Amino Acids (2012) 43:135–142
DOI 10.1007/s00726-012-1271-5

INTRODUCTION

Carnosine and cancer: a perspective

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Received: 23 September 2011 / Accepted: 9 March 2012 / Published online: 28 March 2012

Abstract The application of carnosine in medicine has been discussed since several years, but many claims of therapeutic effects have not been substantiated by rigorous experimental examination. In the present perspective, a possible use of carnosine as an anti-neoplastic therapeutic, especially for the treatment of malignant brain tumours such as glioblastoma is discussed. Possible mechanisms by which carnosine may perform its anti-tumourigenic effects are outlined and its expected bioavailability and possible negative and positive side effects are considered. Finally, alternative strategies are examined such as treatment with other dipeptides or β-alanine.

Introduction

Since its discovery in 1900 (Gulewitsch and Amiradzibi 1900), the dipeptide carnosine (β-alanyl-L-histidine) has been investigated in various laboratories. However, despite many efforts, its precise physiological function(s) remain uncertain up to the present day [for reviews see (Quinn et al. 1992; Hipkiss 2009a, b)]. Anti-neoplastic effects of carnosine were first described by Nagai and Suda (1986). These authors subcutaneously implanted Sarcoma-180 tumour cells into ddY mice. The day after implantation, carnosine was administered subcutaneously 2 cm from the implantation site. Treatment with carnosine (50 mg/kg/day) was continued every second day. When compared to treatment with saline, it became obvious that carnosine significantly inhibited tumour growth and also reduced mortality. Unfortunately, the experiments of Nagai and Suda did not receive their deserved attention, probably because the original manuscript was not published in English. Inspired by the work of Holliday and McFarland (1996) who found that carnosine selectively inhibited the growth of transformed and neoplastic cells, but over 20 years after the Nagai and Suda paper, Renner et al. (2008) showed that carnosine inhibited the growth of cultured tumour cells isolated from human glioblastoma. The effects of carnosine were also studied in nude mice following subcutaneous implantation of cells expressing the human epidermal growth factor receptor 2 (Her2/neu) (Renner et al. 2010b). The animals received a daily intraperitoneal injection of either 500 µl carnosine solution (1 M) or saline as controls. These experiments demonstrated that aggressive tumour growth was significantly delayed by carnosine and that tumour size was also reduced. In addition, tumour cells of animals treated with carnosine were less pleomorphic and exhibited a reduced number of mitotic figures. Consequently, these experiments, the inhibitory effects of carnosine on various neoplastic cell lines (human and rodent) (Holliday and McFarland 1996) and on HCT116 colon cancer cells (Iovine et al. 2012), plus the experiments originally described by Nagai and Suda (1986) raise the hope that carnosine may also be effective for the treatment of other types of tumours.

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The enigma of carnosine's cellular effects

Carnosine has been described as an enigmatic peptide (Bauer 2005). This conclusion is exemplified by carnosine's contrasting effects on tumour cells and differentiated fibroblasts (Hipkiss 2009a), whilst carnosine extends the proliferative potential of cultured human fibroblasts, lengthens their lifespan and suppresses the appearance of the senescent phenotype (McFarland and Holliday 1994), the dipeptide strongly inhibits the growth of cultured tumour cells, as outlined above. It is possible, however, to reconcile this apparent paradox when one considers the metabolic differences between these two cell types, i.e. the Warburg effect. Indeed, it has been suggested that specifically targeting cancer cell metabolism may reveal therapeutic opportunities (Vander Heiden 2011). Most tumour cells are predominantly glycolytic for ATP supply and provision in metabolic precursors for macromolecule biosynthesis, whereas in terminally differentiated fibroblasts, where biosynthetic demands are lower, ATP synthesis is predominantly mitochondrial in origin, as outlined by Warburg so many years ago. The observation suggesting that carnosine may in some ways interfere with glycolysis (Renner et al. 2010a) is entirely consistent with these metabolic differences. It is also interesting to note that carnosine concentration in skeletal muscle of healthy children is reported to increase from 30 to 40 mg% at 5 years of age to 120–140 mg% at 14 years of age (Grinio and Stvolinsky 2011).

