Bioadhesive Delivery of Metformin Using Prosopis Gum with Antidiabetic Potential

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The antidiabetic properties of prosopis gum alone and as a bioadhesive base for the delivery of metformin are presented. The bioadhesive value of the gum was commensurate with those of Carbopol 974-P and sodium carboxymethyl cellulose (NaCMC). The release of the drug was higher from prosopis gum based bioadhesive formulations than from NaCMC and Carbopol 974-P products. This was shown by the shorter time required to reach t_{50} (the time required for 50% of the drug to be released) or t_{20} (time required for 20% of the drug to be released) for the release of metformin. The gum showed moderate antidiabetic properties when used alone. In combination with metformin in a bioadhesive form, the glucose lowering effect was found to be synergistic. The areas under the plasma drug concentration vs. time curves (AUCs) for the bioadhesive combinations were similar to those of the drugs alone in an aqueous system. This shows that the gum did not interfere with absorption of the incorporated drug. However, the areas under the effect vs. time curves (AUECs) were much higher when combined in a bioadhesive form than with the drug alone. The AUECs obtained with NaCMC based bioadhesive formulations were relatively smaller than those of metformin in an aqueous system and the combinations of metformin and prosopis gum.

Key words bioadhesive delivery; metformin; prosopis; antidiabetic potential

Bioadhesive delivery of drugs has recently gained some prominence in recent times as a means of drug administration. Many of the substances used in bioadhesive drug delivery formulations are polymers, and several are polysaccharides. The plant sources of these polysaccharides are known to possess antidiabetic properties and have been used by indigenous peoples for such purposes. It is widely known that many polysaccharides possess antidiabetic properties. It has been shown that materials that contain soluble fibers slow down gastric emptying time, which allows for a gradual increase in glucose levels. This may be associated with the action of these polysaccharides.

Prosopis gum is a xylogalactan extracted from the seeds of Prosopis africana (Fam. Mimosaceae) whose major monosaccharides components are xylose and galactose. It also contains fructose and glucose to a lesser degree. The gum from the plant has been evaluated for different purposes. The seeds from which the gum is extracted are fermented and used for cooking in many African homes even with the advent of semi-synthetic taste enhancers. The plant from which the seeds are obtained has been classified as one of the most valuable agroforestry tree species in Africa. The gum from the seeds is used in the present study as a bioadhesive agent for the delivery of metformin.

Metformin is an antidiabetic drug that has been widely used in the treatment of type 2 diabetes. It belongs to the class of drugs known as the biguanides, and is thought to perhaps have some antiaging effects. It acts by decreasing hepatic glucose production and hepatic glucose output by reducing glycogenolysis and gluconeogenesis. It does not act through insulin and is effective after total pancreatectomy in animals. It increases insulin binding to insulin receptors and increases the sensitivity of the body to insulin. Metformin also slows glucose absorption from the gastrointestinal tract. This action is similar to those of gelling polysaccharides and this is the rational for using the two products in a bioadhesive formulation. The pharmacodynamic and pharmacokinetic properties of the bioadhesive dosage form are presented.

MATERIALS AND METHODS

Materials The following materials were obtained from commercial sources were used. Acetone (Riedel de Haen), Carbopol 974-P (CBC Co., Ltd.), sodium metabisulphite (BDH, Chemicals), acetonitrile (Cica-Reagent), dichloromethane (Nacalai Tesque), and metformin (Sigma). Prosopis gum was obtained from a batch processed in our laboratory as described below. Distilled water was prepared from an-all steel still (model AutostillWS23, Yamamoto), while deionised water was obtained using an Aquarius deioniser (model GS-500, Advantec). Sodium carboxymethyl cellulose (NaCMC), hydrochloric acid, a glucose test kit, streptozotocin, and anaesthetic ether were obtained from Wako Pure Chemicals (Osaka, Japan). Other reagents were prepared according to standard procedures or the manufacturers’ instructions.

Animals Male Wistar rats aged two months with a mean weight of 400 g were purchased from Nippon SLC Co., Ltd. (Hamamatsu, Japan) and fed a standard diet (LabDiets®) made by the same company. All of the rats were kept in standard and conditioned animal houses at Kyoto Pharmaceutical University. After purchase, the rats were allowed to aclimatize to the new laboratory conditions for one week before use. All experiments were conducted according to the ethical standards stipulated in guidelines (No 141, 1987) issued by the Science and Internal Affairs Bureau of the Japanese Ministry of Education, Science, Sports, and Culture.

Methods. Preparation of Prosopis Gum The prosopis seeds were purchased from a local market in Nigeria and washed with water. They were further soaked in water for 24 h and cooked for 4 h using glass containers to prevent the darkening that takes place in metallic containers. The swollen tegmen were collected manually and soaked in glass in an aqueous solution of 0.1% w/v sodium metabisulphite
for 24 h. At the end of this period, the material was homogenised using a Silverson homogenizer. The highly viscous material obtained was passed through a muslin cloth to remove any gritty particles and the filtrate was precipitated with twice the volume of acetone. The resultant woolly precipitate was collected on a Buchner funnel by means of suction using a vacuum pump. The material was dried in a vacuum oven (to prevent auto-oxidation) for 24 h and pulverized using an end-runner mill. This method of gum preparation ensures the reproducibility of the characteristics of this gum. The powder samples were stored in tightly closed containers until used.

**pH Measurements** The pH measurements were carried out on the gum dispersions alone as well as in the presence of the drug. A dispersion of the gum (1% w/v) was used for the study. Measurements of pH were done with a Horiba pH meter (model M-11) using calomel electrodes (model S003). The mean of three determinations was calculated.

**Bioadhesive Studies** Bioadhesive studies were conducted on the compressed discs of the gums. The discs (14 mm in diameter and 0.72 mm thick) were prepared using a Shimadzu hand press (Model SS-P10A) at 0.2 ton. The tablets were glued onto the lower platform of the equipment for mucoadhesive determination (model FGN, Shimpo). An excised rat duodenum measuring 2 cm was attached to the arm of the equipment by means of glue. The mucosa was gently brought into contact with the tablet and adhesion was allowed to take place for 1 h through mucosal hydration of the gum material. At the end of 1 h, the mucosa was gently detached from the tablet disc and the force was directly recorded on a meter calibrated in Newtons. The mean of three determinations was obtained.

**Gum Swelling Studies** Discs of the gums were compressed at 0.2 ton using a hand press (model SS-P10A, Shimadzu). Each disc was 1.5 mm thick and 14 mm in diameter. The initial weight of the disc was recorded and the disc was placed in 500 ml of water and allowed to swell. At regular time intervals, the swollen disc was carefully removed, blotted dry, and the weight gain was recorded. Water sorption was calculated from the difference between the initial weight and the weight at the time of determination. The experiment was repeated three times and the mean was calculated.

**In Vitro Release Studies** Metformin release from the compressed discs (1.5 mm thick and 14 mm in diameter) was assessed in a dissolution apparatus (model NTR-6100, Toyama) according to the method outlined in the Japanese Pharmacopoeia. A 900 ml volume of 0.1 N HCl was used as the dissolution medium. The dissolution medium was maintained at a temperature of 37±0.1 °C throughout the dissolution period. At regular time intervals, a 5 ml aliquot of the dissolution medium was withdrawn and assayed spectrophotometrically at 240 nm using a spectrophotometer (model UV-Visible 1600, Shimadzu). Each amount of dissolution fluid withdrawn was immediately replaced with an equivalent amount of dissolution fluid. The concentration of metformin released during each period was determined from a Beers plot which was previously determined for the drug.

**Induction of Diabetes** Experimental diabetes was induced in the rats using streptozotocin (60 mg/kg). Streptozotocin was dissolved in 0.5 ml of citrate buffer (pH 4.5) and injected intraperitoneally into rats that had been fasted for 18 h but with access to water ad libitum. The extent of diabetic induction was monitored and based on blood glucose levels and weight decrease. Blood glucose levels of up to 500 mg/dl were accepted as the basal level for diabetes. This blood glucose level was achieved after 4 d of treatment with streptozotocin.

**Pharmacodynamic Evaluation** Semi-solid aqueous dispersions of the gum alone or metformin/gum admixtures were prepared at different concentrations and allowed to equilibrate for 12 h. The dispersions were administered to the rats using a gastric tube. At regular time intervals of 1 h, 0.5 ml of blood was sampled from the jugular vein of the rats, centrifuged at 12000 rpm for 70 s, and the serum was collected and kept frozen until analyzed for glucose content using a Glucose-B test kit (Wako Pure Chemicals Industries, Osaka). The amount of drug administered was maintained at two dose levels, 200 and 400 mg/kg body weight, while the gum was maintained at a single dose level of 100 mg/kg body weight. Above this concentration, the gums formed a gel too firm to be administered by gastric intubation. The negative control was normal saline administered to the rats in a similar manner. Free drug at the same concentrations as in the mucoadhesive formulations was used for the positive control. The standard chosen for mucoadhesive comparison was NaCMC. The mean for four rats was calculated.

**Determination of Pharmacokinetic Parameters** Diabetic male Wistar rats were used for this study. Diabetes was achieved in the rats using the method described earlier. After fasting for 18 h, the drug or its formulations were administered to the rats under light ether anaesthesia using a gastric tube. At the predetermined times, 0.5 ml of blood was sampled from the rats after light anaesthesia with ether and centrifuged at 12000 rpm for 5 min. The plasma was collected and analyzed for drug content using a modified method of Chen and Chou. The plasma was collected and kept frozen prior to analysis.

**Extraction and Analysis of Metformin from Rat Plasma** The method of Chen and Chou was slightly modified and applied to the extraction and analysis of the plasma samples. A 0.2 ml aliquot of plasma was acidified with 20 μl of 1 N HCl and then treated with 0.6 ml of acetonitrile. A 100 μl volume of water was added to increase bulk, and then mixture vortexed for 30 s, and centrifuged at 12000 rpm for 5 min to remove the precipitated protein. The resulting supernatant was transferred into a fresh tube washed with 0.6 ml of dichloromethane, vortexed for 30 s, and centrifuged at 12000 rpm for another 5 min. A 50 μl quantity of the upper aqueous layer was injected into an HPLC system (model AS-8020) equipped with a printer (model 5340 HE, Oki Microline). An HPLC system (model AS-8020) equipped with a printer (model 5340 HE, Oki Microline) was used. A Chemcosorb 5Si column (250 mm×4.6 mm 1.D) was used. The mobile phase was a mixture of acetonitrile and 0.03 × 3 mm diammonium phosphate (1:3) (pH 7.0) and was passed through the column at a flow rate of 1.5 ml/min and a constant temperature of 50°C. Ultraviolet detection was done at 236 nm using a Shimadzu detector (model SPD-10A). All concentrations were extrapolated from a Beer’s plot of metformin obtained from spiked plasma samples.

**Data Analysis** The pharmacokinetic and pharmacodynamic parameters were determined using the program Win-HARMONY. The areas under the plasma concentration vs.
time curves (AUC) as well as the areas under the effect vs.
time curves were determined using the trapezoidal rule based
on a noncompartmental pharmacokinetic analysis.

**Statistical Analysis**  The Student’s *t*-test was used to test
the results at the 5% level of significance to see if there were
significant differences between the parameters obtained from
bioadhesive formulations and those of the control experi-
ments. The AUEC of the bioadhesive prosopis preparations
and the AUECs of NaCMC as well as those of the aqueous
preparations were compared. Similarly, the AUCs of the
bioadhesive prosopis preparations were compared with those
of the NaCMC and aqueous preparations.

**RESULTS AND DISCUSSION**

The weight and serum glucose level changes in the rats
treated with 60 mg/kg body weight of streptozotocin were
significant. This is a good way of assessing the extent of dia-
abetes induction in the animals and this approach has been de-
scribed by other workers.16,17) There was an increase in the
serum glucose levels of over 500 mg per dl from an initial
value of 177 mg/dl. This represents more than a 182% in-
crease in the serum glucose level of the rats. The weight of
rats decreased from a mean value of 430 to 405 g.

Table 1 shows the pH values of the various preparations.
The pH determination indicated that metformin solution
alone had a pH value of 6.9, while the pH value was slightly
modified to 6.65 and 6.48 in the presence of prosopis gum
and NaCMC, respectively. However, in the presence of Car-
bopol 974-P, the pH changed significantly to 3.6 with a resul-
tant precipitation of the drug from solution. This is a case of
drug-excipient interaction. It is, therefore, not advisable to
use Carbopol 974-P in the formulation of metformin without
adequate pH control.

