Contemporary Clinical Trials 33 (2012) 260–267

Contents lists available at SciVerse ScienceDirect

Contemporary Clinical Trials

journal homepage: www.elsevier.com/locate/conclintrial

Methylene blue MMX® tablets for chromoendoscopy. Safety tolerability and bioavailability in healthy volunteers

A. Repici [a], A.F.D. Di Stefano [b,*], M.M. Radicioni [b], V. Jas [c], L. Moro [d], S. Danese [a]

[a] Department of Gastreonterology, IRCCS Istituto Clinico Humanitas, Milan, Italy
[b] Cross Research S.A., Via F. A. Giorgioli, 14, CH-6864 Arzo, Switzerland
[c] Analytisch Biochemisch Laboratorium B.V. (ABL), W. A. Scholtenstraat 7 9403 AJ Assen, The Netherlands
[d] Cosmo Technologies Ltd., 42–43 Amiens Street, Dublin 1, D1, Ireland

ARTICLE INFO

Article history:
Received 27 June 2011
Received in revised form 14 October 2011
Accepted 5 November 2011
Available online 11 November 2011

Keywords:
Methylene blue
Pharmacokinetics
Healthy subjects
MMX
Chromoendoscopy

ABSTRACT

Methylene blue-MMX® tablets are proposed as colonic diagnostic staining. Methylene blue taken prior to colonoscopy is expected to provide an effective staining of colonic and rectal mucosa leaving unstained the dysplastic or polypoid areas.

The present single dose, open-label study investigated the safety of methylene blue after single oral doses of 200 and 400 mg in healthy volunteers. The absolute bioavailability was also investigated after the intake of 2 L of bowel cleansing preparation in 2 h and by comparing the dose of 200 mg with a single iv dose of 100 mg in the same subjects.

Only non-serious adverse events occurred. Related events occurred to 8/22 subjects. Most of the events were mild and transient. Abnormal transaminases, gastrointestinal disorders and dysuria frequency were 13.6%. After intake of the laxative and the oral dose of 200 mg, systemic exposure to methylene blue was shown in all subjects with concentrations increasing for 12 h. The peak was reached in a median of 16 h. Peak blood concentration did not increase proportionally with the dose. AUC_{0-t} was 32.94 μg/mL × h after 200 mg and 38.08 μg/mL × h after 400 mg. Half life ranged between 14 and 27 h after the lower dose and between 6 and 26 h after the higher dose. The cumulative excretion was about 40% of the injected dose, 39.67% after 200 mg and 23.48% after 400 mg. Absolute bioavailability of methylene blue calculated as ratio between AUC_{0-t} oral/iv corrected for the dose was on average $F_{abs} = 139.19 \pm 52.00\%$.

1. Introduction

Methylene blue is able to stain tissues without impairing their vital functions.

Currently, methylene blue is used in human medicine in a number of therapies and diagnoses.

Methylene blue MMX® tablets are a new oral modified release formulation manufactured using a multimatrix structure (MMX®, Cosmo Technologies, Ireland). MMX is a modified release technology that ensures a colonic drug delivery. In the MMX structure, microparticles of active ingredient are dispersed in a sequence of lipophilic and hydrophilic matrices. The multimatrix creates a partially hydrophobic environment which slows and controls the drug dissolution process. The outer tablet gastroresistant polymer film begins to disintegrate when the suitable intestinal pH is reached. Thus, the tablets arrive unaltered to the terminal ileum. When the colon fluids interact with the tablet after coating dissolution, the matrix structure forms an outer, viscous gel mass that controls the diffusion of the active ingredient. Whilst the tablet progresses in the colon towards the rectum, debris of the gel mass disaggregate and release the active ingredient in proximity to the mucosa. MMX formulations' gastrointestinal transit and colonic delivery was demonstrated by pharmaco-scintigraphic investigations of various drugs in healthy male volunteers [1,2]. In the study of gastrointestinal transit and distribution of budesonide MMX® tablets, ^{153}Sm-budesonide radioactivity incorporated into tablets was

* Corresponding author. Tel.: +41 91 6300510; fax: +41 91 6300511.
E-mail address: andrea.distefano@croalliance.com (A.F.D. Di Stefano).

1551-7144/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.cct.2011.11.006

followed by means of pharmaco-scintigraphic imaging [2]. The relative percentage of budesonide absorption in the time during which radioactivity was detectable in the target region (i.e. the region comprised between the ascending and the descending-sigmoid colon) was 95.9 ± 4.2%. Budesonide tablets were detected in the ascending colon between 6 and >24 h. The descending colon was left 12 to >24 h post-dosing. Initial tablet disintegration started, on average, 9.48 ± 5.11 h after administration either in the small intestine (n = 2), the ileum (n = 5), the ascending (n = 2), transverse (n = 2) or sigmoid colon (n = 1). Times of tablet residence in the gastroenteric regions were 17–117 min (stomach), 37 min–9.95 h (small intestine), 0.5–12 h (ileum), 3 to >15.5 h (ascending colon), 4 to >17 h (transverse colon), and 12 to >17 h (descending colon) [2].

