl-distamycin derivative or, alternatively, sequentially in any order. Preferably, the schedule treatment first comprises administering the drug to the patient which only subsequently is subjected to radiotherapy exposure.

According to the present invention, the acryloyl distamycin derivative may be also administered with additional antitumor agents such as, for instance, topoisomerase I or II inhibitors, e.g. CPT-11, topotecan, 9-amino-camptothecin, 9-nitro-camptothecin, 10,11-methylenedioxy-camptothecin, doxorubicin, daunorubicin, epirubicin, nemorubicin, idarubicin, etoposide, teniposide, mitoxanthrone, losoxantrone, amsacrine, actinomycin D; alkylating agents, e.g. melphalan, chlorambucil, mechlorethamine, cyclophosphamide, ifosfamide, busulfan, carmustine, lormustine, semustine, fotemustine, decarbazine, temozolide, thitepa, mitomycin C, cisplatin, carboplatin, oxaliplatin, nedaplatin, lobaplatin; antimicrotubule agents, e.g. paclitaxel, docetaxel, vincristine, vinblastine, vindesine, vinorelbine, estramustine; antimetabolites, e.g. metotrexate, trimetrexate, tomudex, 5-FU, floxuridine, ftorafur, capecitabine, cytarabine, azacitidine, gemcitabine; protein kinase inhibitors, e.g. STI571 (Gleevec), ZD-1839 (Iressa), OSI-774 (Tarceva), SU 5416 (Semaxanib), SU 6668, SU 11248; retinoid

derivatives, e.g. cis-retinoic acids, trans-retinoic acids; cyclooxygenase inhibitors such as COX-2 inhibitors, e.g. celecoxib, rofecoxib, parecoxib, valdecoxib; hormonal agents, e.g. exemestane, formestane, atamestane, letrozole, fadrozole, anastrozole.

According to a preferred embodiment of the invention, the use of a α-bromo- or α-chloro-acryloyl-distamycin derivative with radiotherapy also comprises the administration of a platinum alkylating agent, more preferably cisplatin.

PHARMACOLOGY

The remarkable radiosensitisation effect exerted by the α-bromo- or α-chloro-acryloyl-distamycin derivatives, in particular the compounds of formula (I), is shown according to in vitro clonogenic assays on SQ20B (radiation-resistant human squamous cell carcinoma of the larynx) and A431 (human vulval carcinoma) cell lines. In this respect, two different schedule treatments were evaluated either comprising simultaneous exposure to the tested compound of formula (I) and to radiation, or sequential exposure to both these cytotoxic agents in any order, that is drug/radiation or radiation/drug (see details below). As control, the effect of cisplatin in combination with radiotherapy has been tested in the same operative conditions.

To define a Sensitization Ratio (SR), the clonogenic survival of cells being treated with a combination of irradiation and drug exposure (S_{X+D}) was compared with the product of survival for drug alone (S_D) and irradiation alone (S_X), as follows

$$SR = S_{X+D} / S_D * S_X$$

From the above, it is clear to the skilled person that if both radiation and drug exerted their cytotoxic effect independently from each other, SR values would be close to 1 whereas, on the contrary, a radiosensitisation effect indicating a synergism between ionizing radiation and drug is characterized by SR values lower than 1 (SR < 1).

Analysis of the obtained results in any of the experiments being carried out clearly indicate that the tested compound of formula (I) exerts a remarkable and statistically significant radiosensitising effect.

In particular, whilst sensitization is substantially comparable to that of cisplatin on SQ20B cell line, it is unexpectedly and significantly superior than that of cisplatin on A431 cell line, hence indicating a possible widest range of applications for the compounds of formula (I), in combination with radiotherapy.

In addition to the above, we unexpectedly found that the radiosensitisation effect of the compound of formula (I) could be even increased, to a statistically significant extent, when drug exposure occurred before irradiation treatment, according to one of the sequential schedule treatments.

To better illustrate the present invention, without posing any limitation to it, the following examples are now given.

Example 1

Radiosensitisation activity of PNU 166196A in comparison to cisplatin

For both compounds PNU 166196A and cisplatin, exposures were simultaneous to ionizing radiation in both SQ20B and A431 cell lines. The schedule consisted of 2 h drug treatment with a period of irradiation (10 minutes) starting at the beginning of the 2nd hour of treatment.

Four data sets for each of PNU 166196A and cisplatin, in each cell line were obtained (see table 1) comprising duplicates of two different drug concentration chosen to yield cytotoxicity values corresponding to 80% (C_{80}) and 20% (C_{20}) survival for treatment with the drug alone.

