Acta Oncologica Vol. 32, No. 2, pp. 209–215, 1993

THERAPEUTIC USE AND PERSPECTIVES OF SYNTHETIC PEPTIDES IN ONCOLOGY

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Several native peptides can regulate tumour cell proliferation. After binding to specific membrane receptors they have the ability to stimulate or inhibit directly cell growth. Peptides can also control the regulation of endocrine or paracrine growth factor secretion. Agonist and antagonist molecules have been synthesized for therapeutic purposes. Hypothalamic neuropeptides are used in oncology. GnRH agonists lead to biochemical castration which is useful in treatment of hormone-dependent tumours (breast and prostate). Somatostatin analogues are beneficial in the treatment of gut neuroendocrine tumours and have demonstrated an antitumoural effect in experimental studies. Cytostatic agents, such as Gastrin Releasing Peptide antagonists, may be of interest as an adjuvant to chemotherapy or surgery in small cell lung cancer and other malignancies. The role of peptides in antigenic presentation, cell proliferation control and the metastatic process suggests a new therapeutic potential for these compounds. Progress in biotechnology could provide specific tools to screen new molecules and increase the understanding of mechanisms of action. Improvement in drug delivery techniques will allow for more convenient routes of administration.

Peptides are currently among the most promising molecules for cancer treatment; their potential therapeutic use is closely connected to their physiological role as neuropeptides, hypothalamic hormones, intestinal hormones, or growth factors (Table). Peptides can modulate the proliferation of normal and tumour cells by several mechanisms (1) Peptidic regulation may result either from direct action on specific receptors of the target tissues or from the control of other hormonal secretions. These can be summarized as follows:

- Some peptides directly stimulate cell growth, i.e. Gastrin Releasing Peptide (GRP) in lung cancer (2), while some reduce it, i.e. somatostatin (3,4). The presence of peptidic receptors on tumour cell membranes partly reflects tumour differentiation (4) and may explain the key role of neuropeptides in the control of neuroendocrine tumour growth.
- Some peptides exert an indirect endocrine action on other hormone secretion i.e. GnRH analogous modulate the growth of some breast and prostatic cancers which are hormone-dependent (5).
- Some peptidic analogues may play a role in controlling metastasis and cellular invasion (6) by inhibiting cell adhesion or angiogenesis (3, 4). Peptides can also regulate some side-effects related to paraneoplasic syndromes (3).

A clearer understanding of the biological role of natural and synthetic peptides will be achieved with the development of biotechnology, which may use some of the following procedures:

- cloning of genes which code for the natural peptides and their receptors,
- determination of the second messenger pathways,
- structure determination of natural proteins through computer modeling,
- synthesis of new analogues, which will enable a thorough study of the peptides pharmacological properties as well as the development of agonist and antagonist molecules with therapeutic potential.

Received 23 September 1992.
Accepted 29 September 1992.
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Presented at the 3rd International Workshop on Neuroendocrine Tumors held in Lofoten, Norway, June 24–28, 1992.

Table

Examples of neuropeptides that modulate cell proliferation in culture

Neuropeptide	Cell origin
Cholecystokinin	SCLC, colon (40, 62)
Gastrin	Colon, stomach (62)
Gastrin releasing peptide	SCLC, colon, prostate, pancreas (2)
Vasoactive intestinal peptide (VIP)	SCLC (40)
Substance P	Fibroblast, synoviocyte (1)
Galanin	SCLC (40)
α-melanocyte stimulating hormone (αMSH)	Melanoma (77)
Gonadotropin-releasing hormone (GnRH)	Breast (23)
Somatostatin	Neuroendocrine tumours (3, 4)
Angiotensin	Smooth muscle cell (78)
Arginine-vasopressin	SCLC (40)
Endothelin	Fibroblast, smooth muscle cell, some epithelial tumour cell lines (79)

SCLC: Small cell lung cancer

The boundaries of development

Techniques in peptide synthesis (i.e. addition of unusual residues, of lateral chains, and structural restriction) have allowed for the development of more selective, more potent and longer-acting molecules. Two methods are commonly used, namely liquid phase synthesis, which is a complex process mostly suited to the industrial production, and solid phase synthesis, developed in the 1960's by Merrifield and which consists of constructing a peptidic chain on a resin support. In the last phase of synthesis, the peptides are cleaved and purified by high pressure liquid chromatography. Automatized synthesis can produce peptidic chains up to 40 amino acids. For longer chains, genetic engineering is the most appropriate production method.

