British Journal of Pharmacology

British Journal of Pharmacology (2017) **174** 1378–1394 1378

Themed Section: Principles of Pharmacological Research of Nutraceuticals

REVIEW ARTICLE

Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review

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Received 28 May 2016; **Revised** 12 August 2016; **Accepted** 18 August 2016

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In the past few years, increasing interest has been directed to bioactive peptides of animal and plant origin: in particular, researchers have focused their attention on their mechanisms of action and potential role in the prevention and treatment of cancer, cardiovascular and infective diseases. We have developed a search strategy to identify these studies in PubMed (January 1980 to May 2016); particularly those papers presenting comprehensive reviews or meta-analyses, plus *in vitro* and *in vivo* studies and clinical trials on those bioactive peptides that affect cardiovascular diseases, immunity or cancer, or have antioxidant, anti-inflammatory and antimicrobial effects. In this review we have mostly focused on evidence-based healthy properties of bioactive peptides from different sources. Bioactive peptides derived from fish, milk, meat and plants have demonstrated significant anti-hypertensive and lipid-lowering activity in clinical trials. Many bioactive peptides show selective cytotoxic activity against a wide range of cancer cell lines *in vitro* and *in vivo*, whereas others have immunomodulatory and antimicrobial effects. Furthermore, some peptides exert anti-inflammatory and antioxidant activity, which could aid in the prevention of chronic diseases. However, clinical evidence is at an early stage, and there is a need for solid pharmacokinetic data and for standardized extraction procedures. Further studies on animals and randomized clinical trials are required to confirm these effects, and enable these peptides to be used as preventive or therapeutic treatments.

LINKED ARTICLES

This article is part of a themed section on Principles of Pharmacological Research of Nutraceuticals. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.11/issuetoc

Abbreviations

APO, apolipoprotein; BBI, Bowman-Birk inhibitor; Bcl, B cell lymphoma; C33-A, cervical carcinoma cell line; CYPTA, cholesterol 7α-hydroxylase; eNOS, endothelial NOS; HT, human colon adenocarcinoma cell line; Sw480, human colon carcinoma cell line; U87, U87-MG human glioma cells

DOI:10.1111/bph.13608

Tables of Links

TARGETS	
Other protein targets[a]	**Enzymes**[e]
Bax	ACE
Bcl-2	Akt
NPC1L1	Caspase 3
TNF-α	Caspase 2
GPCRs[b]	Caspase 9
D_1 receptor	COX-2
NTS_2 receptor	eNOS
Other ion channels[c]	FAK
Cx43	GSK3β
Transporters[d]	HMG-CoA reductase
PepT1 (PEPT1)	IKK-α
	IKK-ß
	iNOS
	JNK
	PI3K

LIGANDS	
CCL2 (MCP-1)	IL-1β
CCL5 (RANTES)	IL-2
cGMP	IL-5
ICAM-I	IL-6
IFN-γ	VCAM-I

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d,e]Alexander *et al.*, 2015a,b,c,d,e).

Introduction

There is an abundance of bioactive peptides contained in a wide range of food sources (products of plant, animal and marine origin) and generated by fermentation, enzymatic, chemical hydrolysis or gastrointestinal digestion processes from food proteins (Udenigwe and Aluko, 2012). In recent years, there has been a marked increase in the number of publications highlighting their potential effects on blood pressure (BP) and lipid metabolism, in addition to their anticancer, immunomodulatory, antimicrobial, analgesic, antioxidant and anti-inflammatory activity. In particular, interest has been directed towards their effects on hypertension and dyslipidaemia, which are two of the most important risk factors for cardiovascular diseases and represent a major cause of mortality in developed countries. There is now much evidence that many non-pharmacological treatments, including bioactive peptides, have the ability to regulate BP and lipids levels (Cicero and Colletti, 2015a,b).

Together with cardiovascular diseases, cancer is one of the main causes of death in developed countries (Siegel *et al.*, 2013). Conventional chemotherapy remains one of the principle treatments, but it is usually associated with significant side effects, because it is often not selective for cancer cells (Holohan *et al.*, 2013). The anticancer effects of peptides have been extensively explored (Boohaker *et al.*, 2012; Gautam *et al.*, 2014), and there is an increasing number of approved peptide-based drugs (Vlieghe *et al.*, 2010). These drugs have great potential because they exhibit antitumoral activity (Thundimadathil, 2012) by acting on multiple molecular pathways involved in carcinogenesis and are usually not genotoxic (Blanco-Míguez *et al.*, 2016).

Another important field for the application of bioactive peptides is the regulation of immunity and the prevention of infections. An important advantage in the use of bioactive peptides for reinforcing immunity is that they have a large spectrum of activity and have effects on both non-specific and specific immunity (Yang *et al.*, 2009; Wang *et al.*, 2010; Cheung *et al.*, 2015). Bioactive peptides have also been demonstrated to have analgesic, antioxidant and anti-inflammatory activity both *in vitro* and *in vivo* (Chakrabarti *et al.*, 2014).

In this context, the aim of this review is to analyse the results of the studies and clinical trials conducted with bioactive peptides, to evaluate their mechanism of action and their potential role in the prevention and treatment of a wide range of pathologies.

Methods

A search strategy was developed to identify trials in PubMed (January 1980 to May 2016).

Firstly, the authors assessed the major fields of study of bioactive peptides through the MeSH terms (major subject heading) 'bioactive peptides' and 'review' or 'meta-analysis'. Then the search was refined to evaluate clinical trials: the MeSH terms 'bioactive peptides' and 'cardiovascular diseases' or 'hypertension' or 'dyslipidaemia' or 'immunity' or 'cancer' or 'analgesic' or 'antimicrobial' or 'antioxidant' or 'anti-inflammatory' were incorporated into an electronic search strategy. The bibliographies of all the studies and review articles identified were examined to obtain additional studies of interest. The authors reviewed all of the citations

retrieved from the electronic search to identify potentially relevant articles for this review. They subsequently reviewed the potential trials to determine their eligibility. They selected papers reporting comprehensive reviews or meta-analyses, or original *in vitro* and *in vivo* studies and clinical trials on bioactive peptides with an action on cardiovascular diseases, immunity and cancer or with antioxidant, anti-inflammatory and antimicrobial activity. Studies were also selected based on the quality of the methodology, data completeness and extent of sampling.

Blood pressure lowering effect

According to the European guidelines for hypertension management (Zannad *et al.*, 2012), the nutraceutical approach could be a good compromise for patients with borderline values of BP and an adjuvant in combination with antihypertensive drugs in the treatment of moderate hypertension (Sirtori *et al.*, 2015; Borghi and Cicero, 2016). The use of nutraceuticals in the treatment or prevention of hypertension would result in a reduction in the typical side effects of conventional drugs (cough, skin rashes, hyperkalaemia, loss of taste, sleep apnoea, erectile dysfunction and angioedema) and hypothetically have economic savings on health expenditure due to a potential reduction in cardiovascular disease (Houston, 2013).

