St. John’s Wort Extract Induces CYP3A and CYP2E1 in the Swiss Webster Mouse

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Received August 17, 2001; accepted November 2, 2001

This investigation was designed to determine the ability of St. John’s wort (SJW), a readily available antidepressant, to induce various hepatic drug metabolizing enzymes. SJW (140 or 280 mg/kg/day) was administered to male Swiss Webster mice for 1, 2, or 3 weeks. Enzymatic activity was analyzed in hepatic microsomes for all of the following drug metabolizing enzymes: CYP3A, CYP1A, CYP2E1, and UDP-glucuronosyltransferase (UDPGT). The catalytic activity of CYP1A was unchanged from control following any dose or duration of SJW, while both CYP3A and CYP2E1 catalytic activities were increased 2-fold by both SJW concentrations but only following 3 weeks of administration. Results from Western immunoblotting studies supported the changes in catalytic activity, as protein levels for CYP2E1 and CYP3A were increased (2.5-fold and 6-fold, respectively) following 3 weeks of SJW administration. Additionally, the catalytic activity of the conjugation enzyme UDPGT was unchanged from control following all SJW treatments. These results indicate that in the mouse moderate doses of SJW cause an increase in the catalytic activity and polypeptide levels of CYP2E1 and CYP3A but only following 21 days of administration, while the catalytic activity of CYP1A and UDPGT activity remain unaffected.

Key Words: St. John’s wort; UDPGT; CYP3A; CYP2E1; CYP1A.

St. John’s wort (SJW) is an herbal remedy that is used in the treatment of depression (Barnes et al., 2001;Josey and Tackett, 1999). Investigations to determine the bioactive constituents responsible for the therapeutic effect produced by SJW have identified hyperforin, adhyperforin, and hypericin as possible active constituents (Chatterjee et al., 1998; Jensen et al., 2001; Kaehler et al., 1999; Laakmann et al., 1998; Singer et al., 1999). Specifically, antidepressant effects and changes in synaptic neurotransmitter concentrations have been observed in rats administered 10 mg/kg of hyperforin (Chatterjee et al., 1998; Kaehler et al., 1999). Commercially available SJW extracts of European, American, and Far Eastern origin have been found to contain different proportions of the proposed active constituents (Meier, 2001), with hyperforin levels ranging from less than 0.3% to a maximum of 3.3% (Kurth and Spreemann, 1998). Despite this large variation, the hyperforin content in SJW preparations generally remains 10-fold higher than the content of hypericins (Holzl and Ostrowski, 1987). SJW preparations are available over-the-counter and the self-medication with natural remedies is increasing as individuals often perceive them to be safer than synthesized drugs (Wills et al., 2000). However, this perception is not correct as herbal products are not subject to the rigorous approval process applied to new drug applications by the U.S. Food and Drug Administration.

In the last 2–3 years, drug interactions linked to the use of SJW have been reported (Ernst, 1999; Fugh-Berman, 2000; Johne et al., 1999; Nebel et al., 1999; Piscitelli et al., 2000; Ruschitzka et al., 2000). Many of the case studies and clinical trials report interactions with SJW and drugs that are substrates of cytochrome P4503A (CYP3A). However, there is limited direct evidence to support the theory that these drug interactions have resulted from CYP3A induction. Experimentally, there has only been one in vitro study (Moore et al., 2000) and one in vivo study (Durr et al., 2000), and two clinical trials (Durr et al., 2000; Roby et al., 2000) that have provided evidence of CYP3A induction by SJW. Additionally, there is no evidence that SJW alters CYP450 activity following treatment of less than 7 days duration. Specifically, SJW (280 mg/kg/day, 4 days) failed to increase CYP450 isoforms in the mouse (Bray et al., in press) and did not change the metabolism of dextromethorphan or alprazolam when administered to healthy volunteers (300 mg, 3 times a day for 3 days; Markowitz et al., 2000).

Since the majority of the literature to date has reported interactions of SJW with substrates of CYP3A (Ernst, 1999; Fugh-Berman, 2000; