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STUDIES ON FORMULATION AND INVITRO EVALUATION OF DICLOFENAC SODIUM AND BIODEGRADATION BY RAT'S CEACAL MICROFLORA FOR COLON TARGETED DRUG DELIVERY SYSTEM

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ABSTRACT
The main objective of the present work was developing a novel diclofenac formulation for colonic targeted drug release. The proposed delivery system consisted in a polymeric matrix tablet containing a drug central core purposely designed for obtaining a slow release of the drug from the matrix tablet with different natural polysaccharides. It was well known that many of the natural polysaccharides were utilized for colon targeted drug delivery system. The selection of the natural gum depends on the biodegradable capacity of the gum with the colonic micro flora with azoreductase. Tablets containing the drug central core were prepared by direct compression and tested for dissolution properties according to the USP (United state pharmacopoeia) paddle method. For series of formulations, the amount of the drug and the quantity of the natural polysaccharide were varied and reported. The *invitro* release of drug determined initially for 2hrs in 0.1n hydrochloric acid and subsequent six hours in phosphate buffer of pH 7.2 .the results showed that no drug was released during 2hrs time in 0.1N hydrochloric acid from all five formulations. However, formulations containing natural polysaccharides shows better release rate than compared with conventional method of targeting to colon. Also the colonic microflora was characterized for its microbial properties, found to have strict anaerobic condition, gram negative, non motile.

KEYWORDS: guargum; diclofenac sodium; colon targeting; bacteroids; colonic microflora.

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INTRODUCTION

The colon specific drug delivery system have gained prominence not only for the treatment of inflammatory bowel disease (inflammatory bowel syndrome IBS, ulcerative colitis, Chron's disease, various carcinomas and other infections) but also for systemic delivery of proteins and peptide drugs. These systems however, to reach colon in an intact from should surpass the pH barriers in stomach and intestinal environment for which different approaches were studied such as prodrugs coating with pH dependent polymers, coating with pH independent biodegradable polymer and matrices of polysaccharides. Salyers reported that natural polysaccharides such as pectin and xylose will not be digested in human stomach or in small intestine but are degradable in colony by resident bacterial flora[1]. A mixed coating comprising amylose and ethyl cellulose (1:4) has been reported to provide colon specific delivery. Studies with pectin by Ashford indicated that the degree of methoxylation of pectin and calciumcontent of pectin layer influences the solubility of the layer and its susceptibility to enzymatic degradation[2]. A colon delivery system based on the use of mucopolysaccharides, chondroitin, found in human colon from sloughed epithelial cells and dietary meal, has also been reported. The sulphate salt of chondroitin has been reported to utilized as a substrate by bacteria (*Bacteroids* and *B.ovatus*) in large intestine as major energy source[3].

Inulin, a naturally occurring carbohydrate in many plants has been shown to resist hydrolysis and digestion in upper G.I.T but is fermented by the colonic microflora, more specifically by *Bifidobacteria* and *bacteroides*.

Inulin H.P (high degree of polymerization) incorporated in Eudragit RS film has been reported as a possible biodegradable coating for colonic delivery system. The same authors have outlined the sysnthesis and characterization of inulin hydrogels as carriers for colonic drug delivery[4].

Chitosan, a high molecular weight cationic polysaccharide derived from naturally occurring chitin in crab and shrimp shells by deacetylation process has been reported in the form of microcapsules to deliver insulin to the colon. A delivery system based on glassy amylose for colon specific drug delivery was also reported[5, 6].

Other investigators have taken advantage of azoreductase activity of microflora of the colon, to develop bacteria – biodegradable polymer coating. A part from azopolymers, naturally occurring plant hydrocolloids, Galactomannans which will be degrade by glycolitic activity of colonic bacteria have been exploited along with methacrylate solutions to form degradable coating around the drug for colon delivery[7]. Krishnaih et al used Guargum alone matrix for preparing colon drug delivery system for different drugs. Other plant hydrocolloids like Tracaganth and Acacia have not yet been fully studied for their utilization for preparing colon specific delivery system[8].

