ELSEVIER

International Journal of Pharmaceutics 211 (2000) 51–56

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Stability of monoHER in an aqueous formulation for i.v. administration

M.A.I. Abou El Hassan [a,*], D.J. Touw [b], A.J. Wilhelm [b], A. Bast [c], W.J.F. van der Vijgh [a]

[a] *Department of Medical Oncology, Free University Hospital, P. O. Box 7057, 1007 MB Amsterdam, The Netherlands*
[b] *Hospital Pharmacy, Free University Hospital, P. O. Box 7057, 1007 MB Amsterdam, The Netherlands*
[c] *Department of Pharmacology and Toxicology, University of Maastricht, P. O. Box 616, 6200 Maastricht, The Netherlands*

Received 10 July 2000; received in revised form 18 September 2000; accepted 20 September 2000

Abstract

MonoHER is a semisynthetic flavonoid used successfully in modulating the cardiotoxic effect of doxorubicin but not its antitumor activity. The oral bioavailability of monoHER is $< 1\%$. Therefore, it should be prepared as an i.v. formulation for use in clinical trials. The solubility of monoHER in water is highly pH dependent. At $pH \leq 8.3$ the drug precipitates 4 h after preparation. DMSO was tested for enhancing the solubility of monoHER in aqueous solutions. In all DMSO-based aqueous solutions monoHER recrystalized again at $pH < 8.3$ and room temperature within 4 h after preparation. Moreover, the stability of monoHER was lower in a DMSO stock solution than after dilution with an aqueous solution. The stability of monoHER was tested in alkaline solutions (pH 8.3 and 9.5) using an HPLC-DAD procedure to detect all possible degradation products within 10 min after injection. Minor degradation occurred to monoHER in alkaline solutions when exposed to daylight or 1% H_2O_2. MonoHER intensively degraded when exposed to a high temperature (80°C). The stability of monoHER was almost the same in saline or 5% glucose when kept at room temperature and an alkaline pH of 8.3 and 9.5. Under shelf-life conditions the stability of monoHER in 5% glucose (pH 8.4), decreased with about 10% during 48 h after preparation.

Keywords: Stability; Monohydroxyethylrutoside; i.v. formulation; HPLC; ECD; DAD

1. Introduction

Flavonoids are a group of polyphenolic compounds ubiquitously present in fruits and vegetables. 7-Monohydroxyethylrutoside (monoHER), a semisynthetic flavonoid (Fig. 1), is the best antioxidant in the registered HERs mixture Venoruton® (Haenen et al., 1993; Van Acker et al., 1993).

* Corresponding author. Tel.: +31-20-4442632; fax: +31-20-4443844.
E-mail address: m.abulhassan@azvu.nl (M.A.I. Abou El Hassan).

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00587-1

MonoHER was successfully used to modulate the cardiotoxic effects of doxorubicin without interfering with its antitumor effect neither in vivo nor in vitro (Van Acker et al., 1997). HERs were also used safely in the treatment of chronic venous insufficiency (Frick, 2000). These properties make monoHER an interesting compound for clinical applications. Pharmacokinetic studies of monoHER in mice (own unpublished data) indicated that the oral bioavailability was $< 1\%$. Therefore, an i.v. formulation of monoHER had to be developed for early clinical trials.

The solubility of monoHER in aqueous solutions (saline and 5% glucose) is poor and strongly pH dependent. The use of DMSO as solubilizer for quercetin (Liu et al., 1995) encouraged its use as an enhancer for the solubility of monoHER to develop an aqueous i.v. formulation. Firstly, the influence of DMSO on the stability of monoHER was investigated using HPLC with electrochemical detection (ECD). Secondly, we studied the degradation of monoHER in 0.9% saline under various conditions by means of HPLC with diode array detection (DAD) to test whether possible degradation products could be detected by this technique. Because of the reactivity of monoHER as an antioxidant (Husain et al., 1987; Yuting et al., 1990), the stability in saline and 5% glucose was subsequently tested 4 days after preparation. Finally, the shelf-life stability of monoHER, dissolved in 5% glucose, (using three concentrations covering the dose-range expected during the phase I study) was studied during 2 days after preparation.

