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Invited Review

Using microemulsion formulations for oral drug delivery of therapeutic peptides

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Abstract

Peptide drugs are increasingly becoming a very important class of therapeutic agents with the rapid advances in the field of biotechnology engineering. However, these drugs are generally not suitable for oral administration. In this review, the main physico-chemical and biopharmaceutical characteristics of peptides are summarized. The obstacles to peptide drug absorption and the different possibilities for solving these difficulties are listed. Results using this formulation approach for oral drug delivery of peptides are apparently promising with some specific peptides such as cyclosporin. Various mechanisms are only beginning to be understood and further investigations need to be performed in this area to explain the results obtained with some peptides.

Keywords: Peptide; Microemulsion; Oral drug delivery

1. Introduction

Management of illness through medication is about to enter an era of rapid growth in the area of pharmaceuticals that are of peptide and protein origin. Decades of peptide research have created a wide variety of biomedical peptide hormones, synthetic peptides, enzyme substrates and inhibitors (Lee, 1986). The growing interest can be ascribed to the increased understanding of their role in physiology and therapy as well as the established capability of producing a large quantity by sophisticated biotechnological processes (Samanen, 1985). Ailments that might be treated more effectively include cancer (Triptorelin®), mental disorders, hypertension (renin inhibitors), autoimmune diseases and certain cardiovascular and metabolic diseases (Lee, 1986).

One typical example is the discovery of cyclosporin, an established immunosuppressant in the management of organ transplant patients. This peptide is a neutral, lipophilic, cyclic undecapeptide (Kahan, 1989).

Although they are highly potent and specific in their physiological functions, most of them are difficult to administer orally. They are usually not therapeutically active by oral administration. In fact, they have an extremely short biological half-life when administered parenterally and repeated injections are often needed. These compounds

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require parallel development of viable delivery systems to improve their systemic bioavailability (Verhoef et al., 1990).

In reality, the commercial success of peptide drugs for medication will depend on the development of non-parenteral routes of administration such as nasal, oral, and rectal or on the successful development of other novel approaches such as implantable delivery systems, self-regulating delivery systems to overcome the drawbacks associated with parenteral administration. Frequent injections are poorly accepted by patients except those suffering from life-threatening diseases.

The oral dosage form is for several reasons the most popular way of administration. One reason is patient compliance which is important in long-term therapy with potent drugs as peptides. In an attempt to improve patient acceptance, new strategies are used to improve the oral bioavailability of peptides. Bioavailability is the fraction or percentage of a dose that reaches the systemic circulation intact, when not directly injected into the circulation (Devissaguet, 1982).

Bioavailability is clinically important because pharmacologic and toxic effects are proportional to both dose and bioavailability. When bioavailability is very low as in the case of most peptides, inter- and intrasubject variability in bioavailability is magnified and incomplete. Therefore, the major reason for trying to maximize oral bioavailability is to maximize our ability to control drug concentrations and the effect of analysis. The biological potency characteristic of these molecules requires methods that are exquisitely sensitive (Samanen, 1985).

Cost may be another driving force. If bioavailability averages 10%, then 90% of a dose is wasted. For drugs that are expensive to produce, wasting 90% of the material may not be acceptable. It must be considered as an important factor for peptide drugs (Aungst, 1993). In reality, maximizing bioavailability contributes to increasing cost-effectiveness.

To improve oral absorption and bioavailability of these compounds, many different strategies can be used. This review presents first briefly the structural aspects of peptides and their major physico-chemical properties. Then, it examines the principle causes of poor membrane permeation of peptides. Possible solutions and examples of applications are given. As the focus of this review is on the use of microemulsions for peptides, this new drug delivery system will be presented. Therapeutic peptide drugs using this new strategy formulation will be given.

2. Physico-chemical and biopharmaceutical characteristics of peptides

Proteins are the most abundant components of cells and are constructed from the same 20 amino acids. They are macromolecules with molecular weights ranging from approx. 5000 to several millions.

Each protein molecule is a polymer with α-amino acids linked together in sequential manner by peptide bonds. The resulting polymers are called peptides. The term polypeptide refers to the peptides which contain about eight or more amino acids whereas oligopeptides are those peptides which have less than eight amino acids. Polypeptides that contain from about 50 to as many as 2500 amino acids are called proteins (Banga and Chien, 1988). The polypeptide chain of a protein is folded into a specific three-dimensional structure which is referred to as the conformation of the protein.