Table 2 shows the values of the level of adhesion to rat
gastric mucosa. The bioadhesive studies revealed that
prosopis gum has adhesion values comparable to those of
Carbopol 974-P and NaCMC. Similar results were obtained
for the gum, when adhesion to glass beads was used as the
index of adhesion.8) The gum rapidly swells in the presence
of water forming a gelatinous mass.

The water sorption profiles of the gums are shown in Fig.
1. It can be seen that the initial water uptake was the highest
for prosopis compacts. The rate of water sorption affects the
rate of gelation of a gum and hence the rate of release of any
incorporated drug. This is because the amount of water that
penetrates into any compact and the rate of this penetration
are critical for the dissolution of the incorporated drug.
The dissolved drug, then, diffuses outwards into the sink solution.
The level of dissolution is further affected by the solubility of
the drug. From this study, it is clear that the three gums em-
ployed here all have high water sorption capacity and are
suitable for the bioadhesive delivery of water-soluble drugs.

Figure 2 shows the release of metformin from the com-
pacts. The highest release was achieved from prosopis based
compacts. A very low amount of the drug was released from
Carbopol 974-P based compacts. This may be associated with
the incompatibility noticed earlier for the drug and this
adhesive material. In the presence of Carbopol 974-P, met-
formin decreased in solubility and precipitated out of the so-
lution. The *t*50 for the release of metformin was achieved in

![Fig. 1. Water Sorption by the Bioadhesive Materials](image1)

![Fig. 2. Release of Metformin from Mucoadhesive Substances](image2)

<table>
<thead>
<tr>
<th>Material</th>
<th>Bioadhesive value (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosopis</td>
<td>1.24±0.02</td>
</tr>
<tr>
<td>Carbopol-974P</td>
<td>1.21±0.02</td>
</tr>
<tr>
<td>NaCMC</td>
<td>1.14±0.03</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. (*n*=4).

<table>
<thead>
<tr>
<th>Material, Aqueous system, pH</th>
<th>Aqueous system, pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>6.90±0.17</td>
</tr>
<tr>
<td>Carbopol-974P</td>
<td>3.40±0.00</td>
</tr>
<tr>
<td>Carbopol/metformin</td>
<td>3.62±0.03</td>
</tr>
<tr>
<td>Detarium</td>
<td>5.95±0.05</td>
</tr>
<tr>
<td>Detarium/metformin</td>
<td>6.38±0.03</td>
</tr>
<tr>
<td>NaCMC</td>
<td>6.65±0.05</td>
</tr>
<tr>
<td>NaCMC/metformin</td>
<td>6.48±0.09</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. (*n*=4).
parameters at a glance, the macodynamic and pharmacokinetic parameters are presented.

Gum and drug-gum dispersion systems on the blood glucose aqueous solution (200 mg/kg);

Prosopis (100 mg/kg);

Fig. 3. Serum Glucose Level of Rats Given Different Formulations of Metformin

TABLE 3. Pharmacodynamic Parameters for Prosopis Gum and Its Admixtures with Metformin in a Bioadhesive Delivery Form

<table>
<thead>
<tr>
<th>Preparation</th>
<th>AUEC (% h)</th>
<th>AUC (mg h/ml)</th>
<th>% Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosopis gum alone (100 mg/kg)</td>
<td>390.5 ± 4.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Metformin (200 mg/kg)/Prosopis (100 mg/kg)</td>
<td>513.2 ± 7.6</td>
<td>126.8 ± 4.7</td>
<td>63.4 ± 2.4</td>
</tr>
<tr>
<td>Metformin (400 mg/kg)/Prosopis (100 mg/kg)</td>
<td>774.0 ± 17.7</td>
<td>142.6 ± 60.2</td>
<td>35.7 ± 15.0</td>
</tr>
<tr>
<td>Metformin alone (200 mg/kg)</td>
<td>305.2 ± 9.5</td>
<td>125.6 ± 28.3</td>
<td>62.8 ± 14.2</td>
</tr>
<tr>
<td>Metformin alone (400 mg/kg)</td>
<td>403.9 ± 22.4</td>
<td>135.6 ± 31.0</td>
<td>33.9 ± 7.7</td>
</tr>
<tr>
<td>Metformin (200 mg/kg)/NaCMC (100 mg/kg)</td>
<td>289.2 ± 5.8</td>
<td>59.9 ± 10.6</td>
<td>30.0 ± 5.3</td>
</tr>
<tr>
<td>Metformin (400 mg/kg)/NaCMC (100 mg/kg)</td>
<td>407.8 ± 9.3</td>
<td>80.4 ± 4.2</td>
<td>20.1 ± 1.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 4).

