The effect of feeding xanthan gum on colonic function in man: correlation with \textit{in vitro} determinants of bacterial breakdown

BY J. DALY, J. TOMLIN AND N. W. READ

Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AU

(Received 12 November 1991 – Accepted 4 June 1992)

Xanthan gum (15 g/d) was given for 10 d to eighteen normal volunteers. \textit{In vivo} measurements of stool output, transit time, frequency of defaecation and flatulence were compared with a preceding control period of 10 d. At the end of the control and test periods fresh faecal homogenate from each subject was anaerobically incubated with xanthan gum and control solutions to assess the ability of the bacteria to break down the gum. Xanthan gum was found to be a highly efficient laxative agent causing significant increases in stool output ($P < 0.01$), frequency of defaecation ($P < 0.05$) and flatulence ($P < 0.01$) whilst having variable effects on transit time. Before feeding xanthan gum, faecal samples from twelve of the eighteen subjects could reduce the viscosity of the gum \textit{in vitro}. This rose to sixteen of the eighteen with significantly greater amounts ($P < 0.05$) of hydrogen and short-chain fatty acids also being produced, indicating bacterial adaptation in the presence of the substrate. Correlations between the \textit{in vivo} and \textit{in vitro} findings did not substantiate claims that the \textit{in vivo} effect of a given polysaccharide can be predicted from its fermentation characteristics \textit{in vitro}.

**Polysaccharide:** Fermentation: Colonic function

Complex non-starch polysaccharides are widely used as laxative agents, but they differ in their potency. It would seem logical that these differences might be related to the ability of the polysaccharide matrix to retain water (McConnell \textit{et al.} 1974). This is not the case; in fact some studies have suggested an inverse relationship between water-holding capacity and stool output (Stephen & Cummings, 1979). The explanation for this apparent paradox lies in the fact that it is the water-holding capacity after colonic fermentation that is more directly related to the effect of a polysaccharide on stool output, and many of the polysaccharides with the highest water-holding capacities are also rapidly fermented. Polysaccharides such as guar gum that are readily broken down by colonic bacteria have only a small effect on stool output (Cummings \textit{et al.} 1978), while isphagula which resists bacterial degradation is a much better laxative (Prynne & Southgate, 1979). In a previous study (Tomlin & Read, 1988), in which the effects of several different soluble polysaccharides were investigated, it was found that retention of some viscosity was associated with increase in stool weight, while reduction in viscosity and evidence of fermentation was associated with reductions in transit time. Our results suggested that the best natural laxatives were only partially broken down thereby increasing stool weight and reducing transit time.

Xanthan gum has a coiled backbone and tightly packed side groups. The tight packing would tend to resist bacterial penetration and could account for its relatively poor fermentation by colonic bacteria (Osilesi \textit{et al.} 1985). Thus, the maintenance of the polysaccharide structure with retention of the water incorporated in the matrix would
predict that this complex polysaccharide would make a good laxative (Adiotomre et al. 1990). The aim of these studies was to test this prediction by investigating the action of xanthan gum on stool output, colonic transit time and flatulence in eighteen healthy volunteers. The results were compared with the results of in vitro incubation of xanthan gum with colonic bacteria obtained from the subjects’ own faeces. Incubations were carried out at the beginning and at the end of each treatment period to determine whether the bacterial populations were able to adapt to break down the polysaccharide.

**MATERIALS AND METHODS**

**Subjects**
These were eighteen healthy male volunteers who were aged between 19 and 34 years and had a normal weight-for-height, none of whom had taken any antibiotics in the previous 6 months. All volunteers gave written informed consent for the study to be carried out and the protocol was approved by the local Ethical Committee (Sheffield Area, Trent Regional Health Authority).

**The test article**
Xanthan gum (Keltrol T; Kelco International Ltd, San Diego, CA, USA) was supplied to each subject in 5 g individual portions to be taken three times daily with meals. In order to maximize the palatability of the xanthan gum and to simulate its mode of ingestion in foodstuffs the preweighed 5 g portions were stirred into 150 ml boiling water in which three cubes of jelly (Rowntrees Ltd, York) had previously been dissolved. This mixture was made up in 150 ml plastic pots fitted with lids and then placed in refrigeration for 24 h to give a palatable, fully hydrated, flavoured substance with a thick jelly-like consistency.