Over the past few years, researchers have investigated different types of bioactive peptides derived from heterogeneous sources, such as fish, milk, meat and plant derivatives, which have potential antihypertensive activity (Hartmann, Meisel, 2007; Bhat *et al.*, 2015). The antihypertensive action of some bioactive peptides is now known to be due to a competitive and/or non-competitive inhibition of ACE, which is responsible for the conversion of angiotensin I in angiotensin II. Angiotensin II is an octapeptide that increases the peripheral vascular resistance and the preload, inducing a hypertensive action. Furthermore, ACE determines the cleavage and inactivation of bradykinin, a vasodilator peptide. Other putative mechanisms of action are attributed to an increase in the activity of certain vasodilating agents including endothelial NOS (eNOS); the increased production of endothelial NO and the inhibition of renin, which converts angiotensinogen to angiotensin I, increases the substrate of ACE. Furthmore, bioactive peptides can act to reduce the activity of the sympathetic system, inducing vasodilatation (Aluko, 2015).

The clinical efficacy of antihypertensive bioactive peptides depends substantially on two factors: their resistance to degradation by gastrointestinal peptidases and their absorption into the blood stream. The absorption of these peptides may be carried by a transporter peptide [peptide transporter 1(PEPT1), for peptides with a maximum of three aminoacids], by pinocytosis (highly soluble peptides) or by the paracellular (through the aqueous transport) or transcellular routes (Rotimi, 2015). Based on these two factors and the amino acid sequence of the bioactive peptide, the clinical results in terms of BP reduction will be different.

The main bioactive peptides used in the treatment/prevention of hypertension, the source from which they were extracted and their mechanism of action are presented in Figure 1.

One of the richest sources of proteins and bioactive peptides is milk that contains a large number of peptides including the tripeptides valine-proline-proline (VPP) andisoleucine-proline-proline (IPP), and the polypeptides phenylalanine-phenylalanine-valine-alanine-proline-phenylalanine-proline-glutamate-valine-phenylalanine-glycine-lysine (FFVAPFPEVFGK) and tyrosine-leucine-glycine-tyrosine-leucine-glutamate-glutamine-leucine-leucine-arginine (YLGYLEQLLR) peptides; there are numerous clinical trials that have evaluated their effect on the major cardiovascular parameters (Cicero *et al.*, 2013). In particular, the tripeptides VPP/IPP have been shown (at dosages between 5 and 100 mg·day^{-1}) to have a variable clinical efficacy, more evident in Asian subjects (suggesting a possible genetic/population-dependent effect, as already seen for some antihypertensive drugs); in a meta-analysis by our group considering 18 randomized clinical trials, the pooled effect of peptides was a reduction of −3.73 mmHg (95% CI: −6.70, −1.76) for systolic BP and 1.97 mmHg (95%CI: −3.85, −0.64) for diastolic BP (Cicero *et al.*, 2011a). More recent data show that these peptides could also positively modulate pulse wave velocity in mildly hypertensive subjects. No safety concerns were raised (Cicero *et al.*, 2011b, 2016).

Whey proteins are also a rich source of bioactive peptides; they can be converted into peptides through different types of treatments (enzymatic hydrolysis by trypsin, alcalase and pepsin); in particular, the aspartate-arginine-valine-tyrosine-isoleucine-histidine-proline-phenylalanine-histidine-leucine (DRVYIHPFHL), aspartate-arginine-valine-tyrosine-isoleucine-histidine-proline-phenylalanine (DRVYIHPF) and arginine-valine-tyrosine-isoleucine-histidine-proline-phenylalanine (RVYIHPF) peptides (respectively a deca, octa and heptapeptide) have shown antihypertensive activity with an inhibitory action on the renin-angiotensin-aldosterone (RAS) system (Yadav *et al.*, 2015). In general, whey and caseins proteins have significant antihypertensive effects both in normotensive/pre-hypertensive and in obese subjects (Bhat *et al.*, 2015; Nongonierma and FitzGerald, 2015). Moreover, numerous studies have reported that biological active peptides isolated from the whey of cow's milk can affect BP. Studies on animals and humans have shown that α-lactalbumin and β-lactoglobulin, which are obtained from enzymatically hydrolysed whey, are able to inhibit ACE, while lactorphins lower BP by normalizing endothelial function or by an opioid receptor-dependent mechanism (Dong *et al.*, 2013).

Several marine peptides with antihypertensive activity have been detected in species such as bonito, tuna and sardine (leucine-lysine-proline - LKP, isoleucine-lysine-proline - IKP, leucine-arginine-proline - LRP), *Okamejei kenojei* (methionine-valine-glycine-serine-alanine-proline-glycine-valine-leucine – MVGSAPGVL, leucine-glycine-proline-leucine-glycine-histidine-glutamine – LGPLGHQ) and *Styela clava* (alanine-histidyne-isoleucine-isoleucine-ilesoleucine – AHIII); the presence of these bioactive peptides has led to an increase of endothelial NO levels and aorta vasodilatation in rats (Cheung *et al.*, 2015).

Finally, numerous plant species provide a wide range of bioactive peptides and proteins. The intake of plant proteins (in particular, soy, oak, barley and pea proteins) seems to be associated with a mild but significant lowering of BP levels

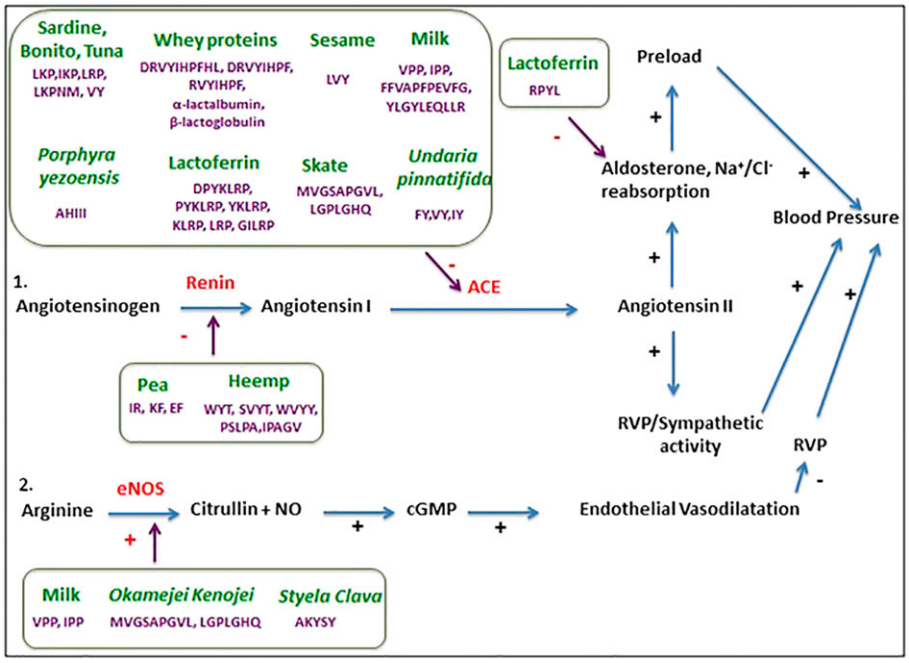

Figure 1

Main bioactive peptides that have been shown to lower blood pressure: proposed mechanisms of action. ACE, angiotensin converting enzyme; eNOS, endothelial NOS; RVP, renal venous pressure; EF, glutamate- phenylalanine; FY, phenylalanine- tyrosine; IKP, isoleucine-lysine-proline; IPP, isoleucine-proline-proline; IR, isoleucine-arginine; IY, isoleucine-tyrosine; KF, lysine-phenylalanine; LKP, leucine-lysine-proline; LRP, leucine-arginine-proline; LVY, leucine-valine-tyrosine; VPP, valine-proline-proline; VY, valine-tyrosine; WYT, tryptophan-tyrosine-threonine.