Based on the conformation, proteins can be classified into two major classes: fibrous and globular. The second class, globular proteins, includes peptide pharmaceuticals whereas the first class forms the basic structural elements in the connective tissue (Banga and Chien, 1988; Samanen, 1991). Unless their N- and C-termini are blocked through cyclization, amide formation, or esterification, peptides and proteins are usually charged and hydrophilic molecules (Lee, 1988). Some exceptions exist such as cyclosporin with a partition coefficient of 991 (in *n*-octanol, pH 7.4) (Kahan, 1989). Peptides can adopt different structures: linear, cyclic, cyclic disulfide and bicyclic (Wieland and Bodanszky, 1991).

Peptides that occur naturally as cyclic structures typically require the cyclic structure for activity. Both somatostatin and vasopressin are

cyclic by virtue of the disulfide bridges between two cysteine residues in the peptides. Analogues of either peptide that lack cyclic structures are inactive (Samanen, 1985).

The oral route presents a severe obstacle for peptide drugs due to the enzymatic and physical barriers of the intestinal tract. The enzymatic barrier is by far the most important barrier. The oral bioavailability of most peptides is less than 10% (Lee, 1988). The exceptions are cyclosporin and thyrotropin-releasing hormone which are resistant to proteolysis (Lee, 1988).

The enzymatic barrier, composed of exo- and endopeptidases, is well designed for the digestion of peptides to a mixture of amino acids and thus it is not surprising that the oral intact absorption of peptides is difficult (Verhoef et al., 1990). Hydrolysis of peptides occurs at several sites: luminally, at the brush border and intracellularly (Lee, 1988).

One way to evaluate whether a compound is degraded by luminal enzymes is via in vitro studies with simulated gastric or intestinal fluids or using washing from the lumen (Aungst, 1993). Intestinal peptidases and their substrate specificities were recently reviewed by Bai and Amidon (1992).

Different features of the enzymatic barrier must be considered in order to understand the poor bioavailability of peptides. First, unlike most small drug molecules that are metabolized in the liver primarily, peptides usually are susceptible to degradation, in the blood and in the kidney (Lee et al., 1991).

Secondly, a given peptide is susceptible to degradation at more than one linkage within the backbone and each locus of hydrolysis is mediated by a certain peptidase. Substance P, for example, an undecapeptide is susceptible to degradation by at least five enzymes (Bunnett et al., 1985). Modification of one linkage still leaves the rest of the peptide drug vulnerable to proteolysis. However, peptides, even if pancreatic proteolysis (trypsin, α-chymotrypsin) is avoided, are subject to the potential attack by brush border peptidases and cytosolic peptidases of intestinal epithelial cells (Verhoef et al., 1990). There is a general tendency for proteins to be degraded by luminal pancreatic enzymes, and for tripeptides and larger peptides to be metabolized by intestinal cytosolic enzymes (Verhoef et al., 1990).

Another important barrier is the physical barrier. A specific peptide to be absorbed must bypass the unstirred water layer, mucous layer, apical and basal cell membranes and cell contents, tight junctions, basement membrane and the wall of lymph and blood capillaries (Van Hoogdalem et al., 1989).

Peptide drugs can pass from the lumen into the bloodstream via two routes: transcellularly, in which molecules are transported into and through epithelial cells and transferred into the systemic circulation. This can occur either as a result of passive, active transport or membrane invagination or intercellularly, in which molecules pass directly into the circulation via the junctions between the epithelial cells (Wilson et al., 1989). Transport via the transcellular route requires that luminal mucus and stagnant water layers must be passed and subsequently at least the apical membrane, the cytoplasm and the basolateral membrane.

In contrast, the paracellular route comprises transport of drugs via the tight junctions and subsequently the interstitial space. It is therefore in principle, a hydrophilic route and transport is mainly limited by the size and/or charge of the tight junctions. In addition, this non-selective route comprises less physical and metabolic barriers than the transcellular route and offers therefore interesting possibilities for peptide drug transport (Smith et al., 1992).

