IN-VIVO CHARACTERIZATION OF HYDROGEL FOR TREATMENT OF CHEMO-RADIO THERAPY INDUCED ORAL MUCOSITIS

P. Jain, R.K. Keservani*, R. Dahima**

School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Bhopal, India-462036 E-mail address-pharmacistdex@gmail.com

*School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Bhopal, India-462036 E-mail address-rajforuonly@gmail.com

**School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Bhopal, India-462036 E-mail address-dahimarashmi@rediffmail.com

For correspondence:
R.K. Keservani
School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Bhopal, India-462036
E-mail address-rajforuonly@gmail.com
Mob: +919893245181

Summary
In the present study, to evaluate the potential effectiveness of topical hydrogel used clinically in buccal cavity, we used a rat model for oral mucositis induced by 5-fluorouracil. Studies have shown that the combination of mild abrasion of the cheek pouch and two doses of 5-fluorouracil (40–80 mg/kg) induces oral mucositis and the reduction of body weight. In a study, the combination of intraperitoneal injections of 5-fluorouracil (40–80 mg/kg) produced a dose-dependent increase in the area of mucositis and a decrease in body weight. This studies shows that the prepared hydrogel is effective in oral mucositis and effective to control oral mucositis. There was significant weight gain was recorded in the L-glutamine hydrogel treated group as compare to control group.

Keywords: Hydrogel, Oral Mucositis, Chemo-Radiotherapy, 5-fluorouracil.

Introduction
Mucositis is a major clinical problem in oncology, caused by the cytotoxic effects of cancer chemotherapy and radiotherapy. Mucositis is a major clinical problem in oncology, caused by the cytotoxic effects of cancer chemotherapy and radiotherapy. Oral mucositis, which is characterized by painful erythematous, erosive and ulcerative lesions of the oral mucosa, is a common complication of many cancer treatments, including myeloablative forms of bone marrow transplant therapy. Between 30% and 69% of patients undergoing bone marrow transplantation experience oral mucositis, and nearly all such patients experience some form of oral complications, including oral mucositis, dysfunction of the salivary glands, infection, dysgeusia, dentinal hypersensitivity and soft-tissue pain.1-3
Hydrogels are of special interest because they have high water contents similar to body tissues and have, in general, high biocompatibility.\textsuperscript{4} Hydrogels have been widely studied for use in medical implants, diagnostics, biosensors, bioreactors and bioseparators and are being used as matrices for drug delivery systems (DDS).\textsuperscript{5-7} For topical oral administration, the conventional formulations like lozenges, troches, gels, oral rinses or mouthwashes would be the simplest dosage forms for delivery of actives components through the mucosa of the oral cavity. However, these conventional dosage forms have the disadvantage of initial burst of activity followed by a rapid decrease in concentration.\textsuperscript{8}

The beginning of modern hydrogel research is considered to be the synthesis of (hydroxyethyl methacrylate) by Wichterle and Lim in 1960.\textsuperscript{9,10} Since then considerable progresses have been made in the synthesis and application of hydrogels. The growth of hydrogel technologies has advanced many fields ranging from food additive to pharmaceuticals to biomedical implants. In addition the development of an ever-increasing spectrum of functional monomers and macromers continue to broaden the versatility of hydrogel applications.

Hydrogels are three dimensional hydrophilic polymer networks capable of swelling in water or biological fluids, and retaining a large amount of fluids in the swollen state.\textsuperscript{11} Their ability to absorb water is due to the presence of hydrophilic groups such as -OH, -CONH- CHN\textsubscript{2}, -COOH, and -SO\textsubscript{3}H\textsubscript{3}. The water content in the hydrogels affect different properties like permeability, mechanical properties, surface properties, and biocompatibility. Hydrogels have similar physical properties as that of living tissue, and this similarity is due to the high water content, soft and rubbery consistency, and low interfacial tension with water or biological fluids.\textsuperscript{12}

Hydrogels are water swollen polymer matrices; with a tendency to imbibe water when placed in aqueous surroundings. This capability to swell, under biological surroundings, makes it an ideal material for use in drug delivery and immobilization of proteins, peptides, and other biological compounds. Due to their high water content, these gels bear a resemblance to natural living tissue more than any other type of synthetic biomaterial.\textsuperscript{13}

Advantages of the buccal absorption route include\textsuperscript{14,15}
- Absence of hepatic first-pass effect.
- Pre-systemic metabolism in the gastrointestinal tract absent.
- Ease of administration.
- Not or minimal painful.
- Buccal mucosa is well vascularized.
- Rapid onset of action.
- No, or little, irritation expected.
- Good Patient compliance.