(Altorf-van der Kuil *et al.*, 2010; Malaguti *et al.*, 2014; Nirupama, *et al.*, 2015). In particular, those peptides extracted from cereals, such as oats and barley, isoleucine-valine-tyrosine (from wheat germ), isoleucine-aspartate-proline show a strong inhibitory action on ACE (Motoi and Kodama, 2003; Nirupama *et al.*, 2015). However, it is not easy to discriminate between the effect of plant proteins and other associated dietary components on BP levels. For example, the isoflavones taken with soy could really be responsible for the soy-related decrease in BP; a recent meta-analysis has shown that in hypertensive patients the intake of soy isoflavones is associated with a decrease in SBP by −5.94 mmHg (95% CI: −10.55, −1.34) (P = 0.01) and of DBP by −3.35 mmHg (95% CI: −6.52, −0.19) (P = 0.04) (Liu *et al.*, 2012).

In conclusion, peptides derived from milk, whey, fish and plants have demonstrated a mild but significant antihypertensive effect in humans, based on the inhibition of the RAS system and/or an increase in endothelial NO levels. These data have been confirmed through further long-term randomized clinical trials in normotensive and pre-hypertensive patients.

Cholesterol-lowering effect

Another important cardiovascular risk factor is represented by dyslipidaemia. The bioactive peptides with the most clinical evidence for inducing a reduction in cholesterolaemia are those derived from soy, lupine and milk proteins (Lammi *et al.*, 2014; Butteiger *et al.*, 2016).

A recent meta-analysis included 35 studies that examined the effects of soy protein (in particular B-conglycinin globulin) on lipid parameters, with treatments that varied from 4 months to 1 year. The results showed that soy proteins have a cholesterol-lowering effect with a reduction in LDL-cholesterol (LDL-C) of 3% (−4.83 mg·L^{-1}; 95% CI: −7.34, −2.31), in total cholesterol (TC) of 2% (−5.33 mg·L^{-1}; 95% CI: −8.35, −2.30) and in triacylglycerol of 4% (−4.92 mg·L^{-1}; 95% CI: −7.79, −2.04); moreover, a significant improvement in HDL-cholesterol (HDL-C) of 3% (1.40 mg·L^{-1}; 95% CI: 0.58, 2.23) was observed. The LDL-C reduction was greater in moderately hypercholesterolaemic patients (−7.47 mg·L^{-1}; 95% CI: −11.79, −3.16) compared with healthy subjects (−2.96 mg·L^{-1}; 95% CI: −5.28, −0.65) (Tokede *et al.*, 2015).

In particular, among the bioactive peptides derived from soy, lunasin, a 43-aminoacid peptide characterized by a RGD sequence followed by eight aspartate residues at its carboxyl-end with high bioavailability and stability (Hernández-Ledesma *et al.*, 2013), showed a potential cholesterol-reducing activity; however, it is important to emphasize that studies conducted with this peptide have hitherto involved animal models and cell lines (Lule *et al.*, 2015). Tests are therefore necessary to assess its efficacy and safety in clinical practice.

The main mechanisms of action whereby soy and lupine peptides reduce blood cholesterol levels can be attributed to an up-regulation of LDL receptors, the regulation of the sterol regulatory element-binding protein 2 (SREBP2) pathway (via PI3K/Akt/GSK3β pathways), the inhibition of the hydroxymethylglutaril-CoA (HMG-CoA) reductase enzyme and an increase in the faecal excretion of bile salts (Marsh *et al.*, 2011; Lammi *et al.*, 2014).Therefore, the up-regulation in the transcription of LDL receptors results in an enhanced

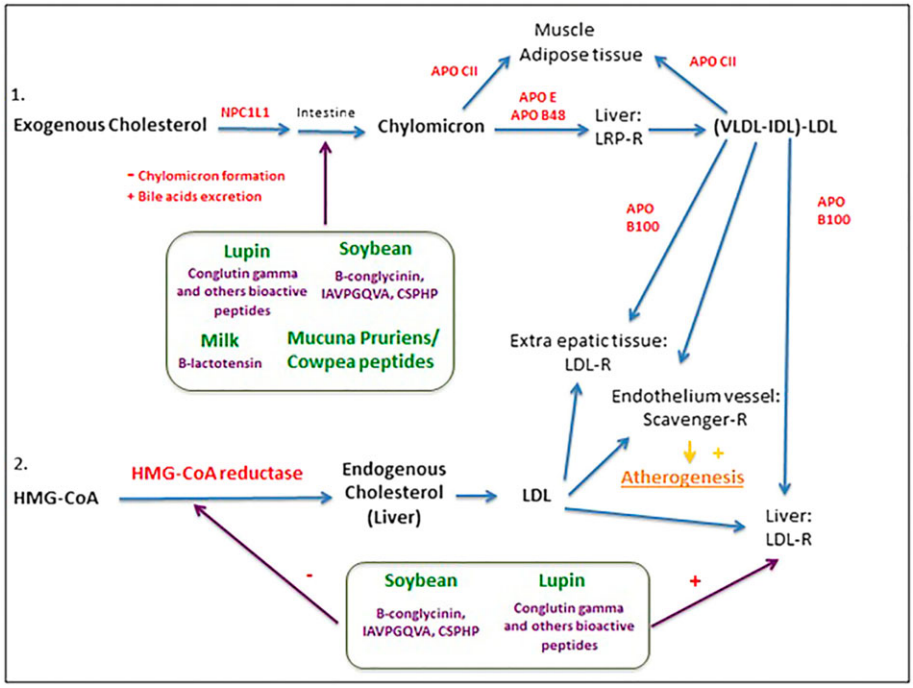

Figure 2

Main bioactive peptides that have been shown to have a beneficial effect on cholesterol metabolism: proposed mechanisms of action. APO, apolipoprotein; HMG-CoA, hydroxymethylglutaril-CoA; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LDL-R, low-density lipoprotein-receptor; LRP-R, low-density lipoprotein receptor-related protein – receptor; NPC1L1, Niemann-pick C1-like 1; VLDL, very low-density lipoprotein.

catabolism or a reduced synthesis of intracellular cholesterol (Cho *et al.*, 2007).