Guar, Tracaganth and Acacia gums are traditionally used in the solid dosage forms as a binder, in liquid oral and topical products as a suspending, thickening and stabilizing agents[9]. Guargum, due to its nonionic nature is compatible with most other plant hydrocolloids such as acacia and tragacanth[10]. So, in the present paper, an attempt has been made to develop and evaluate a colon targeted drug delivery system using a combination of Tragacanth and Acacia gums along with Guargum in different proportions as a carrier for Diclofenac sodium. The investigation also includes, the isolation of a pure organism belonging to genus *Bacteroides* from rats ceacum and to study its ability to biodegrade tragacanth and acacia gums as they are not yet studied fully. So, the main objective of the present work is to provide an alternative method, using the organism in place of ceacal mater for conducting *invitro* drug release studies for colon drug delivery systems

MATERIALS AND METHODS

Diclofenac sodium was a gift sample from M/S ABIC Laborateries Goa. Guargum, Tragacanth and Acacia were of pharmacopoeial grade, procured from Indian gums Bombay, FTM dehydrate from M/S High media, Bombay. Other chemicals, dihydrogen phosphate, hydrochloric acid, nitrofurantidin, hydrogen peroxide, talc, magnesium stearate were of analytical grade.

Preparation of diclofenac sodium matrix tablets

Matrix tablets of diclofenac sodium were prepared by direct compression method. Diclofenac sodium was chosen as a drug for its anti-inflammatory action. The composition of different matrix formulations used in study. Guar, Tragacanth and Acacia gums were used in different proportions, keeping constant the total. Gum content 70% of the total weight of the tablet in each formulation[11,18]. Two hydrophobic lubricants talc and magnesium stearate (4:1) ratio were used as antitacking and antiadhesive additives, the concentration of which were deliberately kept high in the formulations as compared with the conventional levels normally used as lubricants the gum contents along with the drug were mixed well and lubricated with a mixture of talc and magnesium stearate using a laboratory model blender for a period of 30 min this step was found necessary due to inadequate compressibility characteristics of gum combinations. The well coated gum particles were directly compressed to as hardness of 9 kg/cm by using a single station tablet machine using 11mm round flat and plain punches to a weight of 500 mg[12]. The matrix tablets of each composition were tested for their hardness, drug content uniformity and drug release characteristics with a suitable number of tablets for each test.

Determination of drug content

The diclofenac matrix tablets were analysed for drug content. The tablets were finely powdered, and 100mg of the powder was accurately weighed and transferred to 100ml volumetric flask and allowed to stand for 30 min with intermittent shaking to ensure complete solubility of drug and the volume was made up to 100ml with the same buffer, the mixture was centrifuged aliquot of 5 ml samples from supernatant liquid was suitably diluted, filtered through a Millipore filter with 0.22 micron pore size and assayed spectrophotometrically at an absorption maxima of 276nm using double beem shimadzu ultra violet visible spectrophotometer against a reagent blank.[13,14]

The hardness of the matrix tablets was determined by using Monsanto hardness tester.

In-vitro drug release studies

The ability of combined guar, tragacanth and acacia matrix tablets of diclofenac sodium to remain intact in the physiological environment of stomach and small intestine was assessed by conducting drug release studies in 0.1N Hcl for two hours and subsequent six hours in phosphate buffer 7.2 using USP dissolution apparatus with 900ml of the respective dissolution fluids at 37 + or – 0.5 and a stirring speed of 72 rpm.[15,16]

A sample of 5ml was withdrawn at each hourly interval and immediately replaced by an equal quantity of fresh dissolution fluid. The drug content was analysed in the sample withdrawn spectrophotometrically after suitable dilution, at an absorption maxima of 276 nm against a reagent blank.[17] The data was plotted as cumulative percent drug released versus time in hours. The swelling behavior of the matrix tablets was assessed by plotting the data according to peppas exponential equation (Q=ktn)20 as log amount of drug released versus log of time.[18]