Collectively, these observations seem to suggest that high concentrations of carnosine may not be entirely compatible with rapid tissue growth, i.e. where glycolysis predominates, but the dipeptide may assist cell survival of post-mitotic tissue. This speculation is reinforced by some preliminary experiments in yeast; the presence of the dipeptide in the culture medium was partially inhibitory during logarithmic growth, but enhanced cell survival when growth ceased (Cartwright, Hipkiss and Bill, unpublished observations). Thus, carnosine's differential effects towards mammalian cells seem to be reflected in its actions towards cultured yeast cells, and further supports the idea that carnosine may exert some inhibitory effects on glycolysis. Indeed, McFarland and Holliday (1994, 1999) in their 1994 and 1999 papers do comment that the presence of carnosine in the culture medium did result in a slight decrease in fibroblast growth rate. It may also be relevant to note that loss of the E2F-1 transcription factor not only promotes a more oxidative (less glycolytic) phenotype (Blanchet et al. 2011), but also reduces tumourigenesis and extends the lifespan of Rb1(+/−) mice (Yamasaki et al. 1998). These observations are consistent with the view that carnosine's therapeutic potential could be effected via the inhibition of glycolytic metabolism.

Possible mechanisms

The physiological and biochemical mechanisms responsible for the anti-neoplastic activity of carnosine are not known. We will present a more detailed discussion and new hypotheses elsewhere; nevertheless, we would like to raise some topics which we think should be discussed. Carnosine's inhibitory effects towards cancer cell growth have been attributed to interference with tumour glycolysis (Holliday and McFarland 1996; Renner et al. 2010a), although the exact mechanisms explaining how carnosine exerts its action are not known. Some data indicate an influence of carnosine on chaperone activity and hypoxia inducible factor alpha (HIFα) signalling (Asperger et al. 2011); indeed, upregulated hypoxic response activity is a frequent characteristic of tumour cells. A further interesting observation that may hint at a possible mechanism is the finding that some advanced glycation end products (AGEs) inhibit tumour growth (Bartling et al. 2011). In fact, carnosine's ability to react with AGEs and many AGE-inducing agents is one of its likely biological functions [for a recent review see (Hipkiss 2009a)]. Another aspect may be hidden behind carnosine's ability to scavenge reactive oxygen species (ROS) (Gorbunov and Erin 1991) [for review see (Guiotto et al. 2005; Boldyrev et al. 2007; Boldyrev 1993)]. For normal cells, protection from ROS and their products is an advantage, whereas for fast-growing tumour cells it may be deleterious; extracellular signal-regulated kinase (ERK) can be activated by ROS (Guyton et al. 1996a, b). ERK is a member of the mitogen activated protein kinases (MAPK) with at least 180 substrates identified to date [for a review see (Niault and Baccarini 2010)]. Accordingly, ERK signalling regulates many functions including cell survival (Xia et al. 1995) and proliferation in a broad range of human tumours [for review see (Maurer et al. 2011)]. Scavenging of radicals by carnosine may, therefore, reduce ERK signalling in tumour cells followed by impaired survival and proliferation. Evidence supporting the idea of carnosine compromises signalling has been obtained (Jia et al. 2009); carnosine was shown to inhibit mesangial cell proliferation in response to high glucose levels, the cell-cycle being arrested at the transition from G1 to S phase, which was accompanied by decreased phosphorylation of ERK 1/2 and p38 MAP kinase. Evidence that carnosine inhibits phosphorylation of ERK 1/2 and p38 MAP kinase was also obtained by Son et al. (2008) using intestinal epithelial cells. These workers also suggested that carnosine interferes with messenger RNA translation initiation factor eIF4E, perhaps by inhibiting phosphorylation of Akt. It is interesting that rapamycin, an anti-cancer agent, which extends lifespan and suppresses much age-related dysfunction, also inhibits translation initiation via effects on

eIF4E function (Sheaffer et al. 2008). This leads to the speculation that carnosine may be a rapamycin mimic.

Bioavailability

Mode of administration

For carnosine to be considered as a potential anti-neoplastic drug for the treatment of cancer patients, questions about its bioavailability should be addressed. One important aspect for bioavailability is that in several tissues transport of carnosine is mediated by the high affinity, energy-dependent dipeptide transporter, PepT2 (Kamal et al. 2009).