The proteins derived from lupine (50 mg·day^{-1}) demonstrated LDL-C lowering efficacy in a rat model; the conglutin γ (isolated from lupine) was also found to increase the number of LDL receptors in a hepatoma G2 cell line (Sirtori *et al.*, 2012).

Another peptide with cholesterol-lowering activity is derived from the hydrolyzate extracted of *Mucuna pruriens*; it interacts with micelle formation and the absorption of exogenous cholesterol (Herrera Chalé *et al.*, 2016). Peptides from cowpea have also been demonstrated to inhibit cholesterol synthesis and its solubilization into micelles (Marques *et al.*, 2015).

A bioactive peptide derived from milk with cholesterol-lowering action is β-lactotensin; at a dose of 100 mg·Kg^{-1} p.o., it significantly reduced serum cholesterol in mice and increased the excretion of bile acids in the faeces (Yamauchi *et al.*, 2003). Its cholesterol-lowering effect is probably due to its effects on neurotensin NTS_2 and dopamine D_1 receptors, which leads to an increased synthesis of bile acids from cholesterol, enhanced further by the direct action of β-lactotensin on mRNA (induced by increasing levels of CYP7A) (Yoshikawa, 2015) (Figure 2).

It is concluded that the mechanisms of action of lipid-lowering peptides are clear thanks to the numerous studies performed *in vitro* and in animal models; they mainly act by inhibiting the production of endogenous cholesterol and promoting the faecal excretion of exogenous cholesterol. However, it is still necessary to evaluate their pharmacokinetics profiles, bioavailability and long-term effective dosages in humans.

Anti-inflammatory activity

Recent *in vitro* and *in vivo* studies have shown a potential anti-inflammatory activity of several bioactive peptides derived mostly from bovine milk, eggs, soy and fish (Marcone *et al.*, 2016); nevertheless, the mechanisms of action are known for only a few of these peptides. The available evidence suggests that the anti-inflammatory activity of bioactive peptides is mainly due to the modulation of transcription factors, kinases (NF-kB and MAPK) and/or cytosolic compounds (Figure 3). However, the mechanism through which these peptides penetrate the cell is unclear; it is not known if they act directly on the cell membrane or whether they interact with different receptors (Majumder *et al.*, 2016).

IPP and VPP peptides, derived from the bacterial fermentation of casein, show anti-inflammatory activity; they prevent the formation of atherosclerotic plaque by inhibiting the pro-inflammatory JNK-MAPK pathway (Aihara *et al.*, 2009). Another polypeptide (aspartate-methionine-proline-isoleucine-glutamine-alanine-phenylalanine-leucine-leucine-tyrosine-glutamine-glutamate-proline-valine-leucine-glycine-proline-valine-arginine – DMPIQAFLLYQEPVLGPVR) derived from β-casein exerts an anti-inflammatory action through the inhibition of NF-kB pathway (Malinowski *et al.*, 2014). In humans, the administration of proteins derived from milk reduces postprandial inflammation in obese and non-diabetic subjects, as indicated by a reduction in the

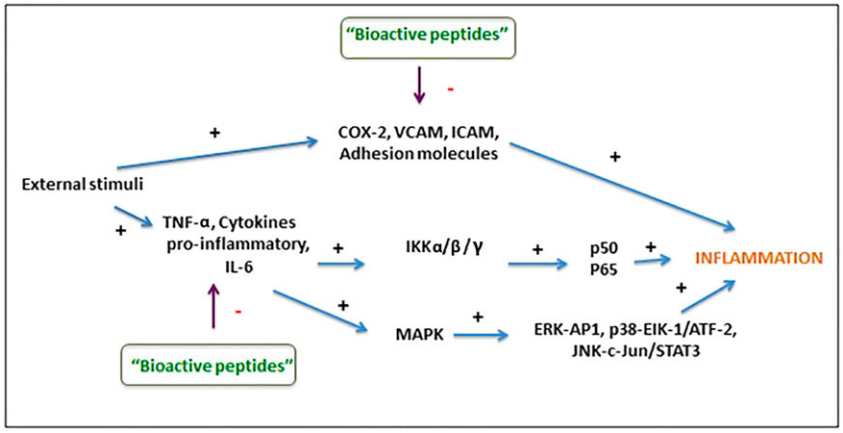

Figure 3

Anti-inflammatory effects of bioactive peptides: main proposed mechanisms of action. ERK-AP1, ERK-activator protein 1; ICAM, intracellular adhesion molecule; IKK, IκB kinase; p38-Elk-1/ATF-2, protein 38-Elk-1/activating transcription factor 2; VCAM, vascular cell adhesion molecule.

serum inflammatory biomarkers monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) and chemokine (C-C motif) ligand 5 (CCL5, also known as RANTES) (Holmer-Jensen *et al.*, 2011).

A tripeptide derived from ovotransferrin, which is present in the albumen of eggs, can reduce the expression of factors such as NF-kB (which inhibits the nuclear translocation of p50 and p65) that mediate the transcription of adhesion molecules [vascular cell adhesion molecule 1 (VCAM1) and intracellular adhesion molecule 1 (ICAM-1)] (Majumder *et al.*, 2013).

Peptides derived from soy, beans and milk have also shown anti-inflammatory effects on intestinal inflammation; among their mechanisms of action, these peptides may inhibit the expression of pro-inflammatory cytokines and chemokines. In particular, ɣ-glutamyl cysteine, a peptide isolated from various food sources including the edible beans, inhibits the phosphorylation of JNK and IkB, unlike valine-proline-tyrosine (VPY) that exerts its anti-inflammatory action by inhibiting the secretion of IL-8 and TNF-α (Majumder *et al.*, 2016).

Lunasin, has shown anti-inflammatory properties, probably due to the inhibition of IL-6 and IL-1β production, activation of NF-kB Akt-mediated (interaction with αVβ3 integrin), COX-2 and inducible NOS expression and PGE_2 production (Dia *et al.*, 2009; Hernandez-Ledesma *et al.*, 2009; Cam and de Mejia, 2012).

Although the anti-inflammatory mechanisms of action of bioactive peptides are not completely clarified, they seem to be related to the modulation of transcription factors and the inhibition of the expression of pro-inflammatory cytokines and chemokines. Data in humans are, however, at the preliminary stage.

Anticancer effect

Bioactive peptides have demonstrated their potential to exhibit cytotoxic activity in numerous cancer cell lines and, consequently, induce possible cancer-selective activity that may be devoid of the side effects of conventional chemotherapy. Peptides may be utilized directly as cytotoxic agents through various mechanisms or may act as carriers of cytotoxic agents and radioisotopes by specifically targeting cancer cells (Thundimadathil, 2012). Their advantages include low intrinsic toxicity, high tissue penetration for their small size, cell diffusion and permeability (Mader and Hoskin, 2006; Otvos, 2008). They are also able to affect one or more specific molecular pathways involved in carcinogenesis, and they are not usually genotoxic (Blanco-Míguez *et al.*, 2016).