It has been suggested that transport via the tight junctions is dynamically regulated in response to physiological, pathological and experimental conditions (Cereijido et al., 1988). The different pathways for a peptide to pass the intestinal membrane have been reviewed by different authors (Wilson et al., 1989; Friedman and Amidon, 1990; Jani et al., 1992).

Absorption is a dynamic process and many factors affect it such as dissolution, ionization, partitioning and blood flow. Poor membrane permeation as one cause of quantified low oral bioavailability can be evaluated in many ways such as by measuring rates of intestinal mem-

brane permeation in vitro and in situ (Aungst, 1993). The distinction between absorption and bioavailability should be noted. Achievement of good absorption may not ultimately translate into good bioavailability if the molecules are subject to rapid metabolism or clearance as is the case for the majority of peptides (Smith et al., 1992).

Artusson and Karlsson (1990) recently demonstrated a proportionality between in vitro permeability coefficients using a cell line derived from a human colon carcinoma (Caco-2), and values for the percentage of oral absorption in humans for 20 drugs and peptides. In general, it is thought that for simple drug molecules, a strong correlation exists between the octanol-water partition coefficient and absorption (Ho et al., 1983). However, this relationship does not seem to apply to the transport of some peptides across Caco-2 cell monolayers (Conradi et al., 1992). It has been shown that within a series of neutral peptides, flux through Caco-2 intestinal membranes in vitro was correlated with the hydrogen bonding tendency of the compound rather than with octanol-water partitioning (Conradi et al., 1992). Burton et al. (1993), have shown an apparent participation of an apically polarized active resistance to peptide transport in Caco-2 cells. Such active polarized systems may serve as an additional barrier to the non-specific uptake of peptides from intestinal lumen.

With in situ methods, an estimation of absorption can be made by measuring drug disappearance from the intestinal lumen and appearance in blood (Swintosky and Pogonowska-Wala, 1982; Schurgers et al., 1986). However, the results might be different from the in vivo data because of factors such as GI motility, bile flow and food intake (Aungst, 1993).

In a closed intestinal loop, the dose is exposed to a limited portion of the intestine and so the specific formulation is more concentrated than it might be in vivo. It is important to measure drug appearance in blood because if only in situ measurements of luminal disappearance are evaluated, false interpretation of the data can be made if the drug binds in the membrane. Nevertheless, in situ methods can help in understanding the mechanisms of absorption.

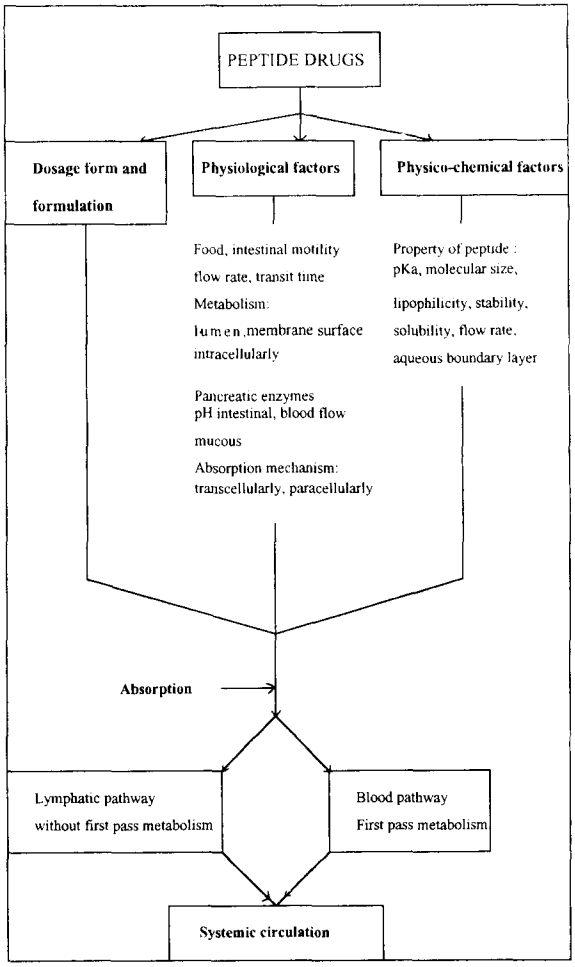

Fig. 1. Main factors involved in peptide drug absorption in the intestine.