The screening of synthetic peptides is a three-step process. The first step involves the determination of the tissue binding site; receptors are characterised from tissues or cell fractions. The second step is determination of the biological effects (7); agonist, antagonist or other properties are defined following in vitro pharmacological testing. In vivo testing is the final step. The half-life in plasma is almost as relevant as the affinity for specific receptors to predict the efficacy of a peptide molecule. Although inter-species metabolic differences may exist, preliminary evidence of action in rodents is a prerequisite for the development of a new molecule. Moreover, in vitro results may be difficult to interpret due to possible endocrine or paracrine interactions between the peptide and various growth factors. In vivo, antitumoural activity is evaluated on human tumour xenografts in athymic nude mice (8). Screening as well as toxicology studies allow the selection of adequate molecules for further development. Therapeutic efficacy can only be determined by clinical trials.

More recently, synthetic peptide combinatorial libraries have been used to generate and select new peptidic analogues; these collections of peptides are tested against a series of target molecules and the active molecules are subsequently sequenced (9). This random screening method is an alternative to the more 'rational' approach, for which a target molecule (receptor) is sequenced and expressed in a cell line; the peptide (ligand) is screened on these cells and they may be synthesized with the help of molecular design programmes.

Today the pharmacokinetic characteristics of the peptides are a major impediment for their development; these compounds cannot be administered per os due to their degradation by intestinal enzymes and their insufficient profiles (10). As a result peptidomimetic molecules, which are devoid of peptidic bounds, have been synthesized (i.e. inhibitors of conversion enzymes). This approach has so far been restricted to small peptidic compounds. In the case of larger molecules, the use of original galenic formulations can facilitate their use. Slow-release forms considerably reduce the strains of parenteral administration (11). Slow-release GnRH analogues allow the delivery of the active molecule for a one-month period following one single injection (11). Other routes of administration are currently being studied, i.e. pulmonary (aerosols), nasal or transdermal administration (10). Bioavailability, though, is further reduced and is roughly proportional to the size of the peptide.

Clinical research on peptides is not subject to specific rules or restrictions. However, optimal dosing is very important for those compounds. The highest dose may not be the most efficient even if it is well tolerated (12).

Major peptidic drugs and indications

GnRH analogues

GnRH was isolated and sequenced by A. Schally in 1971. The hypothalamic pulsatory secretion of this peptide

stimulates the hypophysial secretion of LH and FSH and consequently the synthesis and liberation of sexual steroids (5). GnRH enzymatic degradation sites have been described and molecules with more powerful and prolonged action synthesized by modifying the natural molecule, more specifically on amino-acids 6 and 10. The Triptorelin half-life (D-Trp 6, GnRH) is about 7.5 h whereas the natural GnRH half-life is 8 min. Continuous administration of these agonists, after initial gonadotropic stimulation, leads to hypophysial desensitization and thus inhibits in a paradoxal way the LH and FSH secretion. The consequence of this antigonadotropic effect is a castration which remains totally reversible after the end of the treatment. The GnRH analogues, following extensive experimental tests, have been used in the treatment of hormone-dependent tumours such as prostate and breast cancer.

GnRH analogues have also been tested in hepatoma (EORTC) and in pancreatic cancer (13), with no major clinical efficacy demonstrated to date. Encouraging preliminary results have been obtained in endometrial carcinoma (14).

New GnRH analogues covalently bound to cytotoxic molecules have also shown antiproliferative activity in breast and prostate cancer models (15).