Methylene blue has the property to be selectively absorbed and stain the intestinal columnar epithelium [3–5]. Normal, non-dysplastic mucosa generally exhibits a diffuse, homogeneous, dark blue staining, after methylene blue is absorbed into the columnar cytoplasm and abundant goblet cells. In contrast, severely dysplastic, inflamed or malignant epithelium exhibits decreased cytoplasm and reduced to absent goblet cells. These alterations result in decreased uptake of methylene blue and endoscopic appearance of focal light blue or pink (unstained) or heterogeneously stained (specked) mucosa within the diffusely stained epithelium. This property is applied to the detection of abnormalities of colonic mucosa. During the chromoendoscopy, different dyes, like methylene blue and indigo carmine, are topically applied by a spraying probe [4,6]. Sprayed methylene blue has been used to screen colonic neoplasias at concentrations of 0.1–0.5% [7]. The new tablets would represent a potential alternative to the topical spray. When taken before the colonoscopy, methylene blue tablets are expected to provide an accurate staining in the colon and rectum. The colonic release would increase the contrast of the mucosal cells, lower the polyp detection failure rate and reduce the total time of the colonoscopy by ensuring the mucosal staining before initiating the endoscopy. The excessive prolongation of the endoscopy due to the dye spraying, absorption into the epithelium and excess removal would also be avoided.

The present study aimed at preliminarily investigating safety and absolute bioavailability of methylene blue administered as MMX tablets at doses of 200 and 400 mg to healthy male and female volunteers after the intake of a bowel cleansing preparation. The investigation of the colonic mucosa staining after a single dose of methylene blue tablets is the objective of ongoing trials.

2. Methods

2.1. Study design

The present study was a single ascending dose, open-label, randomised, safety, bioavailability study. Primary objective was the investigation of the safety of methylene blue after single oral doses of 200 and 400 mg as Methylene blue MMX tablets in healthy volunteers undergoing a standardised bowel cleansing preparation. The new formulation was administered for the first time. The study was also aimed at investigating the absolute bioavailability of methylene blue after a single oral dose of 200 mg as compared with a single iv dose of 100 mg of methylene blue 1% and at investigating the PK profiles of methylene blue in the main biological fluids blood and urine. The study consisted of a 1st stage including 2 periods and a 2nd stage including only one period. Subjects enrolled for the 1st stage received a single oral dose of 200 mg and a single iv dose of 100 mg according to a randomised two-way cross-over design to investigate the absolute bioavailability of methylene blue. In the 2nd stage, a 2nd cohort of volunteers was enrolled and administered a single oral dose of 400 mg of methylene blue for the investigation of safety, tolerability and PK profile of the active substance. The blood sampling and the urine collection schedule were planned up to 72 h post-dose considering the recently published data about the PK of methylene blue [8,9] and considering the peculiar delivery profile of the previously studied MMX® formulations [1,2].

Since methylene blue had never been administered orally for the mucosal staining, the selection of doses was made by considering the dose regimen indicated for the marketed injectable and oral products of methylene blue for the treatment of methaemoglobinaemia and urinary infections. Maximal doses for these two indications are 120 mg/day iv and 200 mg/day orally. In the peculiar case of treatment of ifosfamide-induced encephalopathy daily iv injections of up to 300 mg of methylene blue are recommended [10]. On the basis of this safety consideration, single ascending doses of 200 and 400 mg were expected to be safe and tolerable to the healthy subjects. The previously published PK trial of methylene blue in healthy subjects by Walter-Sack et al. was also taken into account. In that study, single doses of 500 mg of methylene blue as a 2.5% aqueous solution were administered without raise of safety concerns [9]. According to Walter-Sack et al., the most frequent untoward effects were nausea and vomiting on one side and temporary blue staining of tissues on the other side. In particular, nausea and vomiting were due to the particular oral solution dosage form used in that study, which had a poor palatability. Less frequent untoward effects were diarrhoea, dysuria and headache. According to this relevant previous experience, the safety assessments of the present study were focussed on the recording of adverse events and on the clinical laboratory assays. A continuous monitoring of ECG and vital signs was regarded as superfluous, since this measurement had been already performed in the Walter-Sack's trial and no significant change had been observed. In the study design, the review of methylene blue tolerability by Clifton and Leikin was also taken into account [11]. Indeed, the expected untoward effect of a dose of 2–4 mg/kg of methylene blue was haemolytic anaemia. Furthermore, the authors reported the untoward effect of blue-green discolouration of urine and faeces after a 4 mg/kg dose. The known expected untoward effects of 7 mg/kg of methylene blue were nausea, vomiting, abdominal pain, chest pain, fever, haemolysis, profuse sweating, dizziness, headache and mental confusion according to the same authors. It is also known that untoward effects may be exacerbated in case of glucose-6-phosphate dehydrogenase deficiency. Therefore, the enzyme was assayed specifically in the present study.

2.1.1. Study population and criteria for inclusion

The study was performed at the Phase I Unit of Cross Research S.A., Arzo, Switzerland.