Fig. 1 summarizes schematically the different elements to be considered in oral peptide bioavailability. What are the different strategies to improve the oral bioavailability of peptides?

3. Different strategies to improve oral absorption of peptides

One of the first strategies is chemical modification to obtain more stabilized and more lipophilic drugs. Different examples exist in the literature. A-72517, a dipeptide renin inhibitor, is a structural relative of A-64662 (a first generation

renin inhibitor) that is intravenously efficacious and has been shown to lack oral bioavailability (Kleinert et al., 1992).

A-72517 is the more lipophilic compound with a log P of 4.6 (in octanol-water, pH 7.4) as compared with A-64662. This compound has oral bioavailability of 8, 24, 32 and 53% in the monkey, rat, ferret and dog, respectively (Kleinert et al., 1992). The P3-site residue of A-72517 imparts proteolytic stability and the sulfonamide moiety improves potency 10-fold as compared with A-64662 (Buhlmayer et al., 1988). A-72517 is under clinical evaluation in human subjects.

Improvement of lipophilicity is reported to be the mechanism by which esterification of the dipeptide angiotensin-converting enzyme inhibitor enalaprilat to the monoester enalapril increases oral bioavailability (Wyvratt and Patchett, 1985).

Another approach is dealing with the co-administration of absorption enhancers such as EDTA, salicylates, surfactants, bile salts, fatty acids, and unsaturated cyclic ureas. Swenson and Curatolo (1992), have reviewed extensively the different types of enhancers. Most of them are able to improve oral absorption but do so by creating disorders in the phospholipid domain in the membrane. Some can cause major tissue damage (Swenson and Curatolo, 1992).

Enhancers may act by transcellular or paracellular pathways or both (Lee, 1990). Although they have been demonstrated to enhance drug uptake (Nakada et al., 1988), their applications are not without problems considering safety and mucosal damage. For example, the co-carcinogenic and co-mutagenic effects of bile salts discourage the development of bile salts containing pharmaceutical formulations (Breuer and Goebell, 1985). However, some absorption enhancers are apparently safe without causing major tissue damage (Hastewell et al., 1994).

Today, the design and search for safe and effective penetration enhancers are becoming an active area of research in peptide drug delivery. The trend appears to be towards enhancers that are based on natural body constituents as glycerides (Swenson and Curatolo, 1992). In fact, the enhancer of choice will be one which enables the reproducible delivery of a therapeutic peptide drug dose in the GI tract with minimal toxicity.

Another strategy is based on the concept that the co-administration of a peptide and an inhibitor of its metabolism can reduce presystemic metabolism resulting in increased fractional absorption. An example of the successful use of a metabolism inhibitor is the increase in insulin absorption in rats and dogs when co-administered with a chymotrypsin (Fujii et al., 1985; Schilling and Mitra, 1991).

The release of a specific peptide from a carrier system to a region of the GI tract where digestive enzyme activity is low, such as colon and where uptake into the lymphatics is maximized, would be most feasible for the peroral absorption of these drugs. However, the presence of bacteria and fecal matter in the colon and their effects on the stability of peptides are not well documented (Kraeling and Ritschel, 1992). Soft gelatin capsules coated with azo-cross-linked co-polymers of styrene and hydroxyethyl methacrylate have been used with insulin and vasopressin (Saffran et al., 1986).

Further investigations need to be performed in this area. Van Den Mooter et al. (1993) have shown that the degradation of azo-polymers by intestinal microflora can be influenced by the type of polymers (their hydrophilicity).

Another important approach to improve oral bioavailability of peptides is the formulation approach. This strategy has been examined extensively to render insulin more stable to proteolysis in the GI tract. The formulations that have been tested, for different peptide drugs, include liposomes (Woodley, 1986; Chiang and Weiner, 1987). Contradiction in results is a problem with liposomes. This is due to the different techniques used by different team researchers.

Many references also exist on the use of emulsions, nanocapsules or nanospheres (Couvreur and Puisieux, 1993), and microspheres to increase the absorption of peptides following oral administration. The combination of different approaches may be interesting as a protease inhibitor combined with microspheres (Morishita et al., 1992). However, none of the above formulation approaches improves oral insulin absorption suffi-

ciently to be of therapeutic benefit. Another strategy is to use microemulsions for oral drug delivery of peptides.