**In vivo experiments**
The study consisted of two 10 d periods separated by a 3 d rest period. The first 10 d acted as the control period for the xanthan-feeding period. The protocol was identical for both periods except that in the control period three cubes of jelly and 150 ml water replaced the xanthan gum mixture.

On the 3 d preceding each study period and on each day of the control and test periods subjects ingested fifteen small radio-opaque markers and kept a diary in which times of meals, exercise, defaecation and episodes of flatulence (passage of wind per rectum) were recorded. Subjects were instructed to maintain similar dietary and exercise patterns throughout both study periods. Ease of defaecation was measured on a visual analogue scale and stool consistency was rated compared with a set of standard photographs (Davies et al. 1986).

All stools passed in the study period were collected in order to calculate stool weight and gastrointestinal transit time using the continuous-marker method (Cummings et al. 1976). On day 10 of each study period subjects provided a fresh faecal sample. Three 10 g portions were freeze-dried to calculate the percentage water of the stool, the remainder of which was used in the in vitro experiments.

**In vitro experiments**
Three cultures were set up containing: (a) 15 ml faecal homogenate (5 g wet weight/100 ml sterile medium) with 15 ml of a solution of xanthan gum in medium (2.5 g/l), (b) 15 ml faecal homogenate in 15 ml medium, (c) 15 ml xanthan gum solution with 15 ml medium.

The medium consisted of NaCl (60 mM), NaHCO₃ (40 mM), KCl (10 mM), and tryptone (10 g/l) which were dissolved in distilled water, then autoclaved at 103 mPa for 20 min.
Hemin (500 mg/l) was dissolved in NaOH solution (100 g/l) then filter-sterilized and added to the culture.

The mixtures were poured into 50 ml stoppered flasks with side-arms and were made anaerobic by bubbling with CO₂ whilst the head-spaces of the flasks were flushed with N₂. The cultures were then incubated anaerobically for 24 h at 37°.

Samples (5 ml) were removed from the cultures immediately after they were mixed and immediately after they were removed from incubation. pH was measured by an electrode (Model 10; Corning Eel, Medfield, MA, USA), viscosity was measured using an Ostwald viscometer and the sample was then frozen at −70° and later analysed for short-chain fatty acids (SCFA) using GLC. The frozen samples were thawed to 4°C, then centrifuged for 20 min at 400 g, frozen again and thawed at 4°C just before analysis. A Varian model 3700 gas chromatograph with a flame-ionization detector (270°) was used to analyse the samples. The column was packed with 10% AT-1200 and 1% H₃PO₄ on Chromasorb WAW 80-100 mesh (column 121°; injection temperature 200°). The carrier gas was pure N₂ (30 ml/min), and the flame gases were H₂ (30 ml/min) and air (300 ml/min). The equipment was calibrated with a standard mixture of 10 mM-SCFA (C₁-C₆); (Supelco, Bellefonte, PA, USA), and both standard and sample were diluted with H₂SO₄ (200 ml/l; 5:1, v/v) before injection of 30 µl into the column. The output was displayed on a chart recorder and peaks measured to yield concentrations of each SCFA.

Gases produced during the incubation were collected in metallized gas bags (Analysis Automation, Oxford) which were connected to the small gas space above the incubation mixture via a three-way tap connected to the side arm of the flask.

Statistical analysis
Results were analysed statistically using the Student’s paired t test and associations between the in vivo and in vitro data examined using the Chi square test and Linear regression analysis.

RESULTS

In vivo results
Stool output. Ingestion of the xanthan gum for 10 d caused a significant increase in stool weight (Table 1) that was composed of significant increases in dry weight as well as stool water, although the percentage of stool water was unchanged. This result was observed in all but three subjects. Stool frequency was also increased as was the average weight of each stool (Table 1).

Transit time
Ingestion of xanthan gum did not cause any significant change in transit time. Nine subjects had increased transit time while nine had decreased values. The net effect of this was a reduction in the spread of results so that subjects with the slowest transit times showed an acceleration with xanthan gum, while those with short transit times showed a delay.

