

Formulation of thermoresponsive and bioadhesive gel for treatment of oesophageal pain and inflammation

Ph.D. Thesis

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1 SUMMARY

Several illnesses related to the oesophagus result in an inflammation which may cause pain, dysphagia, weight loss of the patient. It is essential for us to focus on the effective analgetic and anti-inflammatory therapy, as the availability of the conventional oral administrated dosage forms is limited. Due to its short transit time and the relative impermeability of the stratified squamous epithelium, drug absorption from the oesophagus is not significant in comparison to the other parts of the gastrointestinal tract. However it would be desirable that locally acting agents should be used in the treatment of the pain and inflammation in several cases. In order to reach a considerable drug effect and absorption from the mucosal surface of the oesophagus, contact time should be prolonged by using different methods.

The aim of this study was to formulate a novel thermoresponsive and bioadhesive in situ gelling drug delivery hydrogel system which can adhere to the mucosal surface in the oesophagus, improving the transit time and the bioavailability and decreasing the side effects. In my present work a water-soluble cellulose derivative hydroxypropyl methylcellulose (Metolose[®] 60SH 4000) was used as a thermoresponsive and bioadhesive material. The Metolose[®] 60SH 4000 aqueous system is in sol phase at room temperature, and at a certain temperature it turns into gel phase. The temperature where the gelation can be observed is referred to as thermal gelation temperature (T_2). In normal conditions the gelation temperature of Metolose[®] 60SH 4000 is above body temperature (62 °C), while by using 5% NaHCO₃, this temperature can be shifted to body temperature. The alteration of the pH had no influence on T_2 . Based on my experiments, piroxicam and acetylsalicylic acid proved to have the best permeation and release abilities from the thermal gel. Based on my investigations that were carried out to examine the dependence of these processes on pH, I can conclude that the basic medium is preferable for the release and permeation.

My investigations proved that by using different additives (water-soluble salts, sugar esters) a special behaviour Metolose[®] based drug carrier system can be developed from which the incorporated drug can release up to 100%.

2 INTRODUCTION

Several illnesses related to the oesophagus result in inflammation which causes pain, dysphagia, weight loss for the patient, so it is essential for us to focus on an effective analgetic and anti-inflammatory therapy, as the availability of the conventional oral administrated dosage forms is limited.

In the past few years an increasing number of publications has been issued in the subject of different mucoadhesives which can adhere to different mucosal surfaces, but only a few of these publications focus on the oesophageal drug delivery, however it would be desirable in several cases that locally acting agents should be used in the treatment of the pain and inflammation in the oesophagus.

3 AIM OF THE STUDY

The aim of this study was to formulate a novel thermoresponsive and bioadhesive drug delivery system which can be used in the treatment of several diseases located in the oesophagus. This in situ gelling hydrogel system can adhere to the mucosal surface and contains nonsteroid anti-inflammatory drugs.

4 METHODS

4.1 Preparation of the 2% Metolose[®] gel

Metolose[®] powder of 0.2 g was continuously mixed with 5.0 g of water (70 °C) on a heated magnetic stirrer. 4.8 g of cold water was added to the opaque mixture and was stirred until it became transparent. Drugs and auxiliary materials were added to the prepared gel.

4.2 Rheological determination of thermal gelation temperature

The prepared solution of Metolose[®] was poured into the cup (20 °C) of the HAAKE VT550 rotation viscometer, and rotation was started. The system was first heated from 20 °C to 80 °C than cooled down to 20 °C with a Peltier TC81 thermostate to determine the thermal gelation process. Measuring parameters: sensor SV2, rotation speed G=50 1/sec, time: t=900 s. Heating rate was 4 °C/min.

4.3 In vitro bioadhesion test

In our in vitro adhesion test a specially impregnated membrane (RS Sartorius membrane typ. SM 16754, 25 cm diameter) was used to stimulate the mucosal surface. 2 ml freshly prepared Metolose[®] 60SH gel containing 5 %NaHCO₃ was poured on to the top of the membrane impregnated with saliva (pH= 7,0) and lipophil liquid. The membrane was laying on a glass surface, and it was placed in a temperated (38 °C) chamber in a vertical position. Flow time of the gel flowing down from the top to the bottom of the membrane was determined.