4. Characteristics and different uses of microemulsions

Having remained unknown for a considerable time to the pharmaceutical field, microemulsions can now be certainly considered as a very interesting drug delivery approach for peptides. Microemulsions were first introduced by the British chemist J.H. Schulman in 1943 (Hoar and Schulman, 1943). A microemulsion is considered to be a quaternary system.

Microemulsion systems contain a surfactant/co-surfactant blend which when added to a two-phase hydrophilic/lipophilic mixture, form a stable, optically clear, isotropic, colloidal system apparently thermodynamically stable with ultralow interfacial tension (Borkovec, 1992). The microstructure of these systems consists of micro-droplets of a size less than 100 nm (Bhargava et al., 1987).

A typical diagram of microemulsion is presented in Fig. 2. Polyglycolized fatty acids can be incorporated in a microemulsion formulation with

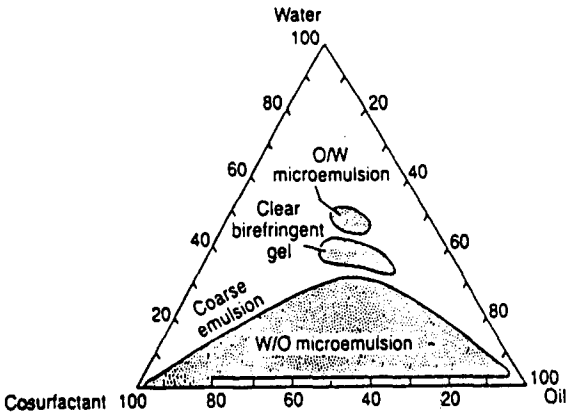

Fig. 2. A phase diagram for an emulsion containing water, oil, surfactant, and cosurfactant. The numbers along the edges indicate the percentages of water, oil, and cosurfactant in the emulsion. The concentration of surfactant is held constant (Bhargava et al., 1987).

Table 1
Microemulsion NF 37, especially for soft or hard gelatin capsules (Gattefosse S.A.)

Raw materials	Source	Type	% w/w
Labrafac lipo®	Gattefosse	lipophilic phase	47
Labrasol®	Gattefosse	'surfactant'	31.3
Plurol oleique®	Gattefosse	'co-surfactant'	13.26
Water		hydrophilic phase	8.44

tensio-active properties instead of some known surfactants (Table 1).

Microemulsions can be used in different areas of applications: optimisation of blood substitutes, assisted recovery of petrol, reactivity in specific media (Rico, 1986) (Berthod, 1983) and also for oral formulation of peptide drugs (Ritschel, 1991).

Among the physical measurements that are useful for identifying microemulsion systems are light scattering, sedimentation, centrifugation, rheology, conductivity and nuclear magnetic resonance (NMR), X-ray and electron microscopy. Each measurement tells us something about the system (Rico, 1986).

The interest to potential users of microemulsions as novel oral drug delivery systems is motivated by different reasons: technologically, microemulsions are interesting because of their spontaneous formation at room temperature, considerable solubilizing properties for active principles, possible sterilization by filtration, and high physical stability (Bhargava et al., 1987).

They are of physiological interest because they can modify the active bioavailability of a specific drug (Ritschel, 1991). They are thus expected to offer several benefits for oral dosage forms such as improved clinical potency and potentially decreased toxicity (Bhargava et al., 1987; Vergnault and Metziger, 1992).

Through the appropriate selection of bioacceptable compounds, oral dosage forms can be designed as in the use of soft gel capsules. An example of a microemulsion designed especially for hard and soft gelatin capsules is presented in Table 1. Based on different references (Tarr and Yalkowsky, 1989), microemulsion formulation has been used with a specific immunosuppressant

peptide, cyclosporin. Due to its very low water solubility (Yee, 1991), the oral dosage form of cyclosporin is an olive-oil based solution (Sandoz, 1993). This was subsequently modified by using maize oil in a soft gelatin capsule. The mean absolute bioavailability of cyclosporin from these formulations (oral solution and soft gelatin capsules) is approx. 30% but varies markedly as has been demonstrated in several transplant patient populations (Ptachinski et al., 1986).