Method of isolation of ceacum from rat

To asses the susceptibility of combined gum matrix tablets to be acted up on by colonic microflora, a pure culture of ceacal organism was isolated from rat's ceacum. This step was carried out because of the similarity of resident bacteria in rat's ceacum and in human intestine. Healthy rat was anaesthetized and dissected for ceacal exposure, which was separated from the other parts of large intestine by typing with thread on either sides. The isolated cecum was opened carefully and its contents were transferred aseptically in to two 500ml conical flask containing 250ml sterile thioglycolate medium. The flasks were plugged and incubated under strict anaerobic conditions using an anaerobic jar for a period of 24hrs from which subcultures were made at suitable intervals using same medium.

Characterizaton of ceacal organism

The organism was characterized for its gram reaction by carrying out grams stain motility was carried by hanging drop technique catalase test was done by following method suggested by dubey and maheswari.[19]

Mean generation time was determined by following I.P method. Thermal death time was determined by following a standard procedure at 60 degree centigrade.[20]

Biodegradation of guar, tragacanth and acacia gums by ceacal culture

Degradation of any polysaccharide by microflora present in colon is prerequisite for their successful utilization as carriers for colon targeting. The ability of the ceacal organism to degrade the gums was next ascertained. For these semiquantitavie experiments, three sets of sterile test tubes, six in each set for three gums were filled with 10ml of freshly prepared FTM (pH 7.2 + or – 0.2). Graded amounts of the three respective gums each at concentrations of 0.2, 0.4, 0.6, 0.8 and 1gm were added aseptically. The tubes were incubated under anaerobic conditions; in each set one tube was similarly inoculated with the organism without any added gum to serve as control. The tubes were shaken gently only to disperse the gum particles and incubated for 48hrs. The tubes were examined visually and their clarity was compared with control.

Determination of phases of growth of ceacal organism in FTM with and with out added gums

These experiments were carried out using only gum acacia and tragacanth as guargum was already known to be degraded by glycolytic activity of ceacal flora. These experiments carried out in FTM by following the method given by kannan[21]. A quantity of 250ml sterilized FTM was taken in two 500ml conical flasks. Another conical flask was inoculated aseptically with 1 ml quantity of ceacal culture and the other one was kept as control. Both flasks after being plugged with cotton were placed on a mechanical shaker under anaerobic conditions. Aliquots of 2ml samples were withdrawn from both flasks at four hourly intervals for a period of 48hrs.The turbidity was measured at 610 nm using colorimeter (systronic make) against a blank sample withdrawn from other uninoculated flask. A plot was between absorbency against time.

The same procedure was used to ascertain the effect of added gums (acacia and tragacanth) on a phase of growth of ceacal culture. In these experiments the blank consists of uninoculated FTM with added gums each at 500mg concentration.

In-vitro release of drug from intact tablets

In this experiment, 100ml of saline phosphate buffer of pH 7.4 was used in a 250ml flask. The intact tablets from *invitro* drug release experiments under physiological conditions were transferred. The buffer was bubbled with carbon dioxide to create anaerobic conditions[18]. The buffer containing flask was inoculated with 1ml quantity of cecal organism (after ten generations) and incubated for period of 24hrs. The contents were filtered using membrane filter of 0.2 micron pore size. Aliquots from the filtrate, after suitable dilution, was analysed for drug content spectrophotometrically at absorption maxima of 276nm against reagent blank using double beam UV spectrophotometer (shimadzu).