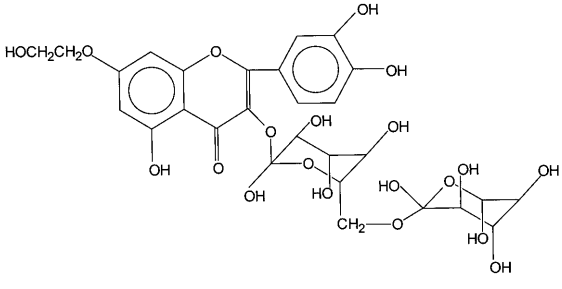

Fig. 1. The structural formula of monoHER.

2. Materials and methods

2.1. Materials

7-Monohydroxyethylrutoside (monoHER) was kindly provided by Novartis Consumer Health (Nyon, Switzerland). Acetic acid, acetone, *o*-phosphoric acid (85%), potassium chloride, silver chloride, DMSO, hydrochloric acid, hydrogen peroxide and sodium dihydrogenphosphate monohydrate were purchased from Merck (Amsterdam, The Netherlands). EDTA from Sigma–Aldrich Chemie (Zwijndrecht, The Netherlands), methanol (HPLC grade) and sodium hydroxide from J.T. Baker (Deventer, The Netherlands) and 0.9% saline and 5% glucose from Baxter (Utrecht, The Netherlands).

2.2. HPLC system

The HPLC system consisted of a Gynkotec solvent delivery system 300, a Spark Basic Marathon autosampler with a cooled sample tray (4°C) and a degasser GT-103 (Separations, H. I. Ambacht, The Netherlands). The separation was performed with a YMC ODS-AQ 3 μm, 150×4.6 mm I.D. reversed phase analytical column (Bester, Amsterdam, The Netherlands) protected by a Chrompack SS 10×2 mm, C18 guard column (Chrompack, Bergen op Zoom, The Netherlands). A mobile phase, consisting of 49% methanol and 51% of an aqueous solution containing 10 mM sodium dihydrogenphosphate, 10 mM acetic acid and 36 μM EDTA (v/v), was used at a flow rate of 0.7 ml/min.

The system was connected to either (A) a Decade electrochemical detector (ECD) provided with an integrated thermostat (set at 35°C) for the column and the cell, a glassy carbon working electrode and an Ag/AgCl reference electrode (Antec Leyden, Leiden, The Netherlands) or (B) a Gyncotek (UVD 340s) (Separations, H. I. Ambacht, The Netherlands) diode array detector (DAD). The electrochemical detector was set at $+0.7$ V vs. Ag/AgCl. The DAD recorded the chromatograms at eight wavelengths in the range of 200–390 nm. During the analysis, each peak was scanned at the middle of its two flanks and at

the top within 5 s over a λ-range from 200 to 595 nm to detect any coincidence with a possible degradation product. The column was kept at 35°C by a separate thermostat (BFO-04, Separations, H. I. Ambacht, The Netherlands).

The chromatographic data were stored and handled by a Dell Dimension XPS p166s computer (Dell, Amsterdam, The Netherlands) provided with a Gynkotec Chromeleon Chromatography Data System (Separations, H. I. Ambacht, The Netherlands).

2.3. Solutions

The solubility of monoHER in all solutions was checked, by visual inspection, every 30 min after preparation.

2.3.1. DMSO-based solutions

A stock solution of 24 g monoHER/100 ml DMSO was prepared and kept at room temperature. This solution was further diluted (eight times v/v) in water, saline or 5% glucose. The pH of these solutions was 4.7. Each of these solutions was incubated at 80°C or exposed to either 1% H_2O_2 or daylight at room temperature. Solutions exposed to 1% H_2O_2 or incubated at 80°C were kept in the dark. The peak area of monoHER (in the stock and in diluted solutions) was measured by HPLC-ECD (Abou El Hassan et al., 2000) immediately, 24 and 48 h after preparation. To check for the effect of pH on the stability of monoHER, the stock solution was diluted eight times in water and the pH was adjusted to either 8.3 or 9.5 with 1 M NaOH and kept at room temperature. The peak area of monoHER in DMSO-based alkaline solutions was determined 4 h after preparation.