4.4 In vitro drug liberation test

2.0 g freshly prepared gel containing NSAID was put into the measuring cell and was covered with a cellulose membrane. The contact surface was continuous without air bubbles. Cells containing drug were put in a PTWS Pharmatest dissolution device container, which were filled with 100 ml saliva simulated buffer solution (pH=7,0, T = 38 °C). Stirring was started at a 50/sec revolution. At appropriate time intervals samples of 5.0 ml were withdrawn through the orifice and the absorbance was determined with a spectrophotometer.

4.5 Examination of in vitro drug release with Franz-type cell

A magnetic stirrer rod was placed at the bottom of the Franz-type cell and pH=7.5 buffer solution (pH of plasma) was poured in it. The liquid surface was covered with a special lipophil (Sartorius) liquid impregnated membrane which could simulate the mucosal surface. The contact surface was continuous without air bubbles and no vortex was formed during stirring. Freshly prepared gel containing drug was placed directly onto the surface of the impregnated membrane and it was fixed. Stirring started and at appropriate time intervals samples were withdrawn through the orifice and the absorbance was determined with a spectrophotometer.

4.6 In vitro release and permeation test

The in vitro release and the permeation tests were carried out with a Sartorius SM 16753 Resorptions modell of two containers. Measuring parameters: Container I: 100 ml buffer solution (donor medium simulating the gastric acid, pH=1,2), container II: 100 ml buffer solution (acceptor medium simulating blood plasma, pH=7,5), temperature: 37 °C. Sample: 10 g gel was put in the first container. A special membran impregnated with lipofil liquid (RS Sartorius membranfilter SM 16754, effective surface: 40 cm²) was placed between the two

medium, and flow began. Samples of 5,0 ml were taken out of each container at appropriate time intervals. The samples were not replaced. The absorbance was determined with a spectrophotometer.

4.7 Morphological test of oesophageal tissue

Male Wistar rats, each weighing 407 ± 15 g, were fed 0.4 ml Metolose[®] 2% 60SH 4000 solution (containing 5% sodium hydrogen carbonate) by a 22G feeding needle, and saline solution were administered into the oesophagus for control reason. Anaesthesia was carried out using urethane 1.3 g/kg intraperitoneal immediately after the feeding. Twelve hours after the administration, the oesophagus was isolated and rinsed with a saline solution. The next step was fixing the oesophagus in a 10% neutral carbonate-buffered formaldehyde and embedding it in paraffin by using an embedding center. As the last step, the oesophagus was cut into slices. The slices were stained with hematoxylin-eosin and observed under a light microscope.

5 RESULTS AND DISCUSSION

In my study a water-soluble cellulose derivative (Metolose[®]) was used as a thermoresponsive and bioadhesive raw material. Three types of Metolose[®] SH (60SH, 90SH, SM) were compared to each other in order to find the most comfortable viscosity level. Based on my rheological investigations, I can conclude that there is no remarkable difference between the three types of Metolose[®] at room temperature, however a significant difference can be observed between the thermal gelation temperature. Metolose[®] SM formulated a solid thermal gel, which can be the reason for an uncomfortable feeling to the patient. Metolose[®] 90SH has a much higher thermal gelation temperature than the Metolose[®] 60SH. In my further examinations only Metolose[®] 60SH was used. Metolose[®] 60SH formulates a low viscosity thermal gel which makes swallowing easier. As the thermal gelation of Metolose[®] 60SH 4000 is above body temperature, I applied different methods and auxiliary materials to reduce the thermal gelation temperature to body temperature. I have examined the different factors which have influence on the thermal gelation temperature like Metolose[®], the Metolose[®] concentration, pH, auxiliary materials, and different drugs.

By increasing Metolose[®] 60SH 4000 concentration, the thermal gelation temperature can be reduced, but the gel structure above 2% is relatively hard at room temperature which makes the oral application difficult. The results demonstrated that Metolose[®] 60SH 4000 2 % solution offers the most optimal gel structure.

The results of my investigation proved that the viscosity of thermal gel and the thermal gelation temperature are independent of the pH value between pH=2-8. The fact that the viscosity of thermal gel was not significantly affected by pH is very important as it means that the gel structure is stable. In the mean time a remarkable difference could be observed in viscosity at room temperature, pH=8 results the lowest viscosity system.

My results showed that by using different alcohols (propylene glycol, glycerin, PEG 400) for auxiliary materials, thermal gelation temperature increased.