In prostate cancer, the GnRH agonists have the same efficacy as surgical castration. They do not exhibit cardiovascular toxicity like oestrogens and offer an alternative to surgical mutilation (16, 17). The initial and transitory stimulation (lasting for about 7 days) of testicular androgen secretion (flare-up reaction) may result in a transitory tumoural growth effect. The addition of an antiandrogen, prescribed at the beginning of the treatment with a GnRH agonist, can prevent this reaction (18). The continuation of treatment by the antiandrogen for more than a month, remains controversial (17). The total blocking of androgen as recommended by F. Labrie et al. (19) can delay the time for avoidance of castration, however without improving the survival in most randomized studies (17). GnRH agonists are not more effective than surgical castration and the existence of a direct effect on tumoural cells has not yet been proved (17).

GnRH agonists have an efficacy comparable to that of castration in metastatic breast cancer (20). A satisfactory response rate (50%) in a selected population of premenopausal women who had metastatic tumours with positive hormonal receptors (21) has been reported. Occasional responses (10%) have likewise been observed in post menopausal patients (22). This suggests a direct antitumoural effect of the peptides. In particular conditions, the peptide was shown to inhibit in vitro cell growth (23). The existence of functional receptors remains controversial and the fixation sites that have been described have a weak affinity for GnRH (24). Larger doses than those used for castration might be necessary to show significant antitumoural effect in postmenopausal patients.

GnRH agonists have also been proposed for ovarian cancer treatment (25). Hormone therapy of these tumours usually results in a low response rate. GnRH analogues, when administered to women who have a growing tumour despite repeated previous chemotherapy, do not appear very efficient (15% objective response). The effect of these molecules, when used as a first-line treatment in combination with chemotherapy remains to be evaluated. The presence of high affinity GnRH receptors in the ovaries (26) may suggest a direct effect of GnRH on this organ.

Somatastatin analogues

Somatostatin (SRIF) is a neuropeptide that inhibits secretion of growth hormone (GH) and many pancreatic and intestinal hormones (3, 4, 27). It decreases the liberation of the Insulin-like Growth Factor (IGF 1), and inhibits angiogenesis. The half-life of somatostatin is short (a few minutes in plasma). Analogues with longer half-life and higher affinity for somatostatin receptors, have been synthesized (3, 4).

Many tumours have receptors for SRIF (4). A variety of receptor subtypes have been identified for these tumours (28). A family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract and kidney have recently been cloned (29). SRIF and its analogues have variable affinity for these different subtypes. Whether or not all the subtypes are responsible for the antiproliferative effects still remains to be verified. The activation of a tyrosin phosphatase (which dephosphorylates growth factor receptors like the Epidermal Growth Factor) may play an important role in the antitumoural effect of somatostatin (30). The growth of cell lines from breast cancer (31), small cell lung cancer, alimentary and prostate cancers is decreased in vitro by SRIF analogues (3, 4). The analogues interact with autocrine, endocrine and paracrine growth factors which, as a result, may lead to cell death by apoptosis (32). SRIF analogues also demonstrate a similar degree of efficacy for the same tumours in vivo (3, 4, 8, 28). Octreotide (Sandoz) is an acknowledged treatment for the symptoms (diarrhoea, flush, etc.) of alimentary endocrine tumours (33) and for acromegaly (34).

Other analogues, such as Lanreotide (Ipsen Biotech) also seem efficient in these pathological conditions (3, 35). Somatostatin analogues can also restrict the growth of many tumours including pituitary adenoma (3, 4, 34), carcinoid (3, 4, 33) and prostatic cancer (36). The doses that exert an antitumoural effect could be higher than the antisecretory doses (37). Clinical studies are on-going testing different doses and therapeutic associations. Tumours with receptors for analogues (4, 38) may be selected in an attempt to confirm clinically the antitumoural effect that has been clearly demonstrated in experimental models. Preliminary results suggest a modest clinical activity in metastatic tumours at doses used for acromegaly treatment.

Studies on SRIF analogues that are specific to particular tumours, given alone or in combination with chemotherapy or as an adjuvant to surgery, may generate an increase in the clinical use of these drugs. Data from clinical (37) and experimental protocols (3) suggest that high doses may be more efficient.

The inhibitory effect on intestinal secretion may be useful in the treatment of diarrhoea as observed in graft-versus-host reaction and 5-fluorouracil toxicity (39). New slow-release formulations may facilitate the treatments by diminishing the injection frequency (35). In many neuroendocrine tumours and in lymphomas, receptors can be detected with labelled analogues (38). This technique allows visualisation of metastases by scintigraphy and a possible selection of tumours which may respond to treatment by somatostatin analogues.

