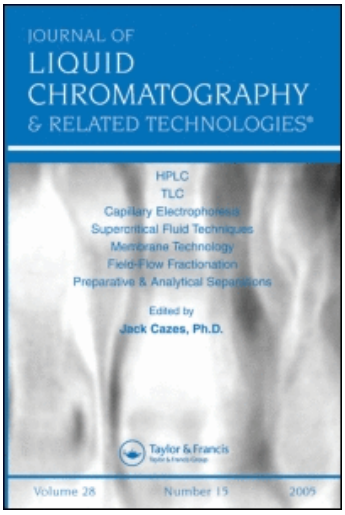

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Abstract: A reversed phased HPLC method with photodiode array detection was used to monitor the stability of opioid growth factor (OGF; [Met^5]-enkephalin) in sterile saline for 1, 2, and 8 weeks, and stored at −17°C, 4°C, and 23°C. Samples maintained at −17°C had 97% of the initial OGF concentration after 2 months, whereas those stored at 4°C had 94% of the original concentration. Preparations housed at 22°C had reductions of 6–12% by the 8th week. These data provide important information about storage of OGF for clinical applications.

Keywords: Degradation, HPLC, Stability, Opioid growth factor, Methionine enkephalin

INTRODUCTION

The native opioid, [Met^5]-enkephalin (termed opioid growth factor (OGF)) (Fig. 1), is a constitutively expressed pentapeptide that interacts with the

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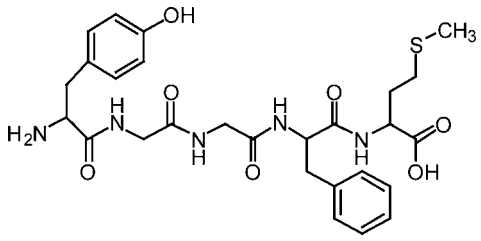

Figure 1. The structure of OGF ([Met^5]-enkephalin.

OGF receptor (OGFr) to regulate growth (both in vitro and in vivo) by inhibitory pathways.[1–3] Administration of exogenous OGF has a potent inhibitory action on neoplasia, cellular renewal, development, and wound healing.[1–3] The action of OGF is stereospecific, non-cytotoxic, reversible, independent of serum, and occurs at physiologically relevant concentrations.[4–9] OGF alters growth in an anchorage-independent fashion.[10] This pentapeptide does not alter cell survival[11] or differentiation.[12] OGF is targeted to DNA synthesis, and is directed toward the G_0/G_1 interface of the cell cycle.[13] OGF and OGFr have been identified in a wide variety of normal and abnormal cells by immunohistochemistry, immunoelectron microscopy, radioimmunoassay, and receptor binding analysis.[14–16]

Clinical investigations using exogenous OGF have been initiated,[17] and phase I trials have been completed showing that this peptide can be safely administered to patients.[17] Both intravenous infusion and subcutaneous routes were utilized in these phase I studies. Although not the primary focus in these initial studies, the efficacy of OGF in the treatment of advanced pancreatic cancer revealed a substantial extension in survival of these patients.

The kinetics of degradation of OGF in aqueous solution have been examined at a variety of pHs and temperatures (25°C, 37°C, and 45°C).[18] However, information about the stability of OGF for purposes of storage and clinical practice have not been reported. This study was designed to begin to define the stability of OGF under different storage temperatures and for time intervals up to 2 months. Our strategy was to prepare OGF in the vehicle (sterile saline) used for clinical practice, and store these solutions in the freezer, refrigerator, or room temperature for 1, 2, and 8 weeks. These solutions were examined with reversed phase high performance liquid chromatography (RP-HPLC) and photodiode array detection (PDA), both qualitatively and quantitatively in order to determine the stability of OGF under these conditions.

EXPERIMENTAL

Materials and Reagents

OGF ([Met^5]-enkephalin) was obtained from Sigma-Aldrich Corporation (St. Louis, MO) and was >97% pure (HPLC).

