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AN ANIMAL MODEL FOR INVESTIGATING MANGANESE ABSORPTION AT VARIOUS REGIONS OF THE GASTROINTESTINAL TRACT

Andrew B.C. Yu, Joel Weiner, Douglas W. Hamel,
King C. Lee
Sterling Winthrop Pharmaceuticals Research
Division, Rensselear, New York, 12144.

ABSTRACT

The absorption of manganese chloride at various sites of the gastrointestinal (GI) tract was studied in rats. Manganese chloride was administered by direct injection into either the stomach (intragastric, i.g.), the duodenum (intraduodenal injection, i.d.), or by simple oral administration (p.o). Serum Mn^{2+} concentrations in the hepatic portal vein were measured using a newly developed atomic absorption method. Administrations of manganese chloride by p.o., i.g. and i.d. routes increased endogenous serum Mn^{2+} concentration by approximately 100%. However, the absorption kinetics of the three routes of administration were different. These results indicated that manganese chloride was readily absorbed after i.d. and i.g. administrations. The absorption of manganese chloride after p.o. administration was delayed, possibly due to passage through the esophagus and stomach into the intestine prior to intestinal absorption. The method was also applied to screening GI absorption of a number of

manganese chelate compounds. The less polar compounds were not preferentially absorbed compared with manganese chloride, which is more ionic in nature.

INTRODUCTION

Manganese is an essential metal for normal metabolic function (4). It has been listed as a vital dietetic element for hospital patients (1,2) and manganese deficiencies have been associated with a number of disease-like states. Manganese has interested the pharmaceutical industry because of its use as a diagnostic agent in nuclear magnetic imaging. Various forms of manganese salt complexes or chelates have been reported in the literature (3,4,5,10-13) for both intravenous and oral use. Several studies have demonstrated that manganese is absorbed orally, based on magnetic resonance imaging or by conventional measurement in animal and man (10,14,15). Literature sources indicate that manganese absorption may be saturable, and may be influenced by food and other nutrients. Ascorbic acid was shown to effect the absorption and retention of manganese (2,4). Characterization of the biopharmaceutic factors influencing absorption and delivery has been an important step toward designing a dosage form for a manganese product, either for diagnostic use or nutritional supplementation. The primary objective of this study was to demonstrate a method and animal model that could readily be used to measure manganese levels in the blood after oral administration. A second objective was to examine the extent of absorption at various sites along the GI tract. Several proprietary compounds were screened for absorption, indicating that the method was a useful screening tool for investigating GI absorption of manganese compounds.

METHOD

The GI absorptions of manganese chloride, a heptadentate complex of Mn^{2+}, and other investigative compounds were tested at 620 μmol/kg in overnight-fasted Sprague-Dawley rats (400-500 g). All research involving animals described in this publication was performed in accord with the Sterling Winthrop Pharmaceuticals Research Division (SWPRD) Policy on animal use and all national and federal ligislation. All SWPRD animal facilities and programs are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The rats were anesthetized with Na pentobarbital (50 mg/kg, i.p.), and anesthetic was supplemented as needed during the experiment. Via an abdominal laparotomy, the manganese compounds were administered by oral administration (p.o.) or by direct injection either into the body of the stomach (intragastric [i.g.]) or into the duodenum immediately below the pyloric sphincter (intraduodenal [i.d.]). A blood sample (3-5 mL) was obtained from the hepatic portal vein from each rat at time points ranging from 0.5 to 3 hours after manganese chloride administrations. Blood samples were centrifuged at 2,000 g at 0 °C. Serum was isolated by aspiration and refrigerated at -4 °C for subsequent assay of Mn^{2+} concentrations.

Analysis of the serum samples was performed by a Varian atomic absorption spectrophotometer equipped with a manganese hollow-cathode lamp and an air-acetylene flame. Atomic absorption was determined at the manganese emission line of 279 nm. Each mL of serum samples was deproteinated by adding 1-2 mL of dimethylformamide, vortexed for 1 minute, and followed by the addition of 1 mL of DMSO plus 1-2 mL of water. The mixture was shaken vigorously for 30 minutes on a Labline multi-wrist shaker

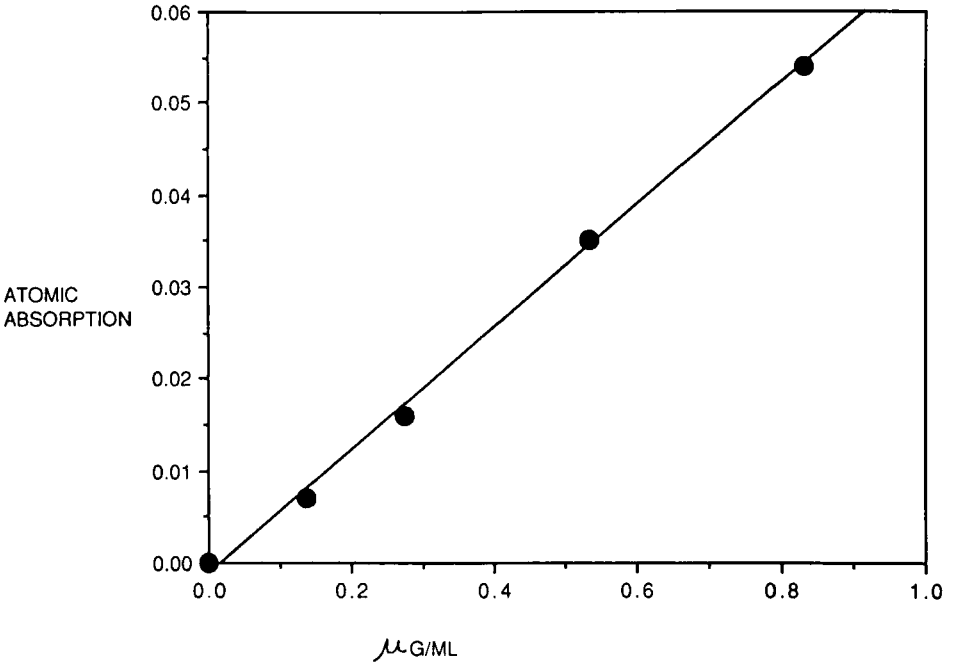

FIGURE 1

A typical standard curve for determination of serum Mn^{2+} concentrations by atomic absorption at 279 nm.