When using water soluble salts (KCl, NaCl, NaHCO₃) as auxiliary material, thermal gelation temperature could be cut down, while applying 5 % NaHCO₃ proved to be the best effect to reduce the thermal gelation temperature to body temperature. To sum it up, it is preferable to use NaHCO₃ as it ensures a basic medium which is favorable in the oesophagus, and irritates less the mucosa. My further investigations were carried out by using 5 % NaHCO₃.

My results proved, that the nonsteroid anti-inflammatory drugs at the given concentration (0,5%) had no important influence on the thermal gelation temperature and on the viscosity of gel at room temperature and at target temperature. In case Metolose[®] 60SH gel contains NaHCO₃ besides the drug, the consistency changes significantly: while the acetyl salicylic acid changed the consistency significantly, the aminopyrin reduced the thermal gelation temperature to a low extent.

The in vitro adhesion test demonstrated that the thermoresponsive gel can adhere to the mucosal surface during up to 60 seconds in an optimal case.

Based on the results of the in vitro release tests, I concluded that there are significant differences between the release capacity of the drugs. During the period of the examination (60 seconds) piroxicam and aminopyrin showed a release capacity of 50 %, while acetyl salicylic acid and ibuprofen proved to have weaker release capacity.

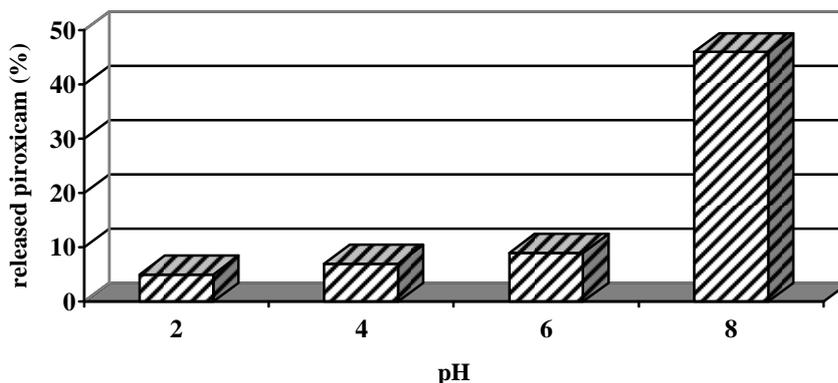
Comparing the in vitro release of different drugs from Metolose[®] 60SH gel with 5 % NaHCO₃, piroxicam, acetyl salicylic acid, aminopyrin, ibuprofen, indometacin showed good results after 60 seconds, and the amount of the released piroxicam was prominently high.

After having examined the permeation ability of drugs from the thermal gel under the studied circumstances, piroxicam and acetyl salicylic acid turned out to have the most favorable permeation ability.

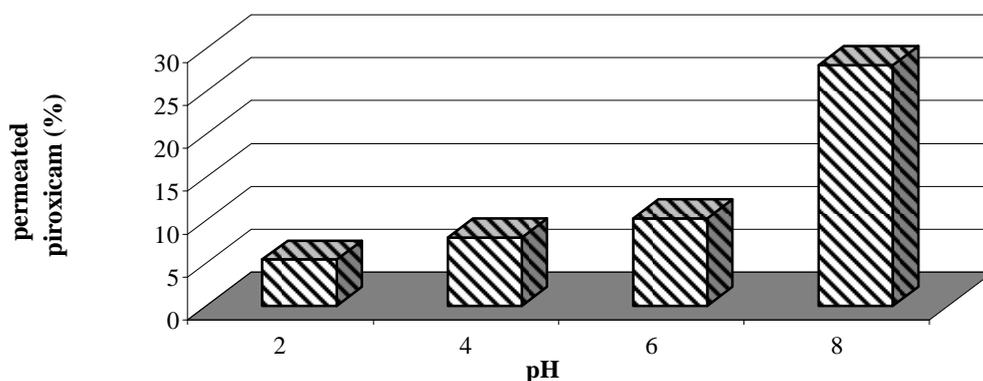
An additional advantage of the piroxicam is the long plasma half-life ($t_{1/2}$ =50 hours) which makes sure the piroxicam has good bioavailability.

In my next investigations I tried to find out to what extent the alteration of the pH of the system could influence on the piroxicam release. The NaHCO₃ (5%) as auxiliary material ensures a basic medium, which has an influence on the piroxicam release and permeation

from Metolose[®] 60SH gel, as the solubility of the piroxicam can be influenced by the pH of the hydrogel.