Preparation of Solutions

Solutions of OGF were prepared by dissolving the OGF in sterile 0.9% saline; the pH of the final solution was 7.4. At 1, 2, and 8 weeks, the solution of OGF was prepared in 4 mL aliquots and stored at $-17.0 \pm 1.0^{\circ}$C (freezer), $4.0 \pm 0.0^{\circ}$C (refrigerator), or $23.2 \pm 0.2^{\circ}$C (room temperature).

Samples were prepared such that they could be analyzed on the same day in order to avoid confounding influences. Samples for HPLC analysis were withdrawn from the stock solutions immediately prior to analysis.

Chromatography

The HPLC system used in these studies consisted of a Shimadzu Model 10A HPLC system equipped with a high pressure gradient, autoinjector, and Model 10 MAV Photodiode Array Detector (PDA), capable of measurements in the UV and visible spectral regions. Wavelengths at 210 and 250 nm were monitored with a 4 nm bandwidth. The HPLC column used was a Kromasil C-8 (4 mm I.D. $\times$ 250 mm) with a mobile phase consisting of 80/20/0.1 (V/V/V) Water/Acetonitrile/TFA flowing at 1.25 mL/min. Authentic OGF was utilized at a concentration of 90 ng/μL. Injection volumes were 10 μL, resulting in an injection amount of 900 ng.

Analysis of OGF Solutions

Solutions at the various temperatures were analyzed by HPLC and compared to authentic OGF standard materials. The peak of interest was scanned from 210 to 350 nm using the PDA detector and data evaluated using standard spectral measurements, peak purity, and derivative spectra. All samples were injected in duplicate.

RESULTS

Using an isocratic HPLC system, the retention time for OGF was 5.02 minutes (Fig. 2). A 15 minute analysis time was required. Evaluation of samples stored at -17°C and 4°C for 1, 2, and 8 weeks showed no changes in the elution profile. At 23°C, the retention times at 1 and 2 weeks were 5.02 minutes, but at 8 weeks the putative OGF peak eluted at 4.97 minutes. In addition, the 8 week sample stored at 22°C had another prominent peak that eluted at 3.52 minutes; no attempt was made to further identify this peak.

The method of HPLC was evaluated for linearity from 40 ng to 2 μg, and a regression coefficient of 0.98 was obtained. Multiple injections (5) of the OGF standard at 90 ng/μL were made to assess reproducibility, with a Cv of 4% obtained for peak height and 3.5% for retention time. The level of

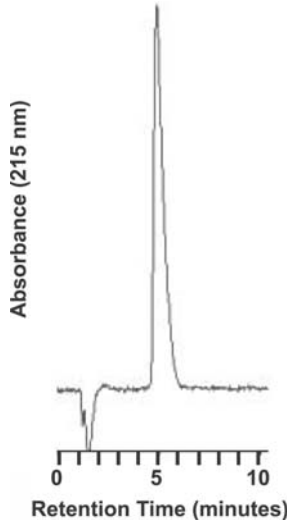

Figure 2. Chromatogram of the OGF standard.

detection (LOD) at 3 times S/N was determined to be 35 ng, while the limit of quantification (LOQ) at 10 times S/N was determined to be 150 ng. Additionally, the method was evaluated for accuracy by analyzing 5 samples of OGF in 0.9% saline at levels of 50, 100, and 200 μg/mL. The results of these studies indicated recoveries of 95%, 96%, and 96%, respectively, with a standard deviation of less than 7% for each sample set. Peak shape and the UV