and then centrifuged at 5000 rpm (about 3900 g) on an American Laboratory Product Centrifuge. The supernatant was removed for analysis. Four standard solutions of manganese chloride ranging from 0.1-0.9 μg/mL were included to ensure linearity within the range of samples tested. A typical standard curve is shown in Figure 1. The samples were read three times and the mean absorbance was used in the calculation of sample concentrations.

RESULTS AND DISCUSSION

After intraduodenal administration of manganese chloride solution, 1-2 μg/mL of manganese was detected in

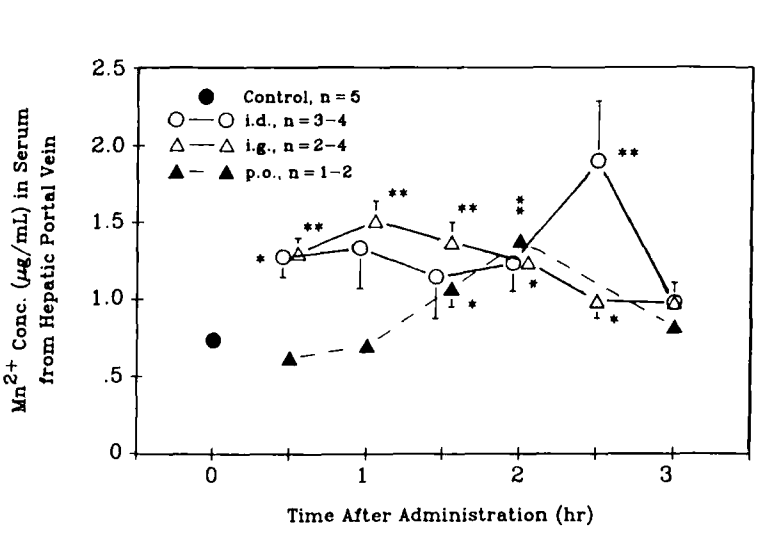

FIGURE 2

Absorption of manganese chloride (reflected by the serum Mn^{2+} concentrations in the hepatic portal vein) after intraduodenal (i.d.), intragastrical (i.g.) or oral (p.o.) administrations. Manganese chloride was not administered to the control rats. One blood sample was collected from each rat at a specific time point after administration of manganese chloride. * = $p<0.05$ and ** = $p<0.01$, compared to control group by ANOVA.

the portal serum at the following time intervals: 0.5, 1, 1.5, 2, 2.5 and 3 hours. Peak concentrations were observed at approximately 1-2.5 hours (Figure 2). Intragastric administration resulted in similar levels, although the peak level at 2.5 hours tended to be lower. Oral administration resulted in generally lower levels, except at 1.5 hours. At 0.5 to 1 hours, the manganese level were similar to that of the control group

indicating that little or no manganese was absorbed during this period.

These results indicate that after oral administration, there was some delay in the absorption of manganese. On the other hand, there was no delay when manganese chloride was administered into the stomach by injection. Since drug solution was emptied from the pylorus into the duodenum periodically and absorbed there, it was not surprising that there was no delay in the absorption by this route compared with intraduodenal administration. The tendency of greater level of manganese absorption via intraduodenal administration is consistent with other reports that manganese was absorbed through the duodenum and jejunum (15,16,19). The exact reason for the delay in manganese absorption observed after oral administration is unknown. There are reports that solid dosage forms can be trapped in the esophageal sphincter resulting in delayed absorption but this is unlikely since manganese chloride was administered in solution state. A possible explanation could be that manganese chloride could have interacted with surrounding enteral material during its esophageal passage and additional staying time in the body of the stomach. Interaction of manganese chloride to form mineral-fiber chelates in the gut was postulated to reduce manganese uptake in a study by Halpin and Baker (17). The fiber complexes may have reduced the gastrointestinal transit time compared to free manganese ion (18). Becker, G., et al has shown that pectin may have increased bile acid excretion and reduced manganese absorption in isolated jejunum segments (19). Thus, it appears that manganese absorption may be affected by a number of dietetic and physiologic factors.

The present study revealed that manganese chloride was able to traverse the lipid GI membrane and undergo

oral absorption despite its dissociation and ionic nature. It was unlikely that absorption occurred via a passive transcellular route due to it's ionic nature. Diffusion by the paracellular route was possible, although the pharmacokinetic profile of the absorption curve does not support an oral single compartment model, which is typical of passive absorption. In a preliminary screening effort involving half a dozen proprietary manganese chelates (unpublished data), the absorption of Mn^{2+} for five chelate compounds were insignificant, The sixth compound resulted in significant absorption although the extent of absorption was lower than that for a comparable dose of manganese chloride. It appears that favorable lipophilicity and partition does not necessarily improve absorption of manganese. Passive transcellular diffusion is unlikely to be a major mechanism of manganese chloride absorption. The primary absorption process in the duodenum and jejunum is currently unidentified. A previous study suggested that Mn^{2+} has a saturable, capacity-limited absorption pattern (16), therefore, its absorption may be mediated by either specific channels or specific carriers. However, the characteristics of the speculated specific channels or specific carriers remains to be investigated.

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