2.3.2. Solutions for the degradation study

MonoHER was dissolved in saline (3 g/100 ml) and the pH was adjusted to either 9.5 or 8.3 with 1 M NaOH. Each of these solutions was exposed to 1% H_2O_2 or daylight at room temperature or incubated at 80°C in the dark. The peak area of monoHER and possible degradation products in all solutions were measured at 0 and 24 h after preparation using HPLC-DAD. The samples were injected onto the HPLC after 1000 times dilution in the mobile phase.

In an additional experiment to compare the stability of monoHER in saline and in 5% glucose, a solution of 3% monoHER was prepared in each of these solutions at pH 8.3 or 9.5 (under aseptic conditions). The stability of monoHER was measured 4 days after preparation.

2.3.3. Solutions for shelf-life stability

Three solutions of monoHER (0.15, 0.4 and 3 g/100 ml) were prepared in 5% glucose. The solutions were adjusted to pH 8.4 using (1 M) HCl, filtered through a 0.2-μm filter and transferred into sterile bottles. Each solution was separately prepared in triplicate. The peak area of monoHER and the decomposition products were measured at 0, 4, 8, 24, 32 and 48 h after preparation. Samples from the 0.15, 0.4 or 3 g/100 ml solutions were injected onto HPLC-DAD after dilution with mobile phase for 100, 200 or 1000 times, respectively.

3. Results and discussion

3.1. Solubility and stability of monoHER in DMSO-based solutions

An HPLC-ECD method, developed in our laboratory (Abou El Hassan et al., 2000), was used to measure the level of monoHER. MonoHER precipitated in all aqueous solutions of pH 4.7 kept at room temperature within 4 h after preparation. Therefore, the stability of monoHER could only be measured in all DMSO-based aqueous solutions kept at 80°C and in the DMSO stock solution. After 48 h the peak area of monoHER decreased with about 5 and 15% in the DMSO-based aqueous solutions (water, saline and 5% glucose) and the DMSO stock solution, respectively. When the pH of the DMSO-based aqueous solutions was adjusted to 8.3 or 9.5 the peak areas of monoHER further decreased with about 10% compared to the DMSO stock solution when standing at room temperature for 4 h. These results indicated that the high pH further reduced the stability of monoHER in DMSO-containing solutions.

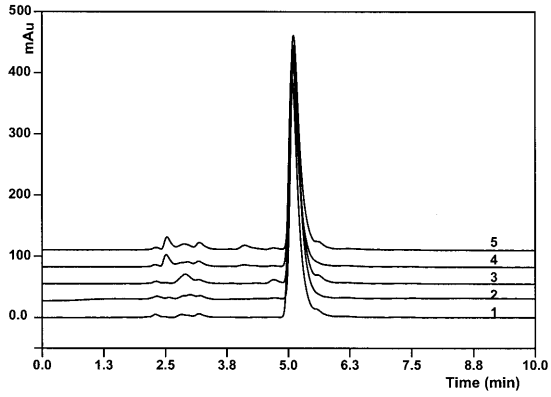

Fig. 2. Chromatograms of monoHER in saline at 0 (1) and after a 24-h exposure to daylight at pH 8.3 (2) or 9.5 (3) or to 1% H_2O_2 at pH 8.3 (4) or 9.5 (5).

Thus far no reports have been published about the formulation of monoHER probably because of its poor solubility in aqueous solutions. DMSO was used by other investigators as a vehicle for different drugs (Liu et al., 1995; Schimizu et al., 1997), however they did not mention the effect of DMSO on the stability of the drugs used. Our case indicates that such a check is necessary.

The rapid decrease in monoHER in alkaline pH may be attributed to the enhanced oxidization of monoHER (Van Acker et al., 1996). Because DMSO did not enhance the solubility of monoHER in aqueous solutions it was decided to prepare monoHER in aqueous alkaline solutions with a pH not less than 8.3 to avoid drug precipitation.