Materials and Methods

Materials:
Carbopol 934 NF, Methyl paraben, Sodium Glycocholate, Glycerin and other chemicals of standard quality were taken from Loba Chem. India. L-Glutamine was procured from Claris Life sciences ltd. India. 5-fluorouracil Inj. from Getwell Pharma ltd. India.
Method of preparation:
Method was accepted with slight modification as proposed by Silva S. Gutter 2003 weighed amount of polymer Carbopol 934 NF (1 %), methyl paraben (0.1%, preservatives), Sodium Glycocholate (5%, Penetration enhancer), glycerin (5%, hydrating agent) and drug (0.5%, L-Glutamine) were placed in the beaker and L-glutamine was dissolved in 10 ml of water with stirring. The final volume was made up to 100 ml with distilled water.\textsuperscript{16}

\textit{In-Vivo} Animal Study:

Animals:

Experiment was performed with 20 male Sprague-Dwaley rats weighing 180-260 g in total. Rats were raised under pathogen-free condition and fed \textit{ad libitum}. They were housed individually and maintained according to the 12 hr light 12 hr dark schedule. The room temperature kept at normal. The one teaspoonful of the L-glutamine hydrogel was applied to the skin of the left whisker pad using toothbrush as well as being applied to the intraoral region using a cotton swab. The quantity of the gel was enough for applying to the inflammatory lesions. The hydrogel was used at room temperature (23°C approximately). Application of the hydrogel to the left whisker pad and intraoral regions was performed after measurement of the mucositis.\textsuperscript{16-27}

Induction of Experimental Oral Mucositis:

Oral mucositis was induced by two intraperitoneal (i.p) administrations of 5-FU (5-fluorouracil) on the first and second days of the experiment (60 and 40 mg/kg, respectively), according to an experimental oral mucositis model previously described (Table 1). In order to mimic the friction to which the oral mucosa is normally subjected, the animal cheek pouch mucosa was irritated by superficial scratching with the tip of an 18-gauge needle on the fourth day, under anesthesia with chloral hydrate (250 mg/kg, i.p.). The needle was dragged twice in linear fashion across the everted cheek pouch until erythematous changes were noted. Ulcers were assessed every other day immediately prior to the application of drugs.

\textbf{Table 1 Study Protocol of Oral Mucositis Model.}

<table>
<thead>
<tr>
<th>S.No</th>
<th>Day of Trial</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5-FU Injection</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Only on day 4</td>
</tr>
<tr>
<td>2.</td>
<td>Scratching</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Application of</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Every Day</td>
</tr>
<tr>
<td></td>
<td>formulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mucositis Score System:

The mouths of the rats in all groups were observed daily to assess the sign of both mucositis. The extent of mucositis was gauged according to the mucositis score system. Score 0 = normal. Score 0.5 = slightly pink. Score 1 = slightly red. Score 2 = severe reddening. Score 3 = focal desquamation. Score 4 = exudation or crusting covering less than one-half of lip. Score 5 = exudation or crusting covering more than one-half of lip (Figure 1). This score was recorded daily from 2 days before irradiation to 15 days after 5-FU application.
Treatment Group:

The rats were randomly divided into 4 groups (each number = 5). All rats were immobilized by anesthetization with pentobarbital sodium (50 mg/kg intraperitoneal). Treatment of the 4 group of rats before and after irradiation were as follows:

**Group A**: Received no treatment.

**Group B**: Application of the L-Glutamine hydrogel to the left whisker pad 2 days before 5-FU administration and continuous application of the L-glutamine hydrogel to the left whisker pad daily for 15 days after administration of 5-FU.

**Group C**: Continuous application of the L-Glutamine hydrogel to the left whisker pad for 15 days after 5-FU application.

**Group D**: Continuous application of Chlorhexidine gel (1% w/w) to the left whisker pad from 2 days before 5-FU application to 15 days after 5-FU application.

The oral mucositis and weight were assessed daily by using the mucositis score system.
Body-Weight Change:

The changes in the body-weight of the rats in each experimental group are recorded. During a 15-day period, a body-weight gain was seen in rats of the control group, whereas a weight loss was found in the animals given 5-FU orally at a dose of 50 mg/kg once daily. There were virtually changes in the weights of rats administered 5-FU with either Chlorhexidine or L-glutamine.

**Result and Discussion**

In a study, the combination of intraperitoneal injections of 5-fluorouracil (40–80 mg/kg) produced a dose-dependent increase in the area of mucositis and a decrease in body weight.

The findings of mucositis begin in 5 of 5 rats in Group A and 4 of 5 rats in Group B on day 3 after application of 5-FU and scratching shown in Figure 2. It began in 3 of 5 rats in Group C and in 3 of 5 rats in Group D on day 5, 4 respectively (Table 2).

![Figure 2 The Time Course of the Mucositis Score After 5-FU Application and Scratching.](image)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>No. of animal used</th>
<th>No. of animal with induced mucositis</th>
<th>Day of induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

In Group A all-5 rats showed score 1.0 on day 5 and score 5.0 on day 15 of 5-FU application and scratching. In Group A the progression of mucositis score started to increase on day 5 and it progressively increase on days 7 it was 2.0 it goes upto 5.0 on day 15. Some rats in Group A have showed exudation or crusting (Table 3).
Table 3 Severity of Mucositis in Different Group.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Days</th>
<th>Score of Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>1.</td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>6.</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>7.</td>
<td>7</td>
<td>2.0</td>
</tr>
<tr>
<td>8.</td>
<td>9</td>
<td>3.0</td>
</tr>
<tr>
<td>9.</td>
<td>11</td>
<td>4.0</td>
</tr>
<tr>
<td>10.</td>
<td>13</td>
<td>4.0</td>
</tr>
<tr>
<td>11.</td>
<td>15</td>
<td>5.0</td>
</tr>
</tbody>
</table>

The time course of mucositis score in Group B started on day 5 of mucositis induction and group B has shown different mucositis progression after 5-FU application and scratching the mucositis score was 2 on day 15 but the progression was not effective as control group.