Peptides under investigation

Neuropeptides and growth of small cell lung cancers

Small cell lung cancer (SCLC) is a neuroendocrine tumour which secretes various neuropeptides (40), some of which, like vasopressine, may cause para-neoplasic syndroms. Gastrin Releasing Peptide (GRP), human homologue of the batratian bombesin, is the neuropeptide for which the growth factor effects have been the best characterised (41). GRP is produced by 20 to 50% of SCLC according to their differentiation (2, 42). Some tumours can transcribe the messenger and produce GRP precursors or the proceeded peptide (2, 43). Lung cancer cells have high affinity receptors for GRP (44, 45). GRP can increase the growth of SCLC cell lines in agar (46) with varying efficacy. The growth of SCLC cell lines that have receptors for the GRP can be delayed in vitro and in vivo by antibodies that are directed against the active part of GRP and that prevent fixation of GRP to its receptor (41). These antibodies are now being studied in phase II clinical trials (47, 48). They may induce antimice human antibodies (which reduce their half-life). Small peptidic GRP competitive antagonists have been synthesized (49). Their efficacy on SCLC cells in vitro and in vivo is at least equal to that of monoclonal antibodies (50, 51). The pharmacological activity is linked to the presence of receptors for GRP (51). A receptor for neuromedine B, a neuropeptide belonging to the GRP family (45, 52), has been found in a large number of tumours. The role of these receptors in SCLC proliferation remains unexplained (53). Other peptides can stimulate SCLC cell proliferation by binding their own receptors (40, 54). This is the case for bradykinin, vasopressine, cholecystokinin, and galanin (54). Their simultaneous incorporation into the culture medium may cause subadditive effects (55).

Molecules which interact with several factors have been synthesized (54, 56). They may be more efficient than monospecific molecules if they could maintain a significant affinity for the different receptors. This does not seem to be the case for the currently available molecules that are active at high doses in vitro and in vivo (54–56). Different antagonists to neuropeptides, for which the tumours have the highest number of specific receptors, should probably be selected in order to treat SCLC successfully with these compounds. Endogenous opioids counteract these stimulating factors and have been shown to inhibit SCLC cell line growth (57). This inhibition can be reversed by naloxone and nicotine. Opioids could act as anti-autocrine growth factors. Methadone can inhibit the in vitro and in vivo growth of SCLC lines (58). The μ and κ opioid receptors have also been described on neuroblastoma, glioma, mammary adenocarcinoma, and melanoma (57).

Neuropeptides play an important role in carcinogenesis. As a result of their contribution to cell proliferation, neuropeptides favour the accumulation of genetic anomalies. It is interesting to note that GRP levels are high in alveolar lavage fluid of asymptomatic cigarette smokers (59) and that CD 10 ectopeptidase (CALLA), which hydrolyses GRP, is inhibited by tobacco components (60). The inhibitory effect of opioids on the SCLC line growth is suppressed by nicotine (57). These findings provide hints for the role of growth factor antagonists in chemoprevention. When tumours are constituted, the use of these molecules will probably not be as efficient in rapidly evolving SCLC, which may not always respond to growth factors. Thus, the C-myc oncogene amplification is conversely linked to the sensitivity of SCLC cell lines to GRP (61). GRP and antagonists could be administered as an adjuvant to chemotherapy. The effect of neuropeptides on lung cancer can be compared to that of the digestive hormones, cholecystokinin and gastrin, on the evolution of colon cancer (62). High gastrin rates are detected in colon cancers (63).