3.2. MonoHER stability in aqueous solutions

DAD was used to detect as much degradation products of monoHER as possible. A representative chromatogram of monoHER, measured immediately after preparation in saline at pH 8.3, is shown in Fig. 2. The retention time of monoHER was about 5.2 min and its peak area represented about 100% of the total peak areas (Table 1).

When the solutions of monoHER in saline at pH 8.3 and 9.5 were treated with daylight or 1% H_2O_2 at room temperature minor peaks appeared after 24 h (Fig. 2) and the percentage of the monoHER peak area to the total peak area represented about 91 and 88% at pH 8.3 as well as 95 and 91% at pH 9.5, respectively (Table 1). When incubated at 80°C (pH 8.3 and 9.5), more degradation peaks appeared after 24 h (Fig. 3) and the monoHER peak represented about 50% of the total peak area (Table 1). The reduced stability of monoHER in alkaline solutions may result from enhanced oxidization of monoHER at high pH (Jovanovic et al., 1994; Van Acker et al., 1996), which is further enhanced by an increase of the temperature.

Spectra in the middle of the flanks and at the top of the monoHER peak recorded immediately after preparation were identical and remained identical during the stability experiment, indicating that no decomposition products were hidden under the monoHER peak in the chromatograms of the stability samples. In all treated solutions the new peaks appeared in the chromatogram

Table 1
MonoHER peak area as percentage of the total area of all the peaks in the chromatogram measured in saline and 5% glucose under different conditions[a]

Vehicle	Treatment	% of monoHER peak area					
		0 h		24 h		96 h	
		8.3	9.5	8.3	9.5	8.3	9.5
Saline	80°C	97.5	99.9	47.7	56.8	NM	NM
	Daylight	99.9	100.0	90.9	94.9	93.9	90.1
	(1%) H_2O_2	97.1	98.0	87.6	90.6	NM	NM
5% glucose	Daylight	100	100	NM	NM	95.4	92.9

[a] NM = not measured.

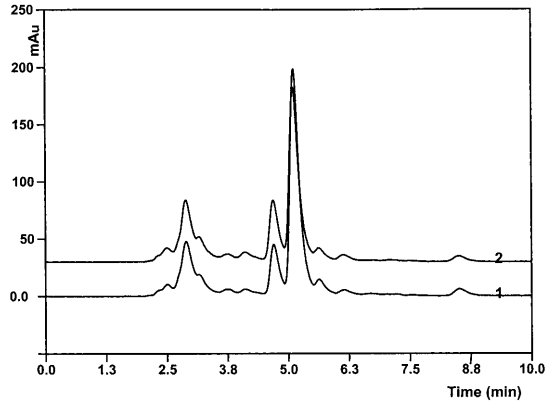

Fig. 3. Chromatograms of monoHER in saline at pH 8.3 (1) and 9.5 (2) 24 h after incubation at 80°C.

within 10 min after injection. The total area of all peaks was comparable to the total peak area measured immediately after preparation. It was therefore very likely that the chromatographic system detected all degradation products of monoHER.

The peak areas of monoHER in 4-day-old solutions in saline or 5% glucose (prepared at pH 8.3 and 9.5 and kept at room temperature) were comparable (Table 1) and thus not leading to a preference of one of these formulations. However, because of the salt load by NaCl and the possibility of blood pressure changes in volunteers and patients the use of 5% glucose is preferred. Also a pH of 8.3 is more preferable than that of 9.5 to reduce the possibility of vascular irritation during an infusion of a monoHER solution into human subjects. Therefore, it was decided to select the monoHER formulation in 5% glucose solution at pH 8.3 for future use.