Flatulent episodes
Xanthan gum caused a highly significant increase in flatulence in all but one subject (P < 0.001).

In vitro results
Incubation of xanthan gum with faecal bacteria from each of the subjects at the end of the control period caused a reduction in viscosity in samples from twelve of the eighteen subjects but viscosity was removed in only seven of these (Table 2). Incubation with
Table 1. *The effect of ingestion of xanthan gum by healthy male subjects* on the in vivo variables measured

(Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Treatment period...</th>
<th>Control</th>
<th>Xanthan</th>
<th>Statistical significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (h)</td>
<td>Mean (h)</td>
<td>Mean (g/d)</td>
</tr>
<tr>
<td>Transit time</td>
<td>46.7</td>
<td>40.7</td>
<td>NS</td>
</tr>
<tr>
<td>Stool wt</td>
<td>190.3</td>
<td>242.8</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Stool water</td>
<td>127.4</td>
<td>175.9</td>
<td>P &lt; 0.008</td>
</tr>
<tr>
<td>Stool dry wt</td>
<td>53.2</td>
<td>63.4</td>
<td>P &lt; 0.035</td>
</tr>
<tr>
<td>Stool wt (g/stool)</td>
<td>169.5</td>
<td>195.8</td>
<td>P &lt; 0.049</td>
</tr>
<tr>
<td>Water in stool (%)</td>
<td>71.3</td>
<td>72.6</td>
<td>NS</td>
</tr>
<tr>
<td>Stool frequency (/d)</td>
<td>1.14</td>
<td>1.31</td>
<td>P &lt; 0.035</td>
</tr>
<tr>
<td>Flatulent episodes</td>
<td>9.6</td>
<td>13.9</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

NS, not significant.

* For details of procedures, see p. 898.

Table 2. *The effect of feeding xanthan gum to healthy male subjects on properties of faeces incubated in vitro*†

(Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Incubation...</th>
<th>Xanthan gum--medium</th>
<th>Xanthan gum--faeces</th>
<th>Faeces--medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Before feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final viscosity (mPa.s)</td>
<td>27.4</td>
<td>17.1</td>
<td>16.4</td>
</tr>
<tr>
<td>Final pH</td>
<td>6.83</td>
<td>0.01</td>
<td>6.74</td>
</tr>
<tr>
<td>H₂ production (µl)</td>
<td>5.4</td>
<td>0.03</td>
<td>31.2</td>
</tr>
<tr>
<td>SCFA production (mmol/kg stool)</td>
<td>0</td>
<td>0</td>
<td>2632</td>
</tr>
<tr>
<td>After feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final viscosity (mPa.s)</td>
<td>35.8</td>
<td>17.0</td>
<td>17.1</td>
</tr>
<tr>
<td>Final pH</td>
<td>6.86</td>
<td>0.03</td>
<td>6.77</td>
</tr>
<tr>
<td>H₂ production (µl)</td>
<td>1.83</td>
<td>1.2</td>
<td>102.5</td>
</tr>
<tr>
<td>SCFA production (mmol/kg stool)</td>
<td>0</td>
<td>0</td>
<td>2656</td>
</tr>
</tbody>
</table>

SCFA, short-chain fatty acids.

Mean values were significantly different from those for xanthan gum--medium: *P < 0.05.

Mean values were significantly different from those for faeces--medium: †P < 0.05.

† For details of procedures, see pp. 898-899.

xanthan gum also reduced the pH of the culture, increased the production of SCFA and produced H₂, but these changes were not significantly different from those seen when the faeces were incubated with the same volume of medium.

After 10 d of feeding the xanthan gum the findings showed that the faecal bacteria had increased their ability to ferment xanthan gum (see Table 2). Samples from all except two of the subjects reduced the viscosity of the xanthan gum and eleven samples removed the viscosity completely. The average reduction in viscosity was greater than that before xanthan gum was fed (50.8 mPa.s v. 24.1 mPa.s; *P = 0.045) and the amounts of H₂ and SCFA produced were now significantly greater than those with the faeces alone (*P = 0.049).
XANTHAN GUM AND COLONIC FUNCTION

Correlations between data sets

Before the subjects were fed on xanthan gum there was an inverse relationship between transit time and stool weight \( (P = 0.001) \). After ingesting the xanthan gum the two variables changed independently of each other \( (P = 0.192) \) but the inverse relationship was still present after the feeding period \( (P = 0.001) \).