Recently, a new oral formulation (Sandimmune Neoral) was developed which incorporates the drug in a microemulsion pre-concentrate containing a surfactant, lipophilic and hydrophilic solvents and ethanol.

A preliminary study showed that a specific faster releasing microemulsion (ME) formulation increased the extent of absorption on average by 49% compared with the soft gelatin capsule in 12 healthy male volunteers (Drewe et al., 1992).

To investigate the influence of a fat-rich meal, on the pharmacokinetics of cyclosporin from this new ME, a randomized, four-way crossover study was conducted in 24 healthy male volunteers. Simple oral doses of 300 mg Sandimmune and 180 mg Sandimmune Neoral (the ME formulation) were each administered after starting a high fat meal. Over a 48 h period, after each administration, whole blood cyclosporin concentrations were determined by a specific monoclonal radioimmunoassay method.

The results demonstrated that the influence of food is less pronounced on the rate and extent of cyclosporin absorption from Sandimmune Neoral and does not perturb the subsequent blood concentration profile (Mueller et al., 1994). In addition, this microemulsion formulation can have a beneficial influence on compliance.

The same doses as in the study before were given in a randomized, sequential bioreplication study in the same number of patients to investigate the inter- and intra-individual variability of cyclosporin pharmacokinetics form Sandimmune Neoral compared with Sandimmune (Kovarik et al., 1994).

The results demonstrated that altering the oral formulation has a considerable and significant impact on pharmacokinetic variability. Reduced inter- and intra-variabilities with ME formulation yield a more consistent and predictable concentration-time profile (Kovarik et al., 1994).

A study was conducted this time in 18 renal stable transplant patients at 6–26 months post-transplant to evaluate the safety and steady-state pharmacokinetics of this new formulation of Cyclosporin. Both formulations with mean doses of 139 ± 27 mg (Sandimmune) vs 120 ± 19 mg (Neoral Sandimmune) are well tolerated (Mueller et al., 1993).

All these results are very encouraging and explain the new interest in microemulsion formulation.

Other peptides such as SKF-106760, a fibrinogen receptor antagonist, have been formulated in microemulsions at pharmacologically relevant levels. Intraduodenal bioavailabilities of SKF-106760 from a solution (S) and the microemulsion (ME) were investigated in the conscious rat. ME administration of SKF-106760 resulted in significant absorption enhancement compared with the solution (21.9 ± 5.7 vs 0.5 ± 0.3 ($n = 3$)) (Constantinides et al., 1993). Furthermore, there were no signs of irritation upon macroscopic observation of the intestinal tract of each rat at 4-6 h post-dosing.

Based on different observations and results, Ritschel (1991) proposed a schematic diagram on the hypothesized mechanism of peptide absorption from microemulsions given orally. To summarize, after by-pass of the aqueous boundary layer, mucins and epithelial cells, peptide drugs can reach the systemic circulation via two ways: lymphatic vessels via the thoracic duct (where first-pass metabolism is avoided) or via mesenteric veins where a first-pass effect may occur (Ritschel, 1991).

According to different results, systemic peptide uptake from ME in the GI tract is dependent on particle size, type of lipid phase of ME, digestibility of lipid used, type of emulsifying agents, pH and shedding of enterocyte cells. Only the most lipophilic compounds (i.e, those with a log octanol-water partition coefficient of more than 5) with good solubility in triglycerides are preferentially absorbed via the lymphatics (Myers and Stella, 1992). This is largely due because the

blood is much more efficient in clearing absorbed compounds than is lymph.

Therefore, targeting a specific lipophilic peptide drug via the lymphatic pathway may be interesting.

5. Conclusion

The specific aim of this review article was to summarize the difficulties in designing efficient oral dosage forms for peptide drugs and to present different strategies. This article emphasizes the new oral formulation strategy, i.e., the use of microemulsion formulations. This formulation approach can help to improve the oral bioavailability of peptides as is the case for cyclosporin. However, examination of different aspects such as the toxicity, safety, and stability of ME needs to be undertaken on a specific peptide. The relatively high surfactant concentration of this micro-emulsified system may result in localized cytotoxic effects. Nevertheless, the results obtained with cyclosporin using this new oral drug delivery system, are very encouraging for the future of oral efficient peptide drugs.

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