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Opioid Growth Factor and its Derivatives as Potential Non-toxic Multifunctional Anticancer and Analgesic Compounds

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Abstract: Despite significant research progress on the pathogenesis, molecular biology, diag-nosis, treatment, and prevention of cancer, its morbidity and mortality are still high around the world. The emerging resistance of cancer cells to anticancer drugs remains still a significant problem in oncology today. Furthermore, an important challenge is the inability of anticancer drugs to selectively target tumor cells thus sparing healthy cells.

One of the new potential options for efficient and safe therapy can be provided by opioid growth factor (OGF), chemically termed Met-enkephalin. It is an endogenous pentapeptide (Tyr-Gly-Gly-Phe-Met) with antitumor, analgesic, and immune-boosting properties. Clinical trials have demonstrated that OGF therapy alone, as well as in combination with standard chemotherapies, is a safe, non-toxic anticancer agent that reduces tumor size.

In this paper, we review the structure-activity relationship of OGF and its analogues. We highlight also OGF derivatives with analgesic, immunomodulatory activity and the ability to penetrate the blood-brain barrier and may be used as safe agents enhancing chemotherapy ef-ficacy and improving quality of life in cancer patients.

The reviewed papers indicate that Met-enkephalin and its analogues are interesting candi-dates for the development of novel, non-toxic, and endowed with an analgesic activity anti-cancer drugs. More preclinical and clinical studies are needed to explore these opportunities.

Keywords: Opioid growth factor, met-enkephalin, analogue, anticancer therapy, multifunctional compound, tar-geted therapy.

1. INTRODUCTION

Cancer is the second leading cause of death world-wide, accounting for an estimated 9.6 million deaths in 2018 (Cancer - World Health Organization) [1]. The conventional treatments for cancer, such as surgery, radiation, and chemotherapy have limited effectiveness in many types of cancer. In addition, the high toxicity usually associated with some anticancer therapy and undesirable side-effects that affect a patient's quality of life increases the demand for novel anti-cancer drugs. Recent *in vitro* and *in vivo* studies have shown that Met-enkephalin (called opioid growth factor (OGF)) inhibits cancer cell growth [2]. Met-enkephalin is a constitutively-expressed pentapeptide that acts through binding specifically to intracellular receptor: opioid growth factor receptor (OGFr) [2]. This receptor has been identified in a variety of human cancers and shows the ability to modulate neoplasia [2-4]. The OGF-OGFr axis inhibits DNA synthesis by upregulat-ing cyclin-dependent inhibitory kinases [5]. Therefore, this endogenous peptide pathway has been proposed as a novel target for non-toxic anticancer therapy. Indeed, preclinical studies have warranted Phase I and Phase II clinical trials using Met-enkephalin infusions as a non-toxic, safe treatment that extends survival in patients with unresectable pancreatic cancer [2]. Moreover, the

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combination of Met-enkephalin with chemotherapy enhances efficacy at reducing tumor size and improves a patient's quality of life [2]. Interestingly, Met-enkephalin has also the potential for use in cancer immunotherapy. It has been found that this peptide stimulates T-cell transformation and augments natural killer (NK) cell cytotoxicity [6]. However, the half-life of Met-enkephalin (IC_{50}) in human plasma is only a few minutes, which limits its anticancer effectiveness and pharmacological application [7].

Therefore, a significant effort has been made to obtain Met-enkephalin analogues with superior pharmacokinetic and pharmacodynamic profiles. For example, it has been documented that the smallest peptide with anticancer and analgesic properties consists of five amino acids [8-12]. Unfortunately, there are still very few analogues and careful characterization of the pharmacological properties of analogues is lacking. Therefore, in this review, we summarize the-state-of-the structure-activity relationship and anticancer potential of Met-enkephalin and its analogues.

2. BIOLOGY OF OPIOID GROWTH FACTOR

An endogenous morphine-like substance, which interacts with morphine receptors, was discovered in 1975 by Hughes *et al.* [13]. After purification, it was named encephalin ('in the head') [14]. Further studies have shown not only the existence of a single compound but a large number of endogenous encephalin-containing peptides, which were named opioid peptides [14]. They are divided into three groups: endorphins, dynorphins, and enkephalins (ENK) [15]. The latter include Met-enkephalin and Leu-enkephalin, which are produced from a propeptide precursor, proenkephalin (proENK). The transformation of propeptide into active peptides requires several endopeptidases: cathepsin L, prohormone convertase 2, aminopeptidase B and E [4,16]. Functional peptides are stored within large dense-core vesicles (LDCVs) near release sites: presynaptic, extrasynaptic or dendritic [17]. Released enkephalins are subject to the level control in order to ensure synchronization of the signal [18]. It has been determined that ENK are degraded in less than a minute after injection in the rat brain [18]. This process is performed by two neuropeptidases: neutral endopeptidase (neprilysin) and aminopeptidase N [19,20].

