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Review

Strategies for the discovery, identification and validation of milk protein-derived bioactive peptides

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ABSTRACT

Background: The workflow used for the discovery of novel milk protein-derived bioactive peptides (BAPs) has significantly evolved over the past number of years. Many of the approaches currently described in the literature for the study of milk protein-derived BAPs have taken similar routes as those conventionally used in drug discovery. However, because milk protein-derived BAPs are prepared from dietary sources and in a food-grade manner, they differ from drugs in that they are generally present in a complex mixture as opposed to drug active compounds which are generally synthetically produced, having a relatively high level of purity.

Scope and approach: This review assesses the utilisation of drug discovery methods for the identification of novel milk protein-derived BAPs. Different methods relevant to drug discovery have already proven effective for the discovery of new BAPs from milk proteins.

Key findings and conclusions: However, reversing the workflow, starting from peptide identification *in vivo* to *in vitro* screening followed by conventional drug discovery approaches may have several merits. These include (a) the design of more targeted studies, (b) the development of novel approaches for the generation of milk protein hydrolysates and (c) the discovery of milk BAPs which may be bioavailable in humans. These BAPs may find applications in the commercial generation of dietary ingredients with health promoting effects in humans.

1. Introduction

Food protein-derived bioactive peptides (BAPs) have been extensively studied in relation to their potential health promoting effects in humans. A large number of studies have been conducted with milk protein-derived BAPs (for reviews, see: Hernández-Ledesma, García-Nebot, Fernández-Tomé, Amigo, & Recio, 2014; Nongonierma & FitzGerald, 2015b).

Despite major advances in medical and pharmaceutical sciences, as well as a broader access to health structures in certain countries, specific metabolic diseases now appear to be more prevalent worldwide. The metabolic syndrome (MetS) is defined as a combination of risk factors including abdominal obesity, insulin resistance along with high cholesterolemia and blood pressure (BP). It is estimated that ~25% of the world's population is affected by the MetS (IDF, 2006). Different strategies to manage the complications of the MetS (i.e., cardiovascular diseases (CVDs), type 2 diabetes (T2D) and obesity) have been proposed. Lifestyle modifications (dietary interventions and participation in physical activity) and/or medication are often recommended as preventative and curative approaches. However, the use of pharmaceutical drugs is sometimes associated with side-effects, for this reason, the search for natural alternatives originating from foods/dietary compounds has increased (Li-Chan, 2015). In this context, scientifically validated milk protein-derived BAPs have the potential to be formulated into foods to provide their health promoting properties to the general population.

The aim of this review was to assess current strategies for milk protein-derived BAP discovery. To date, it appears that numerous methodologies directly arising from drug discovery approaches have been applied to selected BAPs. An attempt to understand the limitations of using a conventional drug discovery approach for studying milk protein-derived BAPs has been carried out herein. Finally, an alternative strategy is outlined which may be beneficial to increasing the rate of discovery of novel BAP sequences targeting specific human disease/health conditions.

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2. General workflow for drug discovery and parallels with milk BAP generation, identification and validation

Modern drug discovery has been described as being mainly based on targeted therapeutics and biomarkers (Qin et al., 2014). Drug discovery comprises several steps (Fig. 1) including the identification of specific targets to allow screening for the selection of potential pharmacological compounds. The promising candidate(s) are then assessed for safety, bioavailability and potency, parameters which are generally determined in cell culture and animal models. The molecules are subsequently evaluated in different human intervention studies before going through an approval phase by the relevant regulatory body. The analogy of a funnel may be applied to the drug discovery process, with the number of promising candidates (>1000) being decreased at each step (i.e., compound selection, screening, selection of target compounds, animal studies, human studies and regulatory approval) of the process (Fig. 1) to finally select one drug.

In terms of milk protein-derived BAPs, only three groups of compounds appear, to date, to have gone through the whole cycle of the drug discovery workflow. These are caseinophosphopeptides (CPPs, e.g., amorphous calcium phosphate (ACP)-CPP), opioid peptides (e.g., β-caseinomorphin 7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile)) and angiotensin converting enzyme (ACE) inhibitory peptides (i.e., the C12 peptide (Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys), α_{s1}-casein (CN) (f 23-34) and the lactotripeptides (LTPs) Ile-Pro-Pro (β-CN (f 74-76) and κ-CN (f 108-110)) and Val-Pro-Pro (β-CN (f 84-86))) (Nongonierma & FitzGerald, 2015c).

The major difference between drugs and milk protein-derived BAPs is in their relative potency and the limited number of chemical structures which are available in terms of BAPs. In addition, differences in purity also exist between pharmaceutical drugs and milk protein-derived BAPs which are generally present as relatively complex mixtures of compounds (i.e., in hydrolysates or fermentates). For this reason, the base of the funnel in Fig. 1 is wider for milk BAPs as compared to drugs. Furthermore, synthetic drugs are normally not present within the human body unless they are intentionally administered. On the other hand, BAPs may be present in humans as they may arise from digestion of food (Boutrou et al., 2013) or from gastrointestinal (GI) endogenous proteins (Dave, Montoya, Rutherfurd, & Moughan, 2014). These differences may bring additional challenges to the discovery of milk BAPs and to the evaluation of their efficacy in humans (Nongonierma & FitzGerald, 2015c).