and 40 min for Carbopol 974-P based compacts, respectively. Adsorption studies showed there was no physical interaction between metformin and NaCMC. Thus, the prolonged and low release rates are not due to adsorption onto the gum but may be suggestive of another interaction mechanism. The long period obtained with NaCMC based compacts led to the choice of using the drug/dispersion of the gums in the study of bioactivity as well as the bioavailability. It should be noted that the faster release rate for the drug from prosopis based compacts is an index of faster penetration of water into the system.

Bioeffect and Bioavailability Studies The effects of the gum and drug-gum dispersion systems on the blood glucose levels of the male Wistar rats are shown in Fig. 3. The pharmacodynamic and pharmacokinetic parameters are presented in Table 3. To illustrate the bioeffect and the bioavailability parameters at a glance, the AUEC, the AUC, and the percent bioavailability are all shown together. From Table 3 it is clear that prosopis gum showed some antidiabetic effect both in the presence and absence of the drugs. The effect was very prolonged when combined with metformin and the serum glucose level had not recovered even after 10 h as shown in Fig. 3. For all of the other agents studied, including metformin aqueous suspension, it is evident that only the bioadhesive formulation with prosopis gum showed an abnormally long blood glucose suppressing activity. This cannot be said to be dependent on the amount available to the systemic circulation alone, since the bioadhesive preparation and the aqueous solution of the drug were almost bioequivalent. It is obvious from the AUEC values that the bioadhesive preparations with prosopis gum were most effective in lowering plasma glucose levels. From Fig. 4 the tmax of the prosopis gum preparation seems high. This is evident from the in vitro release study that resulted in a large amount of drug being released. Thus, there was a correlation between the in vitro release and in vivo availability. In terms of biological availability, the lower dose level of the drug in the bioadhesive preparation was relatively more available than the higher dose level. This was the trend in all the preparations, including the aqueous system containing the drug. This is interesting, as there is a key point that there is a critical value above which the drug is not more bioavailable than the lower doses. There is also a key point in the AUEC values that doubling the dose does not necessarily double the effect. When compared to NaCMC, prosopis gum has greater advantage as a bioadhesive agent in the formulation of metformin as both the bioavailability and biological effects were higher in prosopis gum based formulations than in NaCMC formulations. However, the biological effects in the NaCMC based formulations were significant, when compared to the amount of the drug that was bioavailable. It has been observed that metformin has two points of action in effecting its antidiabetic properties. The first is a presystemic effect in the gastrointestinal tract (GIT), while the second is the systemic effect. Thus, any substance that can delay the effect of the drug will also lead to the presystemic action in the stomach and will achieve effective lowering of blood glucose levels, although the systemic availability may be low. This justifies the effect of NaCMC based preparations in lowering the blood glucose level, although the amount of the drug that reached the systemic effect from its preparations was generally low. For the prosopis based preparations, the mechanism of action is not very clear and is obviously different, since
there seemed to be no delayed absorption when compared to the aqueous preparations. It is generally, however, believed that polysaccharides lower blood glucose levels because of their viscous nature.\(^5\) Gelling undigestible polysaccharides can readily prevent the mobilization of digestive enzymes as well as prevent the absorption through the gastrointestinal wall by coating its surface.

**CONCLUSIONS**

It has been shown in this study that prosopis gum possesses antidiabetic properties alone and resulted in synergistic effects when used in combination with metformin in a bioadhesive formulation. These results are significant because of herbal–drug interactions that may result from such combinations or even food–drug interactions that may result when the drug is taken along with food containing prosopis gum material. The gum should be harnessed for its antidiabetic properties or used to formulate metformin at preferably reduced doses. Such a fixed ratio combination will result in toxicity that may result from the drug when used alone, since the reduced dose of the drug may eliminate some of its side effects that are dose-dependent. One major disadvantage of using biguanides is lactic acidosis. This may be drastically reduced at low dosage levels in combination with prosopis gum.

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**REFERENCES**