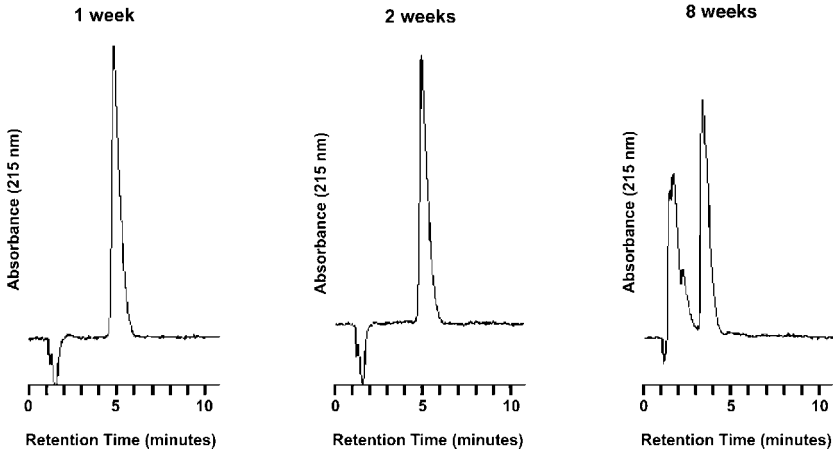

Figure 3. Chromatographs of a solution of OGF stored at 23°C for 1, 2, and 8 weeks.

Table 1. OGF concentration (ng) remaining after injection of 900 ng

Temperature	−17°C	4°C	23°C
Week			
1	900	900	845
2	891	882	832
8	873	842	792

spectra were evaluated at all three temperatures and no degradation of peak shape was seen when compared to authentic OGF standard.

At −17°C, there is less than a 3% loss by week 8, and at 4°C there is a reduction in OGF concentration of 6% on week 8. Analysis of OGF stored at 23°C revealed a decrease of 6% at 1 week from the original concentration (i.e., 900 ng), and by weeks 2 and 8 there was 8% and 12% less OGF.

DISCUSSON

The present study utilized a combination of HPLC and PDA to ascertain the stability of a solution of OGF with respect to temperature of storage, and time, following preparation. The results show, for the first time, that OGF in a sterile saline solution has no loss after 1 week when stored at −17°C or 4°C, and a reduction of 3% and 6%, respectively, from the original concentration up to 2 months after preparation. Data gathered from storage at 23°C revealed a 6% loss within 1 week, and up to a 12% reduction from the original concentration by 8 weeks. Therefore, recommendations for an optimal storage and time of a solution of OGF appear to be either −17°C or 4°C for up to 8 weeks. The relationship between the reductions from original concentrations of OGF, at 23°C, and the functional activity of OGF requires elucidation in order to conclude whether storage at 23°C for short or extended periods disturbs the efficacy of OGF.

Although this is the first report about the stability of OGF at −17°C, 4°C, and 23°C, Chun and Chien[18] have examined the stability of this compound at 25°C, 37°C, and 45°C, for 2–4 weeks. At a pH of 7.07, the half-life of OGF was 117 days and 51 days at temperatures of 37°C and 45°C, respectively; no determinations were provided for a temperature of 25°C. The present data are consistent with the results of this previous report, and extend our base of information in conditions that focus on stability rather than processes of degradation.

Our finding of a second peak in the 8 week sample stored at 23°C raises the question of the nature of this material. The appearance of other peaks may result from cleavage, cyclization,[19] and/or oxidation.[20,21] In fact, the observation of different peaks at temperatures ranging from 25°C to 45°C, and pH

variations from 2 to 10, have been recorded earlier by Chun and Chien,[18] and they conclude from their studies that the degradation pathway(s) of this pentapeptide is(are) very complex and elucidation of the mechanisms may be difficult to decipher.

The motivating factor for our study is that OGF is being used in clinical trials, and information about storage conditions is vital to understanding the best way(s) available to utilize solutions of this peptide. Specifically, Smith and colleagues[17] have published a report on phase I trials with pancreatic cancer patients. Using solutions of OGF formulated with sterile saline, OGF was delivered both by intravenous infusion and subcutaneous injection. The successful completion of these investigations has led to a phase 2 clinical trial. The present study makes the important observation that OGF can be placed into solution and, if stored in a freezer or refrigerator, can be used for up to 2 months rather than being composed fresh on the day of use.

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