There were no statistically significant associations between the reduction in viscosity during fermentation and either the changes in stool weight or the reductions in transit time. A similar percentage of those subjects that increased stool weight and those that failed to increase stool weight after feeding xanthan gum retained some viscosity after fermentation \( (53\% \text{ v. } 66\%) \). Also a similar percentage of the subjects who increased or who reduced transit times showed some reduction in viscosity \textit{in vitro} \( (56\% \text{ v. } 44\%) \). Both the production of \( \text{H}_2 \) and the reduction in viscosity \textit{in vitro} were significantly associated with an increase in stool frequency \( (P = 0.04) \). There were also significant associations between a fall in pH and both decreases in transit time \( (P = 0.041) \) and increases in flatulence \( (P = 0.035) \).

DISCUSSION

The findings from the present study show that xanthan gum is a highly efficient stool-bulking agent causing an average increase in faecal output of about 50 g/d when given at a dose of 15 g/d, confirming previous findings (Eastwood \textit{et al.} 1987). The increase in mass results not only from increased amount of water, but also a significant \( (P = 0.008) \) increase in dry weight. Ingestion of xanthan gum also causes significant increases in the frequency of defeaecation \( (P = 0.035) \) and weight of each bowel movement \( (P = 0.049) \).

The effect on transit time, unlike other polysaccharides (Tomlin \& Read, 1988), was not significant, although on average it was decreased. In fact the xanthan gum appeared to have a normalizing effect similar to that shown by Payler \textit{et al.} (1975) with wheat bran.

The findings from the present study did not support the hypothesis that stool weight and transit time are dependent variables in that there were no correlations between the changes in these factors after feeding xanthan gum. According to previous studies (Tomlin \& Read, 1988) an increase in stool weight without any significant decrease in transit time is associated with maintenance of viscous properties; this suggests retention of polysaccharide structure after fermentation (Tomlin \& Read, 1988). Our results only partly supported this hypothesis. Initially faecal cultures from eleven of the eighteen subjects still retained some viscosity after incubation, and after feeding xanthan gum for 10 d this number had reduced from eleven to seven.

Furthermore, the results only partly supported our previous observations that accelerations in transit were associated with a reduction or abolition of viscous properties, suggesting a partial or complete bacterial degradation. A reduction in viscosity was not associated with an acceleration of transit time, though it was associated with an increased stool frequency. Acceleration of transit time was not associated with production of SCFA and \( \text{H}_2 \) but it was associated with a fall in pH. \( \text{H}_2 \) production was correlated with increased stool frequency.

After feeding the xanthan gum for 10 d significantly greater reductions in viscosity and significantly greater amounts of both \( \text{H}_2 \) and SCFA were produced by the faecal cultures compared with the control; this increase in the physical breakdown of the gum linked to increased amounts of fermentation products indicates a greater degree of fermentation. The increase in breakdown of the polysaccharide structure after a period of exposure to bacteria could simply be due to increasing numbers of bacteria or, more likely, the bacteria may have altered their metabolism in order to derive energy more efficiently from the gum. This increase in enzymic activity has been shown previously with oat bran (Chang \textit{et al}.)
1979) and wheat bran (Bourke & Neale, 1980) on exposing bacteria to the substrate. This ability of the bacteria to adapt may offer one explanation for the loss of efficiency of bulk laxatives that many patients report.

In summary, although the present study confirmed that xanthan gum is a highly effective bulk laxative it does not wholly support the theory that the effect of a given polysaccharide is dependent on the degree to which it is degraded (Tomlin & Read, 1988), and does not substantiate the suggestion that it is possible simply to predict the laxative effect of a polysaccharide from its fermentation characteristics in vitro (Adiotomre et al. 1990).

REFERENCES


Printed in Great Britain