RESULTS AND DISCUSSION

Keeping the total concentration of gum components constant at 70% of the weight of the tablet, five formulations were prepared by changing the proportion of each gum (guar, tragacanth and acacia) using diclofenac sodium as a drug. The *invitro* release of drug determined initially for 2hrs in 0.1n HCl and subsequent six hours in phosphate buffer of pH 7.2 .the results showed that no drug was released during 2hrs time in 0.1N hcl from all five formulations. If three hours is taken as the intestinal transit time, an amount of 12.50, 11.25 and 10.63percent of drug was found to be released at the end of 5h hour from formulation F1, F2 and F3 in which the guargum content is 16, 20 and 30% and tragacanth content is 40, 40 and 30% and the rest of the amount constitute gum acacia. The release from these formulations (F1, F2, F3) were found to be almost same where as the corresponding amounts of drug released in the same period from formulation F4, F5 were found to be 3.35 and 1.50% of the drug. In these formulations the guargum content was 40 and 50% and the tragacanth was 20 and 10 % of the total weight of the tablet. The results indicated that there is a rank order correlation between the degree of drug release retardation and percentage of guar gum. So the results revealed that the slow hydration and gelling of guar gum in acid and alkaline environment has exerted a significant effect on drug release. Further the results showed that an amount of 28. 25, 26.50, 24.70, 12.26 and 6.60 percent of drug was found to be released at the end of 8th hour from all the five formulations (F1, F2, F3, F4 and F5). Insoluble additives talc and magnesium

stearate used in the formulation of matrix tablets, due to an increase in diffusion path way might also have contributed for slow degree of drug. The released data was plotted as drug release Vs time and are shown in figure 1 .The result showed that drug release obeyed 0 order kinetics with release rate constant of 2.78 and 1.18 % /hour respectively for formulation F4, F5 the linearity judged by linear regression coefficient values (0.9977 and 0.9835) for these two formulations .The data was found to fit well in Peppas exponention equation (Q=Ktn) with diffusion coefficient values (M) being 0.6355 and 0.4877 for F4, F5. These values of (N) indicated matrix composition of F4, F5 showed negligible swelling and mechanisum of drug release from these formulation by diffusion.

Characteristics of cecal organisum: The organism isolated from rat's ceacum was found to be gram negative, non-motile, rod shape, and anaerobe, could be grown only in fluid thiglycolate medium immediately after isolation. The organism was highly virulent and ineffective during time of isolation and sub culturing. This tendency of the organism can be attributed by a change to its habitation from rat's ceacum to laboratory media. However, after much generation the organism was found to loss it's virulence and oxygen intolerance and behaved as facultative anaerobe. These results indicate the cecal organism after 10 generations was found to be viable even in saline phosphate buffer in pH 7.4 under anaerobic conditions (bubbled with carbon dioxide). The organism was found to be catalase negative, and sensitive to nitro furantidin. All the three gums are degraded to a quantity of 1gm by the organism when grown in FTM along with gums. The gum degradation has been judged by the clarity of tubes which was found to be comparable with control.

Phases of growth of cecal organisum in FTM with and with out added gums: The phases of growth were determined in FTM and in saline phosphate buffer of ph7.5 (bubbled with carbon dioxide) with acacia and tragacanth (500mg) and compared with continues in both media without added gums. The phases of growth curve with added acacia were also plotted. The results indicated that organism significant increase in its exponential; growth phase in presence of acacia. The ceacal organism has utilized gum acacia as a substrate. Similar results were obtained with tragacanth however; the shape of the phases of growth cure in saline phosphate buffer (ph7.5) gave deviation from normal pattern, but shown viability during the period of experiment (48hrs).The results substantially gave indication that ceacal organism can be used as an indicator organism for *invitro* studies of colon targeted drug delivery systems based on natural gum matrices.

The intact tablets (F4, F5) were found to be biodegraded and utilized when they were transferred to 100ml of saline phosphate buffer (pH7.4) and incubated with organism under anaerobic conditions (bubbled with carbon dioxide) for 24hrs. The remaining drug from in vitro dissolution test under physiological conditions of stomach and environment was accounted in the filtrate by spectrophotometric determination.

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