3.3. Shelf-life stability of monoHER

The selected formulation was further tested for its shelf-life stability. Different concentrations of monoHER covering the expected dose range in the clinical phase I trial were prepared under aseptic conditions. Fig. 4 shows the stability of 0.15, 0.4 and 3 g monoHER in 100 ml 5% glucose at pH 8.4 while standing at room temperature for 2 days after preparation. The observed decrease

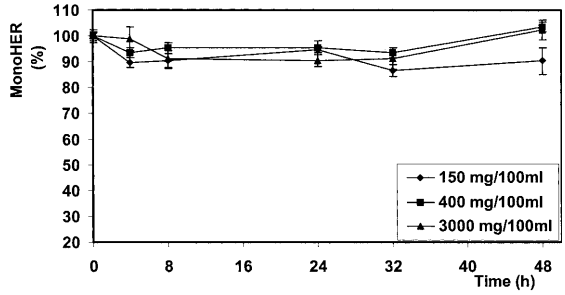

Fig. 4. The stability of i.v. formulations of monoHER at three concentration levels in 5% glucose pH 8.4. Values are mean ± S.D.

was concentration independent and represented about 10% of the original peak within 48 h after preparation during which no precipitate was seen. Part of the decrease may probably be attributed to changes in the sensitivity of the detector. It was therefore concluded that monoHER formulated in 5% glucose of pH 8.4 was well suited to be used in early clinical trials within 24 h after preparation.

4. Conclusion

DMSO did not enhance the solubility of monoHER in aqueous solutions. The stability of monoHER in pure DMSO was less than that in DMSO-based aqueous solutions. The stability of monoHER as measured by HPLC-DAD in saline and 5% glucose (pH 8.3 and 9.5) was comparable. The stability of monoHER in the selected formulation, i.e. 5% glucose of pH 8.4 was concentration independent and a decrease of about 10% was observed during 48 h after preparation when stored under shelf-life conditions. Therefore this formulation can be used for i.v. administration to patients and healthy subjects within 24 h after preparation.

References

Abou El Hassan, M.A.I., Kedde, M.A., Bast, A., Van der Vijgh, W.J.F., 2000. High performance liquid chromatography with electrochemical detection for the determination of 7-monohydroxyethylrutoside (monoHER) in plasma, J. Chromatogr. B. In press.

Frick, R.W., 2000. Three treatments for chronic venous insufficiency: escin, hydroxyethylrutoside and Daflon. Angiology 51 (3), 197–205.

Haenen, G.R.M.M., Jansen, F.P., Bast, A., 1993. The antioxidant properties of *O*-(β-hydroxyethyl)-rutosides of the flavonoid mixture Venoruton. Phlebol. Suppl. 1, 10–17.

Husain, S.R., Cillard, J., Cillard, P., 1987. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 26, 2489–2491.

Jovanovic, S.V., Steenken, S., Tosic, M., Marjanovic, B., Simic, M.G., 1994. Flavonoids as antioxidants. J. Am. Chem. Soc. 116, 4846–4851.

Liu, B., Anderson, D., Ferry, D.R., Seymour, L.W., de Takats, P.G., Kerr, D.J., 1995. Determination of quercetin in human plasma using reversed phase high-performance liquid chromatography. J. Chromatogr. 666, 149–155.

Schimizu, S., Simon, R.P., Graham, S.H., 1997. Dimethylsulfoxide (DMSO) treatment reduces infarction volume after permanent focal cerebral ischemia in rats. Neurosci. Lett. 239, 125–127.

Van Acker, S.A.B.E., Towart, R., Husken, B.C.P., de Jong, J., Van der Vijgh, W.J.F., Bast, A., 1993. The protective effect of Venoruton and its main constituents on acute doxorubicin-induced cardiotoxicity. Phlebol. Suppl. 1, 31–32.

Van Acker, S.A.B.E., van den Berg, D., Tromp, M.N.J.I., Griffioen, D.H., van Bennekom, W.P., Van der Vijgh, W.J.F., Bast, A., 1996. Structural aspects of the antioxidant activity of flavonoids. Free Radic. Biol. Med. 20, 331–342.

Van Acker, S.A.B.E., Boven, E., Kuiper, K., Van den Berg, D., Grimbergen, J.A., Kramer, K., Bast, A., Van der Vijgh, W.J.F., 1997. Monohydroxyethylrutoside, a dose-dependent cardioprotective agent, does not affect the antitumor activity of doxorubicin. Clin. Cancer Res. 3, 1747–1754.

Yuting, C., Rongliang, Z., Zhongjian, J., Yong, J., 1990. Flavonoids as superoxide scavengers and antioxidants. Free Radic. Biol. Med. 9, 19–21.