The way of mucositis score in Group C and D started on day 5 as 1.0 and 0.5 in almost all the animals the there is no significant difference in the mucositis score in Group C and D. As both group showed same progression but the weight change is less in Group C as compare to Group D. The increase in the rates of the mucositis score of group A and group D were significantly higher then those of Group B and C.

During a 15-day period, a body-weight loss with 5-FU was found in-group A (Table 4), the weight loss was significant in control group. The weight gain was significant in case of group B, C, D which was treated with L-glutamine hydrogel (Group B, C) and Chlorhexidine gel (D) respectively (Figure 3). There were more tendencies towards an increase in weight gain in Group B as compare to Group C (Table 5).

Table 4 Weight Changes in Rats Before and After the Treatment.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>No of Animal</th>
<th>Body Weight Before 5-FU Application</th>
<th>Body Weight After 5-FU Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>5</td>
<td>225.2± 6.7</td>
<td>162.7± 8.2</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>5</td>
<td>229.3± 7.2</td>
<td>248.2± 6.7</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>5</td>
<td>238.9± 6.9</td>
<td>254.3± 8.2</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>5</td>
<td>212.2± 4.7</td>
<td>226.5± 7.8</td>
</tr>
</tbody>
</table>
The probable mechanisms of oral mucositis induced by chemotherapy involve complex biological events mediated by a number of inflammatory cytokines, the direct effect of chemotherapeutic drugs or irradiation on the basal epithelium and connective tissue, and the oral microbial environment. Ulcerative mucositis results in the destruction of the oral mucosa as an anatomic barrier. The mouth thus becomes a portal of entry for enteric bacterial, viral and fungal organisms. Thus, ulceration of the oral mucosa results in an increased risk of infection, particularly when there is immunosuppression.

Mucositis viewed solely as an epithelium mediated event, which was the result of the nonspecific toxic effects of radiation or chemotherapy on dividing stem cells. It was believed that direct damage by chemotherapy or radiation therapy to the basal epithelial cell layer led to loss of the renewal capacity of the epithelium, resulting in clonogenic cell death, atrophy, and consequent ulceration.
New research has suggested that mucositis is not just an epithelial process but involves all the tissues of the mucosa, as evidenced by recent data involving morphologic findings, proinflammatory cytokines, platelet aggregation, endothelial and connective tissue injury, and tissue apoptosis.

This finding could be related to the mechanism of cytotoxicity of the chemotherapy agent used in this study. 5-FU is a competitive inhibitor of thymidylate synthetase with consequent thymidine deficiency resulting in inhibition of deoxyribonucleic acid (DNA) synthesis. In addition, incorporation into ribonucleic acid (RNA) interferes with processing and function of RNA, and has been associated with toxicity. In view of this, despite the fact that glutamine is essential for cell proliferation, being an important metabolic substrate for rapidly replicating cells. Different factors may be taken into account to explain the benefits of exogenous glutamine in hastening oral mucosa healing. First, it has been demonstrated that glutamine can activate ornithine decarboxylase, a first and rate-limiting enzyme in polyamine synthesis in a dose- and time dependent manner, thereby enhancing DNA synthesis. In addition, glutamine can activate mitotic signaling pathways, including mitogen-activated protein kinases and transcription factors, leading to proliferative responses. Second, previous studies have suggested that glutamine augments host defenses and may be important in glutathione synthesis thus decreasing the oxidative stress. Accordingly, our data demonstrated that the administration of glutamine increased the mucosal tissue glutathione stores in 5-FU treated rat. It has been demonstrated that glutamine becomes essential during metabolic stress to restore tissue glutathione levels, which have become depleted. Glutathione, the major intracellular antioxidant, is involved in several fundamental biological functions, including free radical scavenging, detoxification of xenobiotics and carcinogens, redox reactions, and biosynthesis of DNA and proteins, being essential to normal cell function and replication. In addition, glutathione has an inhibitory effect on a number of cytokines. Thus, our result suggests that glutamine bioavailability by exogenous administration, restored glutathione level thereby enhancing cell protection, as well as regulating cell proliferation after exposure to 5-FU that generate toxic quantities of free radicals. This in vivo study shows that, there were more weight gain recorded in-group receiving L-glutamine as compare to control and Chlorhexidine treated group. Mucositis was less severe in the L-glutamine treated group as compare to control and Chlorhexidine receiving group. This result indicated that the formulation can be used to treat or prevent chemotherapy induced oral mucositis.

Acknowledgement

Author are thankful to guide Dr. R. Dahima School of Pharmaceutical Sciences, Rajiv Gandhi technological University, Bhopal, M.P., India. for her valuable support and encouragement during the entire research work.

References