Numerous drugs have been successfully developed, validated, and approved for disease management. On the other hand, milk BAPs are not meant to replace pharmaceutical drugs but rather to be utilised as disease prophylactic agents. In contrast, while a number of milk protein-derived BAPs appear to have been scientifically validated in terms of health enhancement, their regulatory approval still represents a major hurdle (Fig. 1).

3. Identification and validation of health targets

The first step in the drug discovery process consists in the identification and validation of specific health targets (Fig. 1). Similar to the process of drug discovery, health targets of milk BAPs are selected using various rationales. These generally take into account the global prevalence of diseases, the requirement for novel compounds targeting the disease (e.g., to increase the potency, reduce the side-effects or improve the sensory characteristics and stability of the molecule) along with economic drivers.

The targets for specific diseases, together with the corresponding drugs, have been compiled in databases (Table 1) such as the Therapeutic Target Database (TTD, http://bidd.nus.edu.sg/group/TTD/ttd.asp) and the Drug Bank (http://www.drugbank.ca/). Additional information on clinical trials conducted with specific drugs are currently available on the web portal ClinicalTrials.gov (https://clinicaltrials.gov/) which comprises the results of >190,000 completed or on-going human clinical trials.

Several milk protein-derived peptides have been discovered using similar targets as those employed in drug discovery. Table 2

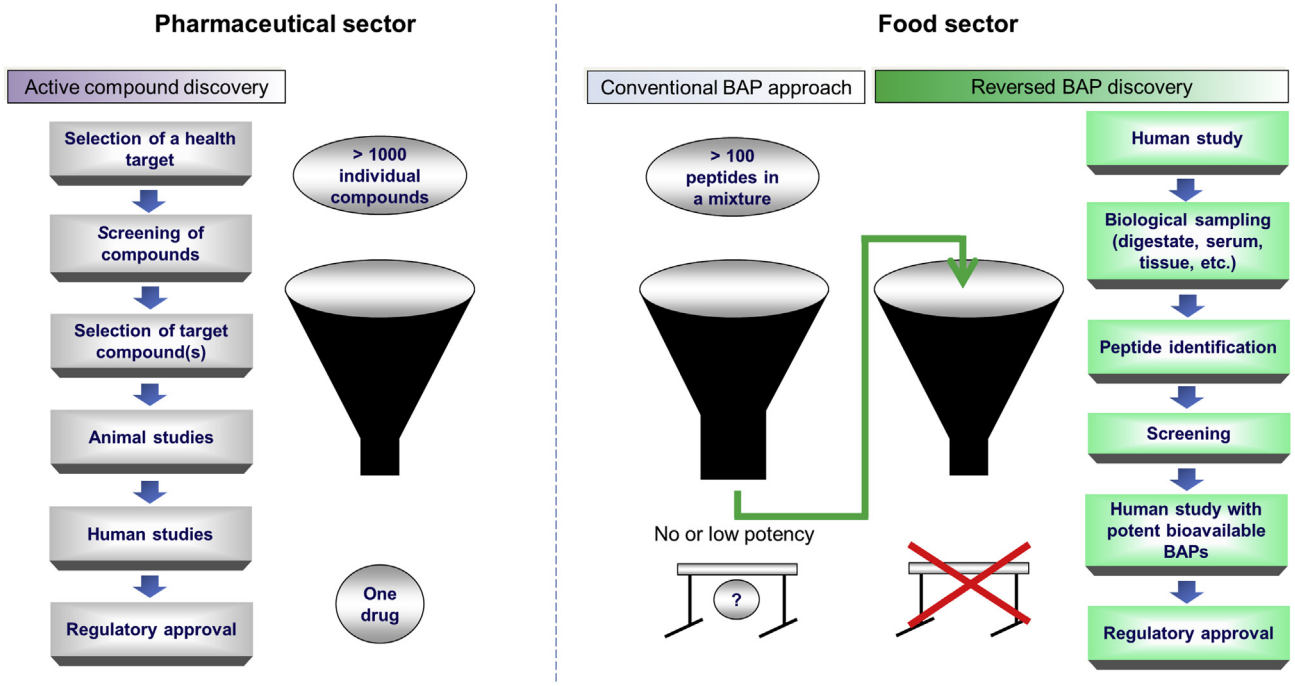

Fig. 1. Schematic of the workflow commonly used in drug discovery and proposed reversed workflow (from peptide identification *in vivo* to *in vitro* screening) for dietary peptide identification and validation.