Their main mechanisms of action are inhibition of cell migration, inhibition of tumour angiogenesis, antioxidant activity, the inhibition of gene transcription/cell proliferation, the induction of apoptosis, and the disorganization of tubulin structure and cytotoxicity (Table 1) (Schweizer, 2009; Tyagi *et al.*, 2015).

Bioactive peptides with potential cytotoxic activity can be derived from plants, milk, eggs and marine organisms. Among the peptides of plant origin, particular interest has been directed towards lunasin that has exerted antineoplastic effects in breast, skin, colon, prostate, leukaemia and lymphoma cell lines with different mechanisms of action (Hernandez-Ledesma and Hsieh, 2015). In mouse fibroblast, NIH 3 T3 and human breast Michigan cancer foundation-7 (MCF-7) cells, lunasin suppresses their transformation, induced by chemical carcinogens, by inhibiting histone acetylation in the presence of the histone deacetylase inhibitor sodium butyrate (Jeong *et al.*, 2002; Hsieh *et al.*, 2010; Jeong *et al.*, 2010); in L1210 leukaemic cells, it has shown a cytotoxic effect, inducing cell cycle arrest in G2/M phase and apoptosis through the activation of caspase-3 (de Mejia *et al.*, 2010). Moreover, in the human colon adenocarcinoma cell line, HT-29, lunasin induced apoptosis by the activation of caspase-3 through the intrinsic apoptotic pathway, as indicated by the induction of B cell lymphoma 2 (Bcl-2)-associated X (Bax) protein and a reduction in Bcl-2 protein levels (Dia and Mejia, 2010); it is also able to inhibit the metastasis of human colon cancer cells by direct binding with α5β1 integrin, suppressing focal adhesion kinase (FAK)/ERK/NF-κB signalling and to potentiate the effect of oxaliplatin in preventing the outgrowth of metastasis (Dia and de Mejia, 2011).

Table 1

Main bioactive peptides with anticancer activity: mechanisms of action

Source	Bioactive peptide	Mechanism of action	Effect	Model	Reference
Soybean	Lunasin	Cell proliferation and cancerous foci formation inhibition	Cytotoxic	7,12-dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene-treated (MCA) fibroblast NIH/3 T3 cells	Hsieh *et al.*, 2010
Soybean	Lunasin	Direct binding with α5β1 integrin suppressing FAK/ERK/NF-κB signalling	Metastasis inhibition	Human colon cancer cells	Dia and de Mejia, 2011
Soybean	Lunasin	Apoptosis through caspase-3 activation	Cytotoxic	L1210 Leukaemia cells	de Mejia *et al.*, 2010
Soybean	Lunasin	Apoptosis through caspase-3 activation	Cytotoxic	HT-29 colon cancer cells	Dia and Mejia, 2010
Soybean	Lunasin	Histone acetylation inhibition	Anticancer	Mice	Jeong *et al.*, 2002
Soybean	Lunasin	Inhibition of cell proliferation and arrest of cell cycle at S-phase	Anticancer	Xenograft MDA-MB-231 or chemically induced breast cancer mice	Hsieh *et al.*, 2010
Soybean	Lunasin	Synergistic effect with IL-2 cytokine in reducing tumour Volume	Reduce lymphoma volume	Xenograft Raji mice lymphoma model	Chang *et al.*, 2014
Soybean	Lunasin	Induction of the apoptotic mitochondrial pathway by modulating expression of Bcl-2, Bax, nCLU,cytochrome c and caspase-2, −3 and −9; Arrest cell cycle at G2/M phase; cytotoxic effects	Delay liver metastasis	Colon cancer KM12L4 cells directly injected into athymic mice	Dia and de Mejia, 2011
Rice bran	Glu-Gln-Arg-Pro-Arg	Cancer growth inhibition	Cytostatic	Colon cancer cells (Caco-2, HCT-116), breast cancer cells (MCF-7, MDA-MB-231), liver cancer cells (HepG-2)	Kannan *et al.*, 2010
Soybean	Bowman-Birk inhibitor	Tumour suppressor gene Cx43 and cell cycle arrest in G1/S phase induction	Cytostatic and cytotoxic	Human osteosarcoma cells and in M5067 ovarian sarcoma mouse model	Chen *et al.*, 2005; Saito *et al.*, 2007; Suzuki *et al.*, 2005.

(continues)

Table 1 (Continued)

Source	Bioactive peptide	Mechanism of action	Effect	Model	Reference
Soybean	Bowman-Birk inhibitor	Apoptosis induction by crossing the membrane of the breast cancer cells and co-localizing with the proteasome in cytoplasm and mainly in nucleus	Cytostatic and cytotoxic	MCF-7 breast cancer cells	Souza Lda *et al.*, 2014
Soybean	Bowman-Birk inhibitor	Unknown	Decrease in serum PSA levels	Humans with benign prostatic hyperplasia	Malkowicz *et al.*, 2001
Soybean	Bowman-Birk inhibitor	Unknown	Clinical effect against oral leukoplakia and effects on potential biomarkers	Humans with oral leukoplakia	Armstrong *et al.*, 2000
Tepary bean	Lectins	Colony formation inhibition	Cytostatic and cytotoxic	C33-A and Sw480 cell lines	Valadez-Vega *et al.*, 2011
Mistletoe	Lectins	Delayed development of colon cancer	Immunomodulatory activity	Mice model	Ma *et al.*, 2008
Mistletoe	Lectins	Improvement of the survival	Immunomodulatory activity	Mice with leukaemia cells	Seifert *et al.*, 2008
Whey	Lactoferrin	Arrests cell cycle at the G1/S transition	Inhibit breast cancer MDA-MB-231 cells growth	Breast cancer MDA-MB-231 cells	Damiens *et al.*, 1999
Whey	Lactoferrin	Suppresses Akt signalling	Inhibit nasopharyngeal carcinoma cells growth	Nasopharyngeal carcinoma cells	Deng *et al.*, 2013
Whey	Lactoferrin	Increase in IL-8 and activation of natural killer and CD8+ T-cells	Anticancer activity	Mice model of head-and-neck squamous cell carcinoma	Varadhachary *et al.*, 2004
Whey	Lactoferrin	Unclear	Improvement of the chemotherapeutic effects of tamoxifen	4 T1 breast cancer xenograft Balb/c mice model	Sun *et al.*, 2012
Whey	Lactoferricin	Induction of apoptosis, modulation of gene expression, prevention of angiogenesis and ability to arrest cell cycle	Cytotoxic	different types of cancer cell lines	de Mejía and Dia, 2010
Whey	Lactoferricin	—	Inhibit spontaneous B16-BL6 melanoma cells growth and to suppress L5178Y-ML25 lymphoma metastases in liver and lung	Mice	Yoo *et al.*, 1997

(continues)

Table 1 (Continued)