Table 1 - Sensitisation ratio of PNU 166196A and cisplatin in combination with radiotherapy

Cell line	Drug	Drug Concentration[a]	Sensitisazion Ratio SR[b]	
			Values for each culture	Mean of duplicates
SQ20B	PNU 166196A	50 (C_{80}) [c]	0.85	**C_{80} 0.85**
		350 (C_{20}) [d]	0.46	**C_{20} 0.50**
		50 (C_{80})	0.84	
		350 (C_{20})	0.55	p=0.017
	cisplatin	0.4 (C_{80})	0.78	**C_{80} 0.80**
		6.5 (C_{20})	0.72	**C_{20} 0.77**
		0.4 (C_{80})	0.82	
		6.5 (C_{20})	0.81	p=0.034
A431	PNU 166196A	20 (C_{80})	0.65	**C_{80} 0.62**
		90 (C_{20})	0.33	**C_{20} 0.37**
		20 (C_{80})	0.59	
		90 (C_{20})	0.40	p=0.029
	cisplatin	0.8 (C_{80})	1.02	**C_{80} 0.96**
		5.0 (C_{20})	1.08	**C_{20} 0.97**
		0.8 (C_{80})	0.84	
		5.0 (C_{20})	0.86	p=0.37

[a] Expressed as ng/ml for PNU 166196A and μM for cisplatin;

[b] SR values lower than 1 (SR < 1) indicate radiosensitisation;

[c] C_{80} drug concentration corresponding to 80% cell survival

[d] C_{20} drug concentration corresponding to 20% cell survival

From the above, SR for PNU 166196A is lower than 1 in both cell lines being investigated; on A431, SR for PNU 166196 is markedly lower than that of cisplatin, hence indicating a superior radiosensitisation effect.

Example 2

Radiosensitisation activity of PNU 166196A under sequential schedule treatment

PNU 166196A was tested in both SQ20B and A431 cell lines, according to two sequential schedule treatments comprising: 2 h drug treatment ending 60 minutes before irradiation (drug-before schedule) and 2 h drug treatment starting 40 minutes after

irradiation (drug-after schedule), the irradiation period being of 10 minutes, in each case. For each cell line, PNU 166196A was tested at the highest concentration (see table 2) to yield cytotoxicity values corresponding to 20% (C_{20}) survival for treatment with the drug alone.

Table 2 - Effect of the sequence of treatment on the sensitisation ratio of PNU 166196 in combination with radiotherapy

Cell line	Drug Concentration (ng/ml)	Sensitisazion Ratio SR [a]	
		Drug-before [b]	Drug-after [c]
SQ20B	350 (C_{20}) [d]	**0.15**	0.62
	350 (C_{20})	**0.47**	0.73
A431	90 (C_{20})	**0.11**	0.87
	90 (C_{20})	**0.13**	0.43
Paired t-test		P=0.043	P=0.074

[a] SR values lower than 1 (SR < 1) indicate radiosensitisation;

[b] 2 h exposure to PNU 16196A before irradiation;

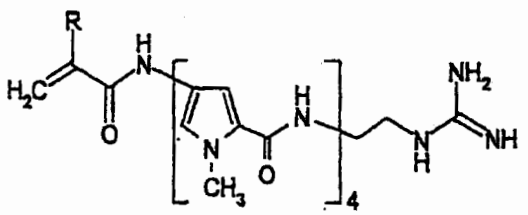
[c] 2 h exposure to PNU 16196A after irradiation;

[d] C_{20} drug concentration corresponding to 20% cell survival

From the above, even if SR values are lower than 1 in both cell lines and according to both schedules, the radiosensitisation activity of PNU 166196A is significantly higher (SR<<1) when the treatment with the compound is carried out before irradiation.

CLAIMS

1. Use of a α-bromo or α-chloro-acryloyl-distamycin derivative for manufacturing a medicament for treating a mammal, including humans, suffering from a neoplastic disease state, by administration to said mammal in combination with radiotherapy in amounts and according to a schedule treatment effective to produce a synergistic antineoplastic effect, wherein the α-bromo or α-chloro-acryloyl-distamycin derivative is of the formula (I)



(I)

wherein R is a bromine or chlorine atom, or a pharmaceutically acceptable salt thereof.

2. The use according to claim 1 wherein, within formula (I), R is a bromine atom.

3. The use according to claim 1 wherein the acryloyl-distamycin derivative is the compound N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl]carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride.

4. The use according to claim 1 wherein the neoplastic disease state is selected from the group consisting of breast, ovary, lung, colon, rectus , kidney, stomach, pancreas, liver, head and neck, esophagus, uterus, cervix , vagina, melanoma and non melanoma skin cancer, as well as sarcomas.

5. The use according to claim 1 wherein exposure to radiotherapy occurs either simultaneously whilst administering the medicament of claim 1, or sequentially, in any order.

6. The use according to claim 1 wherein treating said mammal first comprises administering the α-bromo or α-chloro-acryloyl-distamycin derivative to the patient and subsequently subjecting the said patient to radiotherapy.

7. The use according to claim 1 wherein treating said mammal optionally further comprises the administration of an additional antitumor agent, either separately, simultaneously or sequentially, in any order.

8. The use according to claim 7 wherein the additional antitumor agent is selected from the group consisting of topoisomerase I or II inhibitors, alkylating agents, antimicrotubule agents, antimetabolites, protein kinase inhibitors, retinoid derivatives, cyclooxygenase inhibitors and hormonal agents.

9. The use according to claim 8 wherein the additional antitumor agent is cisplatin.