Intestinal transport

When Gardner et al. investigated the intestinal absorption of carnosine in man they recognized that carnosine crosses the intestine to a huge extent. Up to 14 % of ingested carnosine was recovered in the urine over 5 h after ingestion (Gardner et al. 1991), but rapid post-absorptive hydrolysis was a severe obstacle for the quantification of intact peptide absorption (Gardner et al. 1991). Since carnosine is detectable in urine up to 4 h after ingestion, one may consider administrating carnosine in several daily doses. This was done in a study with 31 autistic children where carnosine was administered in two daily doses of 400 mg carnosine (Chez et al. 2002). Significant changes and behavioural improvements in children given carnosine were reported. This suggests that carnosine can exert neurological effects when ingested orally. Unfortunately, plasma or urine levels of carnosine were not determined and it has been suggested that the improvements in behaviour, etc. could have been due to maturation and educational interventions, as well as other potential confounds that were not addressed in the study design (Levy and Hyman 2005).

Plasma levels of carnosine

Initial attempts to measure plasma levels of carnosine in human probands have not yielded reliable data (Asatoor et al. 1970); it was shown that ex vivo hydrolysis during blood collection resulted in an apparent half-life of carnosine of the order of 1 min (Gardner et al. 1991). Although this does not reflect carnosine's biological half-life in serum, the fact that urinary recovery of carnosine is possible by the fourth hour after ingestion poses a question to the fate of absorbed carnosine before excretion. Whether carnosine is sequestered in serum is uncertain, although given its avidity towards reactive aldehydes and carbonyls one might anticipate permanent or temporary attachment of the dipeptide to serum proteins or lipids, although as far as the authors are aware there have been few studies in this area.

Carnosinase activity

A problem for bioavailability after ingestion may be the presence of serum carnosinase (CN1, EC 3.4.13.20) activity in plasma (Lenney et al. 1982). Gardner et al. (1991) found a highly significant negative correlation between serum carnosinase activity and urinary recovery of intact carnosine after ingestion by human probands. In addition to serum carnosinase activity, hydrolysis of carnosine in liver, kidney and other tissues may also play a role in limiting carnosine bioavailability. Since serum carnosinase has been modelled, it may be possible to design specific drugs to inhibit the enzyme (Vistoli et al. 2006). However, it is uncertain how patients would respond to the inhibition of serum carnosinase. At this point, it should also be noted that it is difficult to extrapolate from animal experiments on carnosine efficacy to humans, since many non-primate mammals (e.g. rat, mouse, rabbit and guinea pig) appear to possess little or no serum carnosinase activity (Jackson et al. 1991). In addition to serum carnosinase, the cytosolic isoform of carnosinase (CN2, EC 3.4.13.3) should not be underestimated with regard to final bioavailability of carnosine in the tissue that is targeted [for a review see (Pegova et al. 2000)]. Since the cytosolic form of carnosinase can be inhibited by bestatin (Peppers and Lenney 1988), adjuvant treatment with this peptidase inhibitor may be considered. Fortunately, there is already information from clinical trials with bestatin available from the literature (Ota and Uzuka 1992).

To our knowledge, it is not known whether a continuously high intake of carnosine induces serum carnosinase activity. Therefore, it is suggested that in the course of a clinical trial, plasma carnosinase activity should be regularly monitored. This will be helpful in order to determine a proper treatment regimen.

That carnosine appears to exert growth-inhibitory effects towards glioblastoma-derived cells immediately suggests that the dipeptide should be explored as a potential therapeutic against intractable glioblastomas, especially as it would be possible to avoid the problem of serum carnosinase by choosing an intranasal administrative route.

The blood–brain barrier

An argument that carnosine may target neuronal tissue comes from the observation that PepT2 mRNA is present in rat brain (Wang et al. 1998) and in astroglia-rich primary cultures (Dringen et al. 1998). More importantly, PepT2 is

expressed and functionally active in epithelial cells in the choroid plexus (Teuscher et al. 2000; Shu et al. 2002), where it appears to be the only transporter responsible for the uptake of carnosine (Teuscher et al. 2004). Since the choroid plexus acts as a barrier between blood and cerebrospinal fluid (CSF), the transporter may facilitate the transport of carnosine between CSF and blood. In fact, dipeptide uptake in the choroid plexus is considerably impaired in PepT2-deficient mice (Shen et al. 2003). It has been suggested, however, that PepT2 on the apical membrane surface of the choroidal epithelial cells may act as an efflux pump in the removal of peptides from the CSF (Bauer 2005). This leaves some uncertainty as to whether orally administered carnosine may enter the brain through the blood–brain barrier. Since the blood–brain barrier is usually not intact in patients suffering from glioblastoma, the penetration of carnosine into the large, often necrotic, glioma cavities is likely as it is the case for other drugs applied to treat this disease (Rieger et al. 1999).

PepT2 may also transport homocarnosine (γ-aminobutyryl-L-histidine) and although carnosine is absent from human CSF, homocarnosine is present; furthermore, CSF levels of homocarnosine have been reported to decline with age in humans by a factor of 7 or more (Huang et al. 2005; Jansen et al. 2006). However, the significance of these observations is uncertain.

Side effects

Negative side effects

When Gardner et al. (1991) performed their experiments to determine intestinal uptake of carnosine, the participants of the study ingested a single dose of 4 g of carnosine. As Gardner et al. reported, no adverse effects were observed aside from a mild and transient digital paraesthesia that some subjects experienced within the first hour. Paraesthesia occurring from about 15–45 min after the ingestion of $\sim$2.5 g of carnosine (0.286 mmol/kg body weight) was also reported by Asatoor et al. (1970).

Positive side effects

As outlined above, it is anticipated that carnosine would not have severe negative side effects, and that beneficial effects for patients treated for certain types of cancers are likely. One example is carnosine-mediated protection from lung injury caused by radiation (Guney et al. 2006). In general, ionizing radiation is still a highly effective therapeutic tool for different types of cancer and is, therefore, frequently employed. Since formation of ROS is one of the major reasons for cellular injury after radiation (Riley 1994), carnosine may protect healthy tissue from damage and inflammation due to its antioxidant (Babizhayev et al. 1994; Chan et al. 1994) or anti-inflammatory properties [for a review see (Nagai 1980)]. The hypothesis of Guney et al. (2006) has not been tested in humans, but 20 years ago Severin et al. (1990) reported that carnosine administered per os (50–200 mg/kg/day) during a period of 20 days prior to irradiation increased survival rates in albino mice subjected to whole-body X-irradiation (5.0 Gy). Comparable experiments were performed by Kurella et al. (1991) with an intake of a single dose of carnosine (50–100 mg/kg body weight). These authors hypothesize that the protective effect was due to an immunomodulating effect of carnosine. Later, Naumova et al. (1992) reported that carnosine, given per os 1 day before irradiation with 7 Gy, considerably decreased the generation of LPO products 1 h after irradiation and fully normalized the decrease in P-450 activity on the fifth day after irradiation (Kudriashov et al. 1999). Strong protection from radiation in mice was recently confirmed by Zainal et al. (2007) who demonstrated that subcutaneous administration of carnosine (2,300 mg/kg) 24 h prior to whole-body irradiation (8.75 Gy, 0.6 Gy/min) resulted in 88 % survival compared with 25 % for the vehicle control animals. To our knowledge, carnosine has been rarely used in clinical trials associated with radiotherapy. However, it is important to note that the goal of radiotherapy is the destruction of tumour cells and protection by carnosine which could, in some cases, reduce therapeutic efficacy. It has, however, been shown that the presence of polaprezinc (a carnosine–zinc complex) suppresses the development of oral mucositis following radiotherapy for head and neck cancer, and no negative effects on the tumour response to radiotherapy were detected (Watanabe et al. 2010). Another protective effect of carnosine in cancer treatment is the observation that carnosine has a nephroprotective effect in mice treated with cisplatin (Fouad et al. 2008). Cisplatin is a highly effective anti-neoplastic drug used for the treatment of a wide variety of tumours, a major adverse effect of which is nephrotoxicity.

In general, the positive effects of carnosine are manifold; there is a substantial literature on its protective effects (Quinn et al. 1992; Hipkiss 2009b), including astroglial cell protection by NO-trapping (Nicoletti et al. 2007) and protection against hypoxia–ischaemia brain damage (Zhang et al. 2011). It has been reported that carnosine reduces the development of inflammation and tissue injury associated with spinal cord trauma (Di Paola et al. 2011); it also protects lung tissue against bleomycin-induced injury (Cuzzocrea et al. 2007) and prevents vascular damage in experimental diabetic retinopathy (Pfister et al. 2011).

Alternative compounds

As an alternative to carnosine administration, treatment of patients with other dipeptides, such as anserine (β-alanyl-L-methylhistidine), D-carnosine (β-alanyl-D-histidine) or homocarnosine (γ-amino-butyryl-L-histidine) may be considered. Other possibilities include administering β-alanine and the development of new derivatives. In the following section, we will briefly discuss some of these possibilities.

Dipeptides

In early experiments performed by Holliday and McFarland (1996), anserine appeared to inhibit the growth of HeLa cells but neither D-carnosine nor homocarnosine were effective. D-Carnosine is not very efficiently absorbed in the intestine, provided it is not chemically modified (Orioli et al. 2011).

β-Alanine

High tissue levels of carnosine can be obtained after the ingestion of β-alanine (Harris et al. 2006). This effect has been intensively investigated in muscle [for a review see (Sale et al. 2010)]. Of course, synthesis of carnosine from β-alanine will be restricted to tissues expressing carnosine synthase [L-histidine:beta-alanine ligase (ADP-forming) (6.3.2.11), formerly also designated 'carnosine synthetase']. At least amongst the tumours originating in the brain, carnosine synthase may exist, as it has been demonstrated that C6 glioma cells (Bauer et al. 1979) and astrocyte-enriched cultures (Bauer et al. 1982) are able to synthesize carnosine. Later, it was demonstrated that only oligodendrocytes produce carnosine, whereas astrocytes possess an efficient carnosine uptake mechanism (Hoffmann et al. 1996) [for a review see (Bakardjiev and Bauer 2000)]. Since normal oligodendrocytes are able to release a significant amount of synthesized carnosine (Bauer et al. 1982; Hoffmann et al. 1996), the possibility that administration of β-alanine may enhance the brain carnosine concentrations should be evaluated. However, it should also be noted that ingestion of high doses of β-alanine can induce quite profound paresthesia (Artioli et al. 2010).

Whether a tumour cell itself is able to express carnosine synthase may be dependent on its origin. Previous experiments by Holliday and McFarland (1996) with HeLa cells and recent experiments with primary cultured human glioblastoma cells did not exhibit an anti-neoplastic response to β-alanine (Gaunitz et al., unpublished results), but it has to be noted that carnosine synthesis, at least in astrocyte-enriched cultures, is highly dependent on media composition (Schulz et al. 1989).

Novel derivatives of carnosine

As outlined above, bioavailability of carnosine is limited by the two isoforms of carnosinase. Therefore, stable derivatives have been described as alternatives to carnosine (Bertinaria et al. 2011; Lanza et al. 2011). First experiments with carnosine amides demonstrate that some are comparable to carnosine with regard to protection against LDL oxidation catalysed by Cu^{2+} ions, and at least one derivative appears to be able to penetrate the blood–brain barrier (Bertinaria et al. 2011). It will be interesting to determine whether these compounds also possess anti-neoplastic activity.

It has been proposed by Babizhayev et al. (1996) that *N*-acetylcarnosine, which is resistant to carnosinase, could be employed as a prodrug as deacetylation probably readily occurs intracellularly, thereby releasing the active dipeptide; such a formulation has been employed topically (in eye drops for treatment of cataracts) but it is uncertain whether this approach has been extensively employed systemically.

Conclusions

The application of carnosine in medicine has been discussed and reviewed in 1992 by Boldyrev (1992). However, as pointed out by Quinn et al. (1992), in the same year many claims of therapeutic effects were not substantiated by rigorous experimental examination nor have they been subjected to double blind clinical trials. During the last 20 years, little has changed and we agree when Quinn et al. (1992) state that "where the evidence is more convincing there is encouragement to undertake further studies to test such claims". We think that the only way to find out whether carnosine will be a useful drug is to test it in rigorous clinical studies with sufficiently large patient numbers. When administered orally, careful monitoring of carnosine's side effects should be monitored along with the physiological markers such as carnosinase activity or serum carnosine concentration. In our opinion, patients with glioblastoma would be the best group to treat since there is currently no cure possible, and even under the best treatment median survival is just 14.6 months (Stupp et al. 2005). Moreover, as noted above, in order to circumvent the serum carnosinase problem, administration of the dipeptide could be carried via the nose (as drops); it is interesting that the olfactory lobe is in fact enriched in carnosine and the loss of a sense of smell is reported to be an early symptom of neurodegenerative disease.

Conflict of interest The authors declare that they have no conflict of interest.

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