Peptidic growth factors and second messengers

Some proteins, such as the Epidermal Growth Factor (EGF) and the Insulin-like Growth Factor (IGF1) are important growth factors. Their proliferative effect can be blocked by antibodies against the protein itself or its receptor (64). Peptides can be used similarly. Antagonists acting on the receptor can also be produced, either by screening or more rationnally by the study of the tridimensional structure of the receptor and its ligand. This may require crystallographic studies and computer analysis (molecular design) in order to build the synthetic molecules. The extra-cellular plasmatic fraction of the receptor can act as a competitive antagonist by neutralizing the 'ligand' (65). Another approach consists of blocking the intracellular second messengers which are common to the activation pathways of different growth factors (G-protein, tyrosin phosphorylation, p21-ras activation,

ion channels) although the proteins that are responsible for the specific differentiation or proliferation messages have not yet been fully classified (66). Some small amphophilic peptides, such as substance P and kinines, enter through the cell membrane and directly activate the G-proteins without any intermediary receptor interaction (67). The effect of synthetic peptides on G-proteins is currently investigated. It is also possible to produce peptides which inhibit the PDGF receptor-kinase interaction (68). The phosphatidylinositol 3-kinase that binds to intracellular fraction of the PDGF receptor transduces the activation of several receptors.

Peptides protection against chemotherapy toxicity

Fast renewing tissues are the most sensitive to the toxicity of antimitotic chemotherapy. Chemotherapy is especially toxic to the bone marrow cells that have entered the cell division cycle when the drug is administered (69). Several peptides have been shown to inhibit haemopoietic stem cell proliferation, i.e AcSDKP (70), MIP 1α (71), and pEEDCK (69). These molecules have a marked protective effect against the toxicity of cytostatic drugs in experimental models. This has been demonstrated for phase-specific (cytarabin) and non-phase-specific (cyclophosphamide) anticancer drugs. AcSDKP and MIP 1α have not yet displayed any significant activity on solid or haemapoietic tumour cells in vitro and in vivo. This suggests that they do not interact with the effects of chemotherapy on cancer cells. Inhibitors of bone marrow stem cell proliferation could be used as an alternative or as a complement to therapy with haemopoietic growth factors (G-CSF, GM-CSF), as the efficacy of the latter diminishes over successive courses of chemotherapy. Some of these inhibitory peptides (AcSDKP, pEEDCK) are currently under phase I clinical investigation.

Peptides and metastasis

The metastatic process is dependent on the adhesion of circulating neoplastic cells, on tissue invasion and on angiogenesis. The peptides GRGDS (72) and YIGSR (73) have been shown to inhibit the adhesion to extracellular matrix proteins, such as laminin (YIGSR) and fibronectin (GRGDS). In murine melanoma models, pulmonary metastatic invasion can be decreased when the tumour cells are treated by these peptides prior to i.v. injection.

Other potential therapeutic applications

A gene coding for a specific tumour antigen which is recognized by cytotoxic T-lymphocytes has recently been cloned from melanoma cells (74). This antigen, also expressed by other melanoma cell lines as well as by tumour cells of other histological types, appears to be presented by the HLA-A1 system. Such a finding may lead to specifically targeted immunotherapy of some cancers. Synthetic peptides could be used as potential vaccines. Neuropeptides play a key role in the modulation of appetite (75), hence some of them may improve the appetite of cancer patients and thus diminish malnutrition while avoiding the deleterious side-effects of steroid treatment. Calcitonin can reduce serum calcium in cancer hypercalcemia. This condition is frequently associated with the production of parathyroid hormone (PTH) like factors by the tumours. Synthetic peptides that block receptor binding to PTH could be used as a specific treatment for hypercalcemia (76).

Conclusions

Most synthetic peptides in clinical use today are hormones. Among hypothalamic hormones, GnRH analogues are useful for the treatment of metastatic prostate and breast cancer. Somatostatin analogues offer an efficient symptomatic treatment of endocrine tumours and of severe diarrhoea. Somatostatin and some specific growth factor (GRP, EGF) inhibitors are cytostatic agents and may be used as adjuvant to surgery and conventional chemotherapy or as chemopreventive agents.

The role of peptides in antigenic presentation, cell proliferation control, and metastatic process suggests new therapeutic perspectives for these compounds. Progress in drug delivery techniques will allow for more convenient routes of administration. Biotechnology will also play an important role: the discovery and development of therapeutically active molecules. In conclusion, it can be postulated that peptides will play a vital part in the advancement of new therapeutic treatments for oncology with their complex mechanisms of action probably being elucidated in the near future.

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