Source	Bioactive peptide	Mechanism of action	Effect	Model	Reference
Casein	β-Casomorphin 7 and β-Casomorphin 5	Arrest cell cycle	Cytostatic	Breast cancer cells	Hatzoglou *et al.*, 1996
Casein	β-Casomorphin 7 and β-Casomorphin 5	Induce apoptosis	Cytotoxic	Intestinal tumour HT-29 and AZ-97 cells	Perego *et al.*, 2012
Whey	α-lactalbumin	Activation of apoptosis in tumour cells, but spares healthy cells	Anticancer activity	40 different lymphoma and carcinoma cell lines	Fast *et al.*, 2005
Egg	Egg yolk hydrolyzates	Antioxidant activity	Inhibit tumour cells proliferation of colorectal cancer	Rats	Azuma *et al.*, 2000
Egg	Lysozyme	Indirect action mediated by the induction of host responses.	Reduce the formation of lung metastases of B16 melanoma	Mice bearing B16 melanoma	Sava, 1989
Egg	Ovomucin	Slight activation of the immune system.	Inhibit tumour growth	Mice	Watanabe *et al.*, 1998
Egg	Lysozyme	Immunostimulator	Improve the effectiveness of chemotherapy treatments on primary tumor growth and on lung metastasis formation and particularly on the postsurgical survival time	Mice bearing advanced mammary carcinomas and treated with 5-fluorouracil	Sava *et al.*, 1995
Marine s ponge	Hemiasterlin	Spindle microtubule dynamics inhibition (Hemiasterlin A produced abnormal mitotic spindles), arrest in mitotic metaphase	Cytotoxic	MCF-7 human mammary carcinoma cells	Anderson *et al.*, 1997
Dolabella auricularia	Dolastatin 10	Tubulin polymerization inhibition and growth of L1210 murine leukaemia cells inhibition	Cytostatic	Murine leukaemia cells in culture	Bai *et al.*, 1990
Red Sea Moses sole	Pardaxin	Induction of c-FOS	Cytotoxic	Cancer cell lines	Ting *et al.*, 2014
Oyster	Hydrolysates	Unclear	Cytostatic	BALB/c mice (sarcoma-S180)	Wang *et al.*, 2010
Tuna dark muscle	Hydrolysates	Unclear	Cytostatic	Human breast cancer cell line MCF-7	Kuo-Chiang Hsu *et al.*, 2011

(continues)

Table 1 (Continued)

Source	Bioactive peptide	Mechanism of action	Effect	Model	Reference
Giant squid	Esperase hydrolysate	Radical scavenging and metal chelating capacity	Cytotoxic	MCF-7 (human breast carcinoma) and glioma cell lines	Alemán *et al.*, 2011
Bullacta exarata	BEPT II and BEPT II-1	Induction of apoptosis	Cytotoxic	Prostate cancer cells	Ma *et al.*, 2013
Arca subcrenata Lischke	Polypeptide P2	Proliferation inhibition of HeLa and HT-29 cell lines	Cytotoxic	Sarcoma S-180-bearing mice	Hu *et al.*, 2012
Reniochalina stalagmitis	Reniochalistatins A-E	Unclear	Cytotoxic	Several tumour cell lines (RPMI-8226, MGC-803, HL-60, HepG2 and HeLa)	Zhan *et al.*, 2014
Aplidium albicans	Aplidine	Cell proliferation inhibition, apoptosis induction and cell cycle arrest	Anticancer	Breast, melanoma and lung cancer cells in humans	García-Fernández *et al.*, 2002

In vivo studies have confirmed the anti-carcinogenic effects of lunasin. For example, in mice models lunasin has been shown to act against chemically-induced breast cancer (Hsieh *et al.*, 2010), to reduce lymphoma volume (Chang *et al.*, 2014) and to delay liver metastasis of colon cancer KM12L4 cells (Dia and de Mejia, 2011).

Besides lunasin, a pentapeptide Glutammate-Glycine-Arginine-Proline-Arginine, extracted from rice bran, demonstrated to cause the 84% inhibition of the growth of colon cancer cells (Caco-2 and human colorectal adenocarcinoma cell line, HCT-116), 80% of the growth of breast cancer cells (MCF-7, MDA-MB-231) and 84% of that of liver cancer cells (HepG-2) at a dose of 600–700 $\mu g \cdot mL^{-1}$ (Kannan *et al.*, 2010).

Bioactive peptides are also contained in legume seeds, which are a source of protease inhibitors, for example the Bowman-Birk inhibitor (BBI). BBI has a well-characterized ability to inhibit trypsin and chymotrypsin activities and seems to have a preventive effect against prostate, breast and colon cancers (Park *et al.*, 2005a,b) and to be an effective suppressor of carcinogenesis. Its mechanism of action is based on its ability to induce the tumour suppressor gene connexin 43(Cx43) and cell cycle arrest in the G1/S phase, as demonstrated in human osteosarcoma cells and in the M5067 ovarian sarcoma mouse model (Chen *et al.*, 2005). Moreover, BBI demonstrated a cytostatic and cytotoxic effect in MCF-7 breast cancer cells by means of apoptosis; it is able to cross the membrane of the breast cancer cells and co-localizes with the proteasome in cytoplasm and mainly in the nucleus (Souza Lda *et al.*, 2014).

The use of BBI concentrate in clinical trials as a New Investigational Drug was approved by the Food and Drug Administration (FDA). Human trials in patients with benign prostatic hyperplasia (Malkowicz *et al.*, 2001) or oral leukoplakia (Armstrong *et al.*, 2000) have also shown its safety and tolerability over a prolonged period of time.

Another group of plant-derived bioactive proteins is represented by lectins, which are able to recognize specific carbohydrate moieties displayed by malignant cells or tissues. For example, tepary bean (*Phaseolus acutifolius*) lectins have demonstrated antiproliferative and cytotoxic effects on the cervical carcinoma cell line, C33-A, and human colon carcinoma cell line, Sw480, by the inhibition of colony formation (Valadez-Vega *et al.*, 2011). Lectins derived from mistletoe have been studied in a mouse model and demonstrated an immunomodulatory effect that delayed the development of colon cancer (Ma *et al.*, 2008) and improved the survival of mice with leukaemia cells (Seifert *et al.*, 2008).

The most studied peptides of animal origin derived from milk, eggs and marine species.

Milk-derived peptides showed interesting chemopreventive effects. In particular, lactoferrin, a whey protein, has been demonstrated, *in vitro*, to inhibit the growth of breast cancer (MDA-MB-231) and nasopharyngeal carcinoma cells by, respectively, arresting the cell cycle at the G1/S transition and suppressing Akt signalling (Damiens *et al.*, 1999; Deng *et al.*, 2013). Studies in mouse model *in vivo* confirmed its anticancer activity against head-and-neck squamous cell carcinoma (Varadhachary *et al.*, 2004) and demonstrated an improvement in the chemotherapeutic effects of tamoxifen in 4 T1 breast cancer (Sun *et al.*, 2012).

Lactoferricin has been demonstrated to act against different types of cancer cell lines through the induction of apoptosis, the modulation of gene expression, the prevention of angiogenesis and its ability to arrest the cell cycle (de Mejía and Dia, 2010). Studies in mice *in vivo* demonstrated that it is able to inhibit spontaneous melanoma cells growth and to suppress lymphoma metastasis in the liver and lung (Yoo *et al.*, 1997).

Casein-derived peptides, β-casomorphin 7 and β-casomorphin 5, are able to arrest the cell cycle in breast cancer cells (Hatzoglou *et al.*, 1996) and to induce apoptosis in intestinal tumour HT-29 and AZ-97 cells (Perego *et al.*, 2012). Moreover, a whey protein, α-lactalbumin, showed chemopreventive properties *in vitro* through the activation of apoptosis (Fast *et al.*, 2005).

Interesting results have been reported with egg-derived peptides, in particular, hydrolysates from egg yolk protein, lysozyme and ovomucin. Studies in rats showed that hydrolysates from egg yolk protein are able to inhibit the proliferation of tumour cells in colorectal cancer, probably through its antioxidant activity, while lysozyme reduces the formation of lung metastases of B16 melanoma; also, ovomucin has been demonstrated to inhibit tumour growth (Azuma *et al.*, 2000; Sava, 1989; Watanabe *et al.*, 1998). Moreover, lysozyme has been reported to improve the effectiveness of chemotherapy treatments (Sava *et al.*, 1995).

Many bioactive peptides are derived from different marine species, for example, from ascidians, molluscs and sponges. Among the bioactive peptides identified in these species, dolastatins, mainly dolastatin 10 and 15, isolated from *Dollabella auricularia*, and hemiasterlin exert a cytotoxic action by blocking tubulin polymerization, arresting the cell cycle and triggering apoptosis (Anderson *et al.*, 1997; Bai *et al.*, 1990). Pardaxin, a 33-aminoacid peptide, induced apoptosis by targeting the endoplasmatic reticulum and activating c-FOS (Ting *et al.*, 2014).

Many marine peptides induce antiproliferative activity against different cancer types; *in vitro* studies showed that peptides from tuna dark muscle had an antiproliferative effect on human breast cancer cells (Hsu *et al.*, 2011), while peptides from squid gelatin had cytotoxic activity on human breast carcinoma (MCF-7) and glioma cell lines (Alemán *et al.*, 2011). Other peptides, for example, BEPT II e BEPT II-1 from *Bullacta exarata* have also demonstrated apoptotic activity towards prostate cancer cells (Ma *et al.*, 2013). Furthermore, the peptide reniochalistatin E from the sponge *Reniochalina stalagmites* showed a cytotoxic activity towards several tumour cell lines (Zhan *et al.*, 2014).

These results have been confirmed *in vivo*. The polypeptide P2 of *Arca subcrenata* has been demonstrated to have an antiproliferative action on HeLa and HT-29 cells in S-180 tumour-bearing mice (Hu *et al.*, 2012) and peptides extracted from oyster (*Crassostrea gigas*) showed an antiproliferative effect in mice against sarcoma-S180 (Wang *et al.*, 2010).

Aplidine, a cyclodepsipeptide isolated from the tunicate *Aplidium albicans*, has also been tested in phase I clinical trials in humans and was shown to have anticancer activity against different cancer cell lines, such as breast, melanoma and lung cancer cells, induced by inhibiting cell proliferation, apoptosis induction and cell cycle arrest; phase I clinical trials confirmed its efficacy in humans, and phase II are ongoing (García-Fernández *et al.*, 2002).

In summary, many studies have demonstrated the cytotoxic and anti-tumoural activity of bioactive peptides in different tumoral cell lines (but not in non tumoral ones). Nevertheless, again, the translation of the numerous preclinical data to the clinical setting has to be thoroughly investigated before any kind of optimistic conclusion can be reached.

Immunomodulatory activity

Bioactive peptides of animal origin may also improve immune responses, as observed in several studies conducted *in vivo* and *in vitro* (Table 2).

Peptides from casein and whey proteins possess immunostimulatory activity. Phosphopeptides derived from α_{S1}-casein as well as β-casein are reported to stimulate phagocytes, the production of IgG in lymphocytes and the proliferation of T-lymphocytes (Lahov and Regelson, 1996; Hata *et al.*, 1998). Peptides derived from fish have shown strong immunomodulatory effects in animals, that may be due to enhanced macrophage activity and lymphocyte proliferation, natural killer cell activity and cytokine regulation (Yang *et al.*, 2009; Cheung *et al.*, 2015);for example, these peptides modulate gut-associated non-specific immunity by enhancing phagocytic activity and the number of IgA-secreting cells in the mouse small intestine lamina propria (Duarte *et al.*, 2006). Among the marine hydrolysates, the one from Atlantic cod promotes the oxidative burst of leukocytes and enhances the bactericidal power of phagocytes (Gildberg *et al.*, 1996), while the one from Chum Salmon (*Oncorhynchus keta*) increases the lymphocyte proliferation induced by the mitogen concanavalin A, the number of plaque-forming cells, natural killer cell activity, the percentage of CD4+ T helper cells in spleen and the secretion of cytokines in mice (Yang *et al.*, 2009). Oyster hydrolysates are able to enhance the activity of natural killer cells, the spleen proliferation of lymphocytes and the phagocytic rate of macrophages in mice (Wang *et al.*, 2010). Immunomodulatory peptides derived from tryptic hydrolysates of rice and soybean proteins stimulate the production of superoxide anions (ROS), which trigger non-specific immune defence systems (Kitts and Weiler, 2003).

In conclusion, bioactive peptides have an immunostimulant effect on both non-specific and specific immunity *in vitro* and in animal models, but these effects need to be confirmed in humans.

Other biological activities

Bioactive peptides from many sources such as wheat gliadin, pea, soy proteins (Wang *et al.*, 2007; Pownall *et al.*, 2010; Malaguti *et al.*, 2014) and also egg yolk proteins, porcine myofibrillar proteins and aquatic by-products have a protective effect against oxidative damage (Pihlanto, 2006), acting as free radical scavengers and metal ion chelators against enzymatic and non-enzymatic peroxidation of lipids and essential fatty acids. Among them, lunasin has been demonstrated to scavenge both peroxyl and superoxide radicals, confirming its antioxidant properties *in vitro*. Moreover, it has been shown to protect cell viability and antioxidant defences of human Caco-2 cells treated with hydrogen peroxide and tert-butylhydroperoxide (Garcia-Nebot *et al.*, 2014).

Table 2

Main bioactive peptides with demonstrated activities on the immune system

Source	Bioactive peptide	Mechanism of action	Effect	Model	Reference
Bovine milk	s1-casein	Mitogenic activity, immunoglobulin production enhancement	Humoral immunostimulator	Cell cultures	Hata *et al.*, 1998
Fish	Fish protein hydrolysate	Phagocytic activity enhancement and number of IgA-secreting cells increase	Gut-associated non-specific immunity modulator	Mice	Duarte *et al.*, 2006
Oyster (*Crassostrea gigas*)	Hydrolysates	Activity of natural killer cells, spleen proliferation of lymphocytes and phagocytic rate of macrophages enhancement	Immunostimulator	Sarcoma S180-bearing mice	Wang *et al.*, 2010
Atlantic cod (*Gadus morhua*)	Medium size (3000 d > M_w > 500 d) peptides	Oxidative burst reactions promotion in leucocytes	Immunostimulator	Head kidney leucocytes from Atlantic salmon	Gildberg *et al.*, 1996
Chum Salmon (*Oncorhynchus keta*)	Oligopeptide preparation	Enhancement of lymphocyte proliferation induced by the mitogen concanavalin A, number of plaque-forming cells, natural killer cell activity, percentage of $CD4^+$T helper (Th) cells in spleen and the secretion of Th1 (IL-2, IFN-γ) and Th2 (IL-5, IL-6) type cell cytokines.	Immunostimulator	Female mice	Yang *et al.*, 2009

Carnosine and anserine, which are the most abundant antioxidant peptides in meat, are reported to have a role in the prevention of stress-related diseases (Hipkiss and Brownson, 2000). Other antioxidant peptides have been identified in marine organisms such as oysters, shrimps, squid and blue mussels (Harada *et al.*, 2010).

In addition to antioxidant activity, some bioactive peptides possess (in experimental models) analgesic activity, thanks to their affinity with opiate receptors. Among them, α-lactorphin and β-lactorphin are opioid peptides found in α-lactalbumin and β-lactoglobulin; they are liberated during the *in vitro* proteolysis of bovine whey proteins and have shown pharmacological activity at μmolar concentrations (Pihlanto-Leppälä, 2000). Bioactive peptides behaving like opioid receptor ligands can also be found in wheat gluten, rice albumin, in possible constituents of bovine meat, such as serum albumin or haemoglobin, and even in vegetables like spinach (Teschemacher, 2003).

Another interesting field of action of bioactive peptides is related to their antimicrobial effects. For example, antimicrobial peptides can affect the activity of bacteria, viruses and fungi; they are also well tolerated and do not induce pathogen resistance (Wang *et al.*, 2010). The best investigated antimicrobial peptide is the fragment 17-41 of lactoferrin, more commonly known as lactoferricin; its bactericidal activity is due to its binding to the lipid A part of bacterial lipopolysaccharides, with an associated increase in membrane permeability. It is active not only against bacteria but also against fungi, protozoa and viruses (Orsi, 2004).

Moreover, four peptides derived from bovine meat (GFHI, DFHING, FHG and GLSDGEWQ) have also shown antimicrobial activity against Gram-positive and Gram-negative bacteria, for example *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli* and *Pseudomonas aeruginosa* (Jang *et al.*, 2008).

Discussion

The current progress in bioactive peptides is an exciting and growing research field. Their potential should not be surprising, because amino acid sequences are responsible for the control, and direct all aspects, of cellular function and coordinate most intercellular communications (Craik *et al.*, 2013). However, as yet, the evidence available on bioactive peptides has several limitations.

Firstly, the mechanistic processes that regulate the putative activities of individual peptides should be more thoroughly investigated. Many peptides appear to act through more than a single mechanism of action and, therefore, to possess pleiotropic activities. Nevertheless, often it is difficult to attribute the mechanism of action to a single peptide, because a complex of the protein or peptides as a whole is studied. Individual components of the complex peptide-rich hydrolysates should be characterized to find out their actions and the specific receptors and signalling pathways involved in mediating some of their beneficial effects. Then, extraction procedures should be standardized. A solution could be made by applying *in vitro* activity-guided fractionation, where analytical separation of protein-digested fractions is combined with *in vivo* evaluation of specific biological activity, to identify the peptide responsible for the effect (Sato *et al.*, 2013).

Secondly, solid pharmacokinetic data is needed to determine proper dosage and frequency of administration, analysing the variability in intake and biological effects (Rutherfurd-Markwick, 2012; Yoshikawa, 2015). In order to get consistent pharmacokinetic data, it is important to study biopharmaceutical aspects of these peptides; the particle size, the mode of administration (fed or fasted phase, with or without water) and the dosage form (suspension, powder, micelle, emulsion, etc.) are only some examples of how the bioavailability of the same peptide can differ because of these variables.

The chemical structure of the peptide must be also considered: processes such as digestion can modify the peptide's bioavailability and activity turning an apparent bioactive peptide into an inactive one and *vice versa*; moreover, the active peptide could be degraded during digestion, not be absorbed or reach the target tissues at a concentration sufficient to exert its effect (Malaguti *et al.*, 2014). Therefore, some peptides may require protection from gastrointestinal enzymes when administered orally using non-conventional dosage forms.

It is important to highlight the fact that the specific duodenal peptide transporter responsible for their bioavailability absorbs the major part of bioactive peptides too; therefore, any mechanism of competition for the same intestinal transporter should be investigated.

Finally, another aspect to consider in humans is inter-individual variability (concomitant therapy, age, sex, diseases, etc.) that can greatly influence the final effectiveness of the nutraceutical, as shown for lactotripeptides (genetic/population-dependent effect on Asian subjects) (Cicero *et al.*, 2011a).

In general, the main limitation in evaluating the clinical effects of these peptides is their short half-life (less than 2 h) and their low plasma concentrations ($pmol \cdot mL^{-1}$); consequently, it is not easy to measure their bioavailability after oral intake (Iwai *et al.*, 2005; Ichikawa *et al.*, 2010) but also not impossible if the appropriate methodology is applied (Shigemura *et al.*, 2009).

Although many studies conducted *in vivo* have demonstrated the effectiveness of bioactive peptides, most of them are at an early stage, especially as regards clinical data. Currently, the best evidence in humans is related to the BP and lipid-lowering peptides that confirm their efficacy and their optimal tolerability and safety; antihypertensive peptides have also been demonstrated to modulate the pulse wave velocity in humans, which is a reliable prognostic parameter for cardiovascular morbidity and mortality (Cicero *et al.*, 2011b, 2016). However, even if they seem to be safe, the presence of immunogenic proteins and peptides within the protein hydrolysates could induce or exacerbate allergic reactions (Franck *et al.*, 2002).

Anti-inflammatory peptides have been shown to lower inflammation parameters in humans, but the data are poor and their mechanism of action needs to be clarified. The immunomodulatory, antioxidant, analgesic and possible microbial activity of many of the peptides have been evaluated in numerous studies. In addition, the effects of numerous of these peptides have been investigated on a variety of cancer cell lines (but not in non-tumoral ones) and in animal models. However, once again, a limited number of phase I clinical trials

has been performed and more studies are needed to confirm their efficacy, safety and tolerability in humans.

In conclusion, so far the results obtained from *in vitro* and *in vivo* studies of bioactive peptides are encouraging and have shown they have potential as treatments of numerous diseases or risk factors. However, studies on humans are needed to better understand the pharmacokinetic profiles of these compounds and to test them for potential immunogenicity. Therefore, further middle/long-term randomized clinical trials will be necessary to confirm the effects of these peptides in disease-preventing/health promoting activities and their potential therapeutic usefulness, with the objective of using them to effectively control the growing burden of chronic illnesses with minimal side effects.

Conflict of interest

The authors declare no conflicts of interest.

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