Piroxicam release from the 2% Metolose[®] gel in the function of the pH
(n=3, RSD \leq 3%)



Piroxicam permeation from the 2% Metolose[®] gel in the function of the pH
(n=3, RSD \leq 3%)

Based on the results of my in vitro drug absorption tests, I could conclude that the thermal gel swallowed accidentally can absorb from the stomach, thus has systematic effects which have further beneficial impacts.

In order to improve the drug release and permeation ability of the model drug as a special surfactant auxiliary material, sugar esters were used. Based on my research I can conclude that by using L 1695 SE (HLB=16), the incorporated drug can release up to 100%.

A morphological test was carried out by using Metolose[®] 60SH 5% containing NaHCO₃. In our in vivo test the possibility of a damage on the oesophageal mucosa was investigated. In the histological investigation mucosal irritation could not be observed neither in the oesophagus slices of the treated rat, nor in the oesophagus of the control rat, so this Metolose[®] solution had no tissue-damaging impact on the oesophageal mucosa even after 12 hours.

6 CONCLUSION

Based on my investigations I have concluded that:

1. Due to its better viscosity, 2% Metolose[®] 60SH 4000 was chosen as a raw material of a thermoresponsive system for an oesophageal use.
2. The thermal gelation temperature of Metolose[®] solution is above body temperature so this temperature should be reduced by different methods and auxiliary materials.
3. 5% NaHCO₃ has the best results among water soluble salts in reducing the thermal gelation temperature to body temperature.
4. NSAIDs at a given concentration did not have a remarkable influence on the thermal gelation temperature and the gel structure.
5. Aminopyrin and piroxicam proved to have the best drug permeation ability from the thermal gel.
6. In vitro adhesion test showed that the gel can adhere to the mucosal surface during up to 1 hour.
7. In vitro drug release and permeation depends largely on the pH of the hydrogel.
8. Piroxicam proved to have the best release and permeation results in case of a gel prepared with a buffer of pH=8.
9. By using 5% NaHCO₃ as an auxiliary material ensures a basic medium (pH=8), which influence the solubility of the piroxicam.
10. As the prepared drug containing gel is used as a systematic acting agent, piroxicam is the best model drug, because of the long plasma half life.
11. By using sugar esters of different HLB value as surfactant auxiliary material, incorporated drug release capacity can be largely increased.
12. By using L-1695 sugar ester as a surfactant auxiliary material, piroxicam released up to 100%.
13. Metolose[®] gel containing 5% NaHCO₃ has no tissue damaging effect in rat oesophagus during 12 hours.

In my study a water-soluble cellulose derivative (Metolose[®] 60SH 4000) was used as a thermoresponsive and bioadhesive material. The thermal gelation temperature of Metolose[®] solution is above body temperature so this temperature should be reduced by different methods and auxiliary materials. This hydrogel system is in sol phase at room temperature, but at body temperature it turns into gel phase which results a better adhesivity to the mucosal

surface, which improves the bioavailability. The consistency of the sol and the thermal gel system is comfortable for the patients, easy to swallow and less irritates the mucosa. Sugar esters as a special surfactant auxiliary materials are able to improve the drug liberation and permeation, so not only the incorporated drug amount, but also the side effects can be reduced.

7 SCIENTIFIC REPORTS, PRESENTATIONS AND POSTERS:

I. Á Makó, G Csóka, E Pásztor, S Marton, G Horvai, I Klebovich. (2009) Formulation of thermoresponsive and bioadhesive gel for treatment of oesophageal pain and inflammation. Eur J Pharm Biopharm, 72: 260-265.

II. G Csóka, A Gelencsér, Á Makó, S Marton, R Zelkó, I Antal, I Klebovich. (2007) Potential application of Metolose[®] in a thermoresponsive transdermal therapeutic system. Int J Pharm, 338: 15-20.

Posters:

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Bioadhezív és termoreszponzív gélek formulálása a tápcsatorna felső szakaszán előforduló daganatos betegségek tüneti kezelésére

Makó Á, Csóka G, Horvai G, Klebovich I.

MFOE 40. Siófoki Jubileumi Kongresszus (2008):

Bioadhezív és termoreszponzív gélek formulálása a tápcsatorna felső szakaszán előforduló betegségek tüneti kezelésére II. Új eredményeink bemutatása

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