Enkephalins, as a part of the neuropeptidergic system, act through the opioid peptide receptors (OPr): μ opioid peptide receptor (MOPr) and δ opioid peptide receptor (DOPr) [21,22]. However, ENK have a slightly higher affinity for DOPr than MOPr as well as they can bind to the κ opioid peptide receptor (KOPr) [23]. Enkephalins are distributed among the central and peripheral and autonomous nervous systems. Neurons producing ENK were found in the piriform, entorhinal, and medial prefrontal cortex. Most nuclei of the hypothalamus are shown to contain ENK neurons [24,25]. In the hippocampus, enkephalins are present in granular cells and mossy fibers [25]. Moreover, ENK were detected in their target organs (skin, liver, lungs, bones) and endocrine tissues (endocrine pancreas, adrenal medulla) [26]. Interestingly, proenkephalin mRNA levels are shown to be higher in the heart than in the brain; however, amounts of extracted proenkephalin-derived peptides are much lower in the heart [27]. MOPr and DOPr distribution are similar to that of ENK projections. They are also expressed throughout the central nervous system [28].

ENK are involved in the vast extent of physiological processes including: thirst and feeding, analgesia, gastrointestinal functions, respiration, and cardiovascular system [29]. Furthermore, they play a part in emotional behaviors such as fear conditioning, anxiety, and stress response [30]. Enkephalins appear to play an important role in heart failure, development and ageing, ischemic preconditioning, hypertension, and hypertrophy [27].

In the early 1980s, the hypothesis that endogenous opioid peptides, particularly [Met5]-enkephalin, are involved in growth regulation of normal and abnormal cells and tissues was advanced [31-33]. In order to distinguish function as a growth factor from that of a neuromodulator, [Met5]-enkephalin has been termed opioid growth factor (OGF). This peptide is identified in tissues from all 3 dermal derivatives: endoderm, mesoderm, and ectoderm; however, with different expression levels and are shown to be autocrine and possibly paracrine produced [34,35]. Importantly, the biological mechanism of action of OGF is stereospecific, pharmacological characteristic and can be blocked by naloxone [36]. As it has been found that [Met5]-enkephalin has a high affinity for δ opioid peptide receptor and littler lower to μ opioid peptide receptor, it has been assumed that action on growth processes is transduced by these receptors. However, growth function, subcellular nuclear-associated location, tissue distribution (neural and non-neural), ligand specificity, and competitive inhibition profile are in strong contrast with classical opioid receptors [37]. The receptor for OGF was originally named the zeta (ζ) opioid receptor; however, further molecular studies confirmed that the proteomic, as well as the genomic nature of this receptor, is different from classical opioid receptors [38].

Consequently, the receptor has been named opioid growth factor receptor (OGFr) and was originally isolated from developing rat cerebellum/brain and mouse neuroblastoma tissue [39,40]. In further studies, OGFr gene expression was determined in the human fetal brain, liver, kidney, and lung as well as the adult brain, liver, heart, skeletal muscle, pancreas, and kidney [38]. The opioid growth factor receptor is an integral membrane protein associated with the nuclear envelope [41]. The OGF ligand enters the cell by active transport through clathrin-mediated endocytosis, and not requiring endosomal or Golgi pathways [42]. The complex of OGF-OGFr has shown to be associated with karyopherin and moves through the nuclear pore in order to binds to the inner nuclear matrix or heterochromatin [37]. The OGF-OGFr axis is in a tonic equilibrium, which allows for maintaining homeostasis of proliferating cells and tissues [37]. The regulation of protein and receptor levels results in increased or decreased cell proliferation. The activity of the OGF-OGFr axis is most pronounced in epithelial tissue of the retina [43] and cornea [44] as well as the gastrointestinal system (tongue and intestines) [45].

OGF exerts a rapid biologic effect and dependent on intrinsic rhythms of the cell (*e.g.*, circadian rhythm) [37]. The cellular mechanism consists of the delay of the cell cycle in G1/S interphase by modulating cyclin-dependent kinase inhibitory (CKI) pathways [46]. It has been determined that [Met5]-enkephalin increases p16 or p21 depending on the presence of specific CKIs [47]. In human umbilical vein endothelial cells, human dermal fibroblasts, mesenchymal stem cells, and human epidermal keratinocytes, OGF depresses replication through modulation of p16INK4a and p21WAF1/CIP1 pathways; however, does not change expression of p15, p18, p19, and p27 proteins [37]. Moreover, [Met5]-enkephalin decreases the phosphorylation of retinoblastoma protein (Rb) as well as reduces Cdk4 or Cdk2 kinase activity but without decreasing level of proteins [47].

The variety of conformational structures of enkephalins and receptors enables their participation in many processes that take place in physiological systems, including the immune system, and in communication between other systems. Met-enkephalin has shown to be involved in humoral and cellular immune reactions. [48] Cytotoxic CD8+T lymphocytes are essential for the immune system, regardless of antitumor activity and antiviral defense [49]. The specific response of T lymphocytes is associated with multiple expression of immunological molecules such as CD28, CTLA-4, PD-1, FasL, and granzyme. B. Jiao *et al.* [49] *showed* that Met-enkephalin may play a role in the immune system through the precise regulation of opioid receptor subunits. Furthermore, the opioid receptors MOR and DOR are presented in CD8+T cells at both RNA and protein levels. OGF also increases the level of surface molecules such as CD28, PD-1, CTLA-4, FasL, and intracellular granzyme B. Additionally, the results *showed* that OGF can promote the proliferation and functions of CD8+T cells through up-regulating μ and δ opioid receptors, and this effect can be cancelled while one or both of the receptors are blocked [49]. It has been confirmed that OGF increases the serum level of the cytokines IL-5 and IL-10, with simultaneous decreases TNF-α and IL-2 level [49]. Interestingly, Met-enkephalin significantly decreases the plasma glucose level and increases the serum insulin concentration in type 2 diabetes mellitus rats [50].

3. THE ANTICANCER POTENTIAL OF OPIOID GROWTH FACTOR

The OGF has been identified in almost all neoplasia, tumor explants and cancer cell cultures [51]. A large number of studies using human cancer cell lines as well as animal models confirmed that the progression of neoplasia can be inhibited by exogenous OGF as well as increase expression of OGFr [37]. The mechanism of the antiproliferative effect of [Met5]-enkephalin in cancer cells has been established as acting through cell cycle disruption: increase the number of cells in the G0/G1 phase with simultaneous reduction cells in S and G2/M phases [46,52]. Interestingly, blocking OGFr with naltrexone (NTX), an opioid antagonist, increases the rate of growth, length of DNA synthesis, mitotic phase as well as decreases doubling time in cancer cells [37]. Moreover, OGF modulates the *in vivo* angiogenesis process. It has been shown that OGF decreases number as well as the total length of blood vessels, and concomitant use of naltrexone removes this effect [5]. Using OGF in therapy is in various stages of advancement with regard to the type of cancer.

Pancreatic cancer is characterized by one of the lowest five-year survival rates, which is 5-6% [53]. Therefore, a new therapeutic approach based on underlying mechanisms of disease is needed. OGF exhibits ubiquitous effect on pancreatic cancer cells at different stages of differentiation [2]. Furthermore, OGF shows synergistic activity in combination with gemcitabine, which is the standard of care for advanced pancreatic cancer as well as with 5-fluorouracil [54]. Additionally,

the combination therapy reduces tumor volume in mice model by approximately 83%, whereas tumor volume is reduced 45% for OGF alone and 56% for gemcitabine alone [54]. An important issue for pancreatic cancer is also a level of OGFr expression. It has been determined that the OGF-OGFr axis provides tonic, homeostatic regulatory control of pancreatic neoplasia. In nude mice, who were transplanted with OGFr overexpressing cells, tumor incidence was reduced up to 50% compare with animals inoculated with wild type cell lines [55]. Moreover, tumor volumes were decreased by 70% [55]. The most interesting and valuable results have been shown in clinical studies. Patients with unresectable advanced pancreatic adenocarcinoma were treated with the maximum tolerated dose, which was established at 250 μg/kg in a phase I clinical trial [56]. From patients surviving more than eight weeks, 62% showed a decrease or stabilization in tumor size. The median survival time was increased three times compared with untreated patients [57]. Importantly, no adverse effects including blood values, cardiac rhythm, neurological status, and other laboratory tests were reported [57]. Moreover, quality of life surveys suggested improvement with OGF.

Carcinoma of the head and neck is the third most common neoplasia in developing countries and more than 90% of cases are squamous cell carcinomas (SCCHN) [53]. It has been confirmed that OGF and its receptor are present in human SCCHN from tissue culture, xenografts, and surgery [58,59]. Furthermore, molecular studies have shown that the OGF influences the G0-G1 phase of the cell cycle in HNSCC cancer cells through increases cyclin-dependent kinase inhibitor p16 protein expression and reduces Cdk4 kinase activity [60]. Additionally, the combination of OGF with paclitaxel as well as carboplatin are markedly inhibitory to SCCHN proliferation and the effect is greater than either of the individual compounds [58,59]. Animal studies confirmed that OGF modulation of SCCHN may have clinical application. Nude mice treated by opioid growth factor with xenografts of SCCHN have reduced tumor size compared to controls and display delays in tumor appearance [58]. By contrast, research-based on tissues from 64 patients with SCCHN revealed that OGFr is defective in SCCHN on translation/post-translation level of OGFr protein [61]. It has been suggested that restoring the OGFr function by gene therapy could provide a useful treatment for inhibiting tumor progression.

Moreover, OGF represents a promising drug candidate for the treatment of hepatoblastoma. Hepatoblastoma is the most common liver malignancy in children, and the 5-year overall survival rate has been improved to 75–80%. However, chemotherapy is associated with high toxicity [62]. Two cases of infants treated by experimental OGF therapy showed [Met5]-enkephalin as an effective and non-toxic therapy for the treatment of hepatoblastoma [63].

Furthermore, the potential of the Met-enkephalin as a new cancer immunotherapy agent has been documented. The OGF delays the development of tumors in the sarcoma mice model with S180 through down-regulating T-cells (Tregs) [9]. Likewise, studies in cancer patients have shown that Met-enkephalin has a strong inhibitory effect on Treg cells as well as significantly stimulates the proliferation of other lymphocyte subpopulations. This finding indicates that the OGF, as an immune booster, can maximize therapeutic *effectiveness* and minimize side effects for cancer patients, whose immune systems are damaged by chemotherapy and radiotherapy [64].

4. MET-ENKEPHALIN ANALOGUES AND FUNCTIONS

Met-enkephalin has multifunctional anticancer potential with a favorable safety profile. However, its short half-life in human plasma significantly limited its pharmacological efficacy and clinical application [7, 65]. This has prompted researchers to obtain appropriate analogues that are more stable and more cytotoxic to tumor cells. Several publications can be found in literature in which the anticancer potential of met-enkephalin derivatives was checked *in vitro* by extending the peptide chain, shortening it, or combining the peptide with other compounds.

For example, Zagon *et al.* [66] presented analogues of opioids and their influence on the proliferation of mouse neuroblastoma cells. They found that met-enkephalin exerted the strongest effect on cell proliferation and at a concentration of 10^{-10} inhibited the growth of cells in a stereospecific and reversible manner by naloxone. However, [Met5, Arg6, Phe7]-enkephalin, [Met5, Arg6, Gly7, Leu8]-enkephaline, and [Leu5]-enkephalin also showed inhibitory properties on neuroblastoma cell proliferation. Importantly, peptides shorter than Met-enkephalin: Tyr-Gly, Tyr-Gly-Gly, Tyr-Gly-Gly-Phe, Gly-Gly-Phe-Met did not affect the proliferation of tumor cells.

Horvat *et al.* [67] synthesized 18 new enkephalin analogues containing various non-natural amino acids of the adamantane type (Aaa1 and Aaa2). These derivatives were tested for their cytotoxic activity by the

MTT test and compared to Met-enkephalin (or its shorter analogues). Only derivatives that contained amino acid residues of the dialkylated glycine CRR (Aaa1) or alkylated glycine CR (Aaa2) exhibited cytotoxic activity against tumor cells: melanoma, larynx, colon, and colon metastasis cell line. Tyr-Aaa2-Gly-Phe-Met peptide proved to be the most effective analogue especially against the most anticancer drug-resistant larynx carcinoma and colon metastasis cell line. Moreover, apoptosis as a programmed cancer cell death was observed after exposure to this pentapeptide.

Interestingly, Gredicak *et al.* [68], using computer modeling, evaluated antiproliferative cancer cells activity of 390 analogues of enkephalins along with adamantine. Based on the obtained data five peptides: Met-enkephalin (1), Tyr-Aaa-Gly-Phe-Met (2), Tyr-Aaa-Gly-Phe-Phe (3), Phe-Aaa-Gly-Phe- Phe (4), and Phe-Aaa-Gly-Phe-Met (5) were synthesized and their potential antitumor properties *in vitro* were tested. The results clearly demonstrated the antiproliferative activity of investigated compounds on three tumor cell lines: breast cancer (MCF7), colon cancer (SW-620), and cervical cancer (HeLa). The highest cytotoxic activities showed the hydrophobic Phe-Aaa-Gly-Phe-Phe (4) and Phe-Aaa-Gly-Phe-Met (5) peptides.

Bajpai *et al.* [6] investigated the immunological properties of Met-enkephalin and its more stable synthetic analogues Tyr-D-Ala-Gly-MePhe-Met-NHC3H7-iso (1), Tyr-D-Ala-Gly-MePhe-Gly-HC3H7-iso (2), and Tyr-D-Ala-Gly-MePhe-Gly-NHCH2C6H5-iso (3). The T-cell transformation was investigated using *in vitro* lymphocyte transformation assay and natural killer (NK) cell cytotoxicity was evaluated. The results have shown that the stimulatory effect on T cells possessed Met-enkephalin and analogues 1 and 2. These peptides also showed an increase in NK cell activity. Analog 3 had no effect on T cells and NK cell cytotoxicity. The immune system has a huge role in combating cancer. Its weakness causes a faster progression of the disease.

Szweda *et al.* [69] proposed to obtain a Met-enkephalin nanocarrier with molecules targeting using thermoactive polymers. The article presents a path leading to obtaining Met-enkephalin nanocarriers from external crusts containing the targeted arginine-glycine-asparagine acid peptide. The formation of a controlled-shape mesoglobulin is obtained by the sudden heating of the aqueous solution of the bioconjugate. The mesoblobulins thus obtained are stabilized by coating them with a crosslinked double layer coating. The fluorescent marker (carboxyfluorescein) is added to the targeting peptide which is connected to the nanocarrier. The presence of glutathione results in the distribution of the coating and the release of Met-enkephalin. Precisely obtaining protective nanoparticles can become a new platform in the treatment of cancer, and can also be used in many other biomedical applications.

During the treatment of cancer, it is also important that the drug has analgesic properties. It has been shown that the peptide chain of five amino acids is the smallest, active structural unit [8-12] and the N-terminal removal [12] or a C-terminal amino acid [8,70,71] leads to complete or almost complete loss of analgesic activity.

The synthesis of analogues of Met-enkephalin containing chemically modified tyrosine, which is responsible for interaction with the receptor [72,73], allowed to investigate its effect on the biological activity of peptides. It has been noted [70,74] that removal of the amino group from tyrosine led to the disappearance of the analgesic properties of Met-enkephalin. The enkephalin analogues with the methylated N-terminal amino group showed a slight reduction in analgesia [12,75]. In addition to the amino group, the biological activity of enkephalins determines the presence of the hydroxyl group of tyrosine. Removal of it from the molecule as a result of replacing tyrosine by phenylalanine resulted in a complete loss of activity [76]. Methylation of the hydroxyl group [76] led to the same effect.

The necessity of an aromatic, hydrophobic phenylalanine residue confirmed the receipt of inactive derivatives, in which this amino acid was replaced with other *e.g.* histidine, tyrosine, or leucine [12]. The distance between tyrosine and phenylalanine is also important. The removal of one of the glycyl residues or the introduction of additional caused a decrease in biological activity, which probably results from the impossibility of creating a suitable spatial conformation during the reaction with the receptor [12]. The presence of the free carboxyl group of the C-terminal amino acid is not a prerequisite for maintaining biological (analgesic) activity. Among the synthesized analogues of Met- and Leu-enkephalin with a blocked carboxylic group, significantly differing in composition from parental peptides showed no difference in the activity of the analogue [75, 77-80]. The analogues of enkephalins with proline or its amide as a C-terminal amino acid, which also contains in the 2-position D-alanine, D-methionine, or D-norleucine [81], were particularly

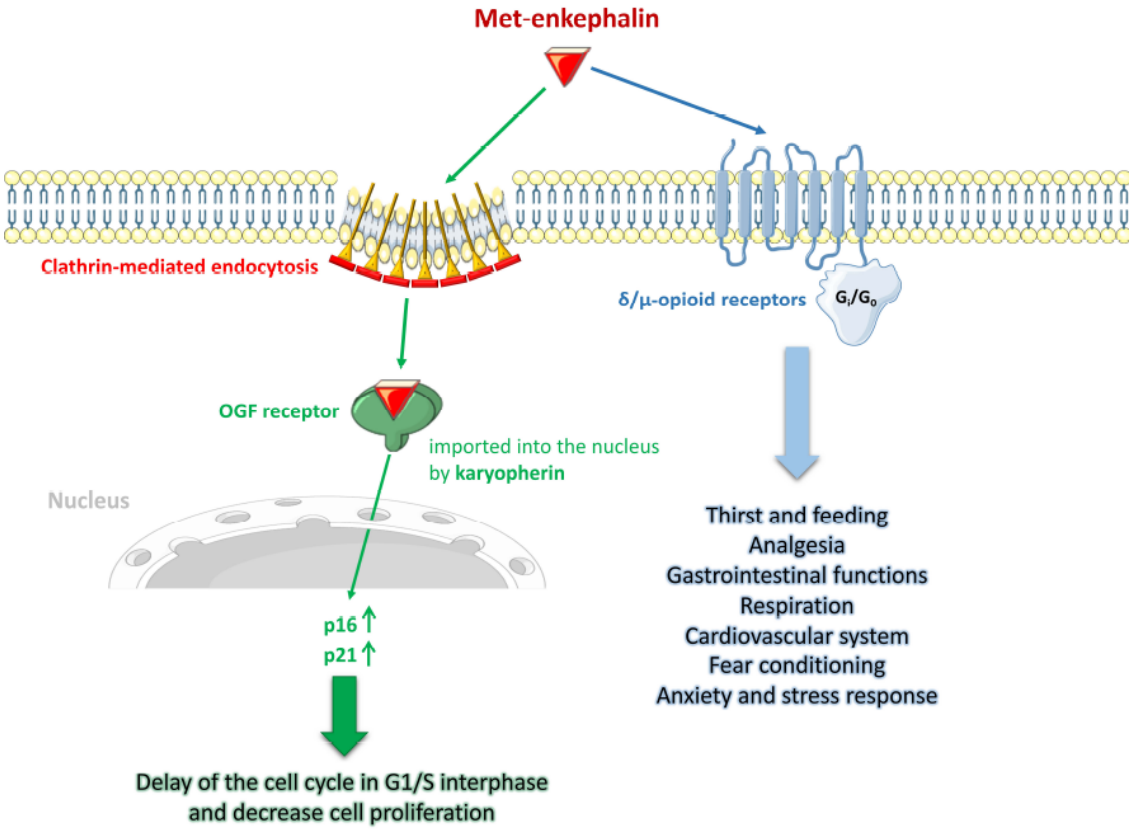


Fig. (1). The biological function of Met-enkephalin. Elements of this illustration were provided by Servier Medical Art (http://smart.servier.com/)

active, which underlines the key role of tyrosine for the biological activity of enkephalins.

Leaving the hydrophobic part of tyrosine and lengthening the chain does not deprive the activity of the compound. Stefano *et al.* [82] presented that the attachment of arginine and phenylalanine in positions 6 and 7 (Fig. **1**) showed activity comparable to Met-enkephalin. Furthermore, since the neutral endopeptidase (enkephalinase, CD10/NEP) was found in the invertebrate immunocytic membranes, it has been demonstrated that a specific inhibitor, phosphoramidamidamide, potentiates the effect of the heptapeptide on the induction of conformational changes in both humans and invertebrate granulocytes. Glycosylation resulted in a slight decrease in affinity to the delta-opioid receptor, and a mixed effect on binding to the mi-opioid receptor.

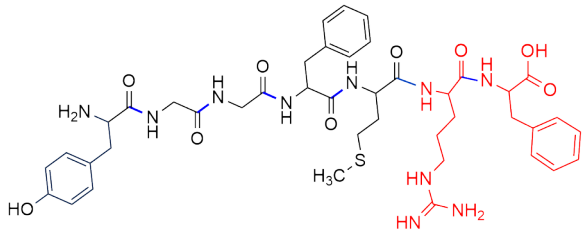
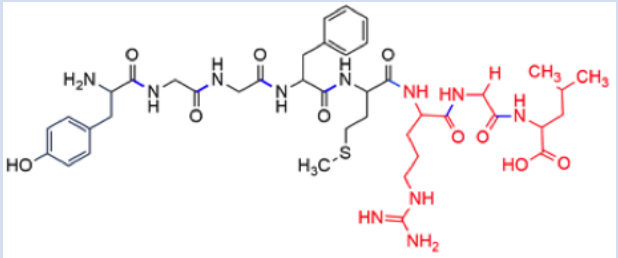
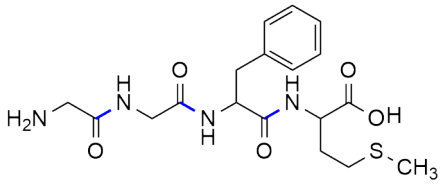
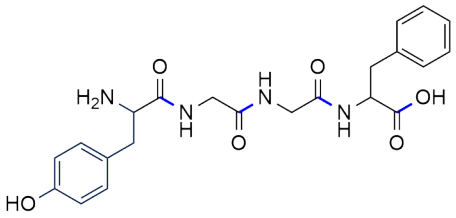
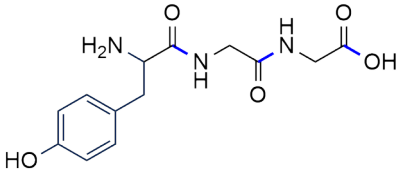
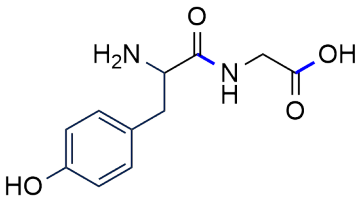
The blood-brain barrier prevents the penetration of many peptide drugs into the brain, thereby reducing the effective treatment of CNS tumors. Egleton *et al.* [83] investigated the effect of glycosylation on the cyclic opioid: [D-Cys, Ser, Gly] encephalin and delivery to the CNS. They found that the O-glycosidic bond caused a decrease in affinity to the delta-opioid receptor and different effects on binding to the μ-opioid receptor. Reduced lipophilicity with a glycosidic linkage resulted in reduced binding to bovine albumin. *In situ* study of brain perfusion showed that opioid uptake was improved by up to 98%.

Table **1** presents the structures and basic functions of selected Met-enkephalin analogues. The changes in the structure of the analogue were compared with the parent peptide and indicated by red color. The peptide bonds that exist between the amino acids in the peptide structure have been marked in blue.

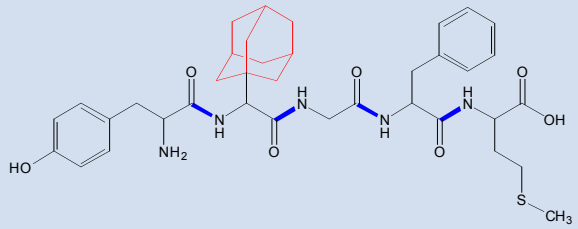
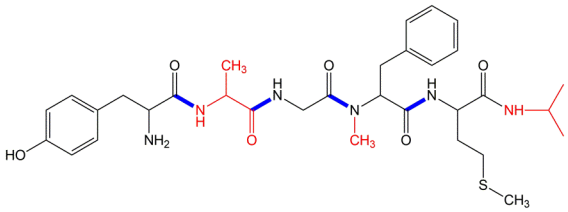
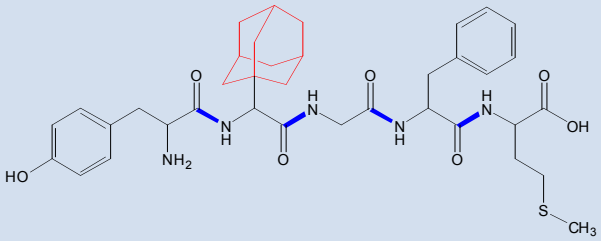
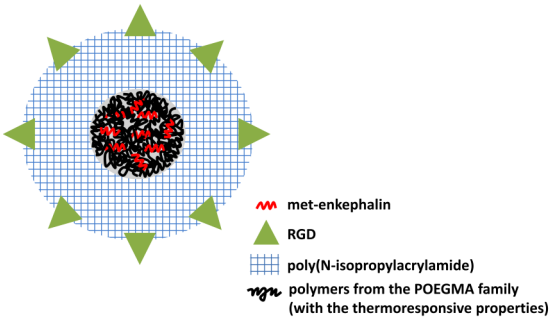
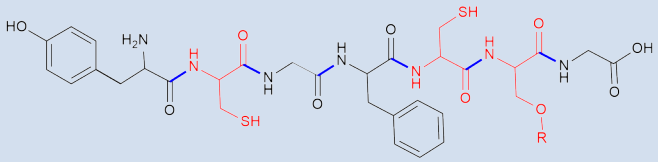
CONCLUSION

Using the met-enkephalin (OGF) creates interesting opportunities to obtain a new generation of anticancer drugs. This article describes the properties and mechanism of the antitumor activity of the peptide in the context of its multifunctional activity. Met-enkephalin has limited properties to inhibit the proliferation of cancer cells, which is why studies are being lead to obtaining more effective opioid analogues. The above work presents the collected Met-enkephalin derivatives and describes their changes in biological properties - structure-activity relationships. The proposed strategy

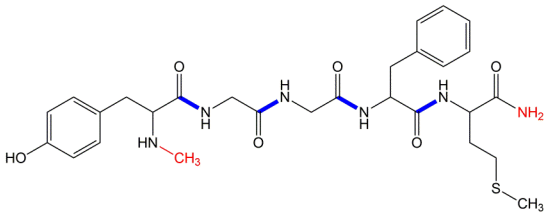
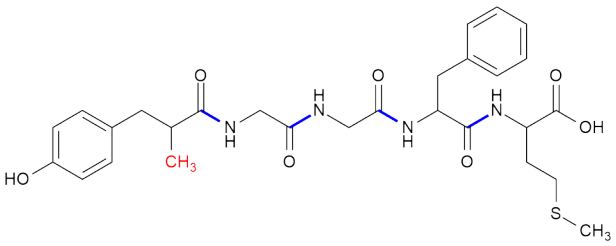
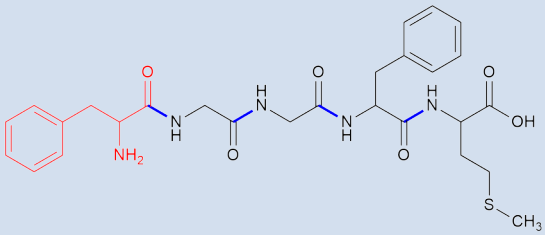
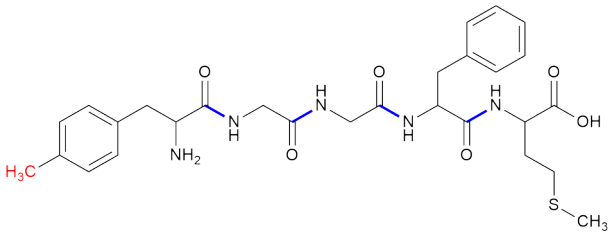
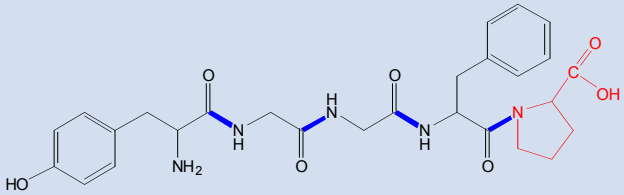
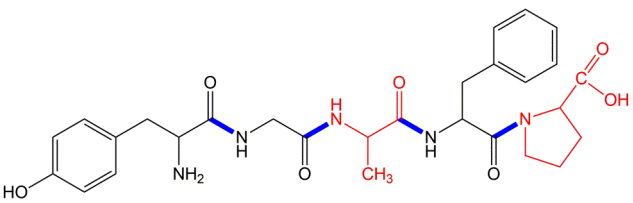
Table 1. Selected Met-enkephalin derivatives. (green: cancer cell lines studies; purple: immunomodulatory activity).

S#.	Chemical Structure	Activity	Refs.
1.	Tyr-Gly-Gly-Phe-Met-Arg-Phe 	**Chain extension with arginine and phenylalanine.** **Inhibitory properties on mouse neuroblastoma cell proliferation (S20Y).** **Stimulating conformational changes and locomotory activity in human and invertebrate granulocytes as capable as OGF.**	[66,82]
2.	Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu 	**Chain extension with arginine, glycine, and phenylalanine.** **Inhibition of S20Y cancer cell line (neuroblastoma) proliferation.**	[66]
3.	Gly-Gly-Phe-Met (**1**)  Tyr-Gly-Gly-Phe (**2**)  Tyr-Gly-Gly (**3**)  Tyr-Gly (**4**) 	**Shortening of the peptide with: tyrosine (1), methionine (2), methionine and phenylalanine (3), methionine, phenylalanine, and glycine (4).**	[66]

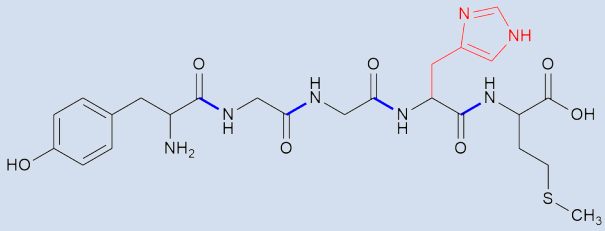
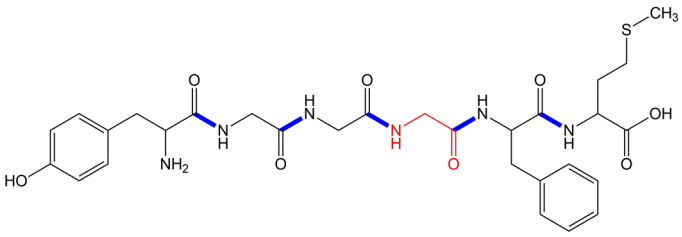
(Table 1) contd….

S#.	Chemical Structure	Activity	Refs.
4.	Tyr-(*R,S*)-Aaa2-Gly-Phe-Met 	**The adamantane-containing amino acids.** **Increased cytotoxic activity (*in vitro* model) on human cervical adenocarcinoma cells (HeLa), larynx cancer (Hep-2), colon cancer (HT-29, Caco-2), on colon cells (SW-620), melanoma cells (HBL). Induction of apoptosis in Hep-2 and SW-620 cell lines.**	[67]
5.	Tyr-D-Ala-Gly-MePhe-Met-NH*C_3H_7-*iso* Tyr-D-Ala-Gly-MePhe-Gly-NH*C_3H_7-*iso* 	**Replacing glycine with alanine and lengthening the methionine chain.** **Stimulation of T cells transformation and increase of NK cell cytotoxicity (almost as active as OGF).**	[6]
6.	Tyr-Aaa-Gly-Phe-Met Tyr-Aaa-Gly-Phe-Phe Phe-Aaa-Gly-Phe-Phe Phe-Aaa-Gly-Phe-Met 	**Enkephalin analogues with (1-adamantyl) glycine.** **Cytotoxic effects on cancer cells of breast cancer (MCF7), colon cancer (SW-620) and cervical cancer (HeLa).**	[68]
7.	Nanoparticles of met-enkephalin with RGD 	**Nanocarriers of met-enkephalin with outer shells containing targeted RGD (arginine-glycine-aspartic acid) which is specifically recognized by the overexpressed integrin receptors on the cancer cells.** **In the presence of glutathione, the whole shell is completely degradable and the met-enkephalin conjugates are released.**	[69]
8.	Tyr-D-Cys-Gly-Phe-D-Cys-Ser(R)-Gly R-glucose 	**Cyclization of the peptide by cysteine's residues and addition of serine connected with glucose.** **Higher bioavailability (the blood-brain barrier penetration).** **Glycosylation reduces the affinity of the peptide for the δ-receptor and has a mixed effect on the μ-receptor affinity.**	[83]

(Table 1) contd….

S#.	Chemical Structure	Activity	Refs.
9.	N-methyl-Tyr-Gly-Gly-Phe-Met amide 	**Peptides were stabilized against enzymatic degradation by blocking one or both termini with N-methyl and C-amide groups.** **A prolonged period of the analgesic action.**	[75]
10.	desamino-Tyr(CH_3)-Gly-Gly-Phe-Met 	**Replacement of tyrosine amino group with methyl. Reduction of analgesic properties.**	[74]
11.	Phe-Gly-Gly-Phe-Met 	**Replacement at the *C*-terminus of tyrosine with phenylalanine.** **Total loss of analgesic functions.**	[76]
12.	(CH_3)Phe-Gly-Gly-Phe-Met 	**The methylation of the phenylalanine's benzyl group.** **Total loss of analgesic functions.**	[76]
13.	Tyr-Gly-Gy-Phe-Pro 	**Block the *C*-terminal with proline.** **Decrease of metabolic stability, however, increase analgesic potency in comparison to the parent peptide (after intravenous and central administration).**	[82]
14.	Tyr-Gly-D-Ala-Phe-Pro 	**Blocking the *C*-terminal with proline or its amide and replacing glycine with D-alanine.** **Increase of metabolic stability and analgesic activity in comparison to the parent peptide (after intravenous administration).**	[82]

(Table 1) contd….

S#.	Chemical Structure	Activity	Refs.
15.	Tyr-Gly-Gly-His-Met 	**Replacement of phenylalanine with an amino acid without a hydrophobic moiety.** **No biological anxiety and stress response and analgesia of the analogue activity.**	[12]
16.	Tyr-Gly-Gly-Gly-Phe-Met 	**Introduction of another glycine to the peptide.** **Substantial loss of analgesia activity.**	[12]

shows derivatives of the OGF as a potential new, more effective multifunctional anticancer, and analgesic drugs without the harmful side-effects caused by conventional anticancer therapies. Having regard to successful treatment applications, further studies on the OGF analogues are necessary, including animal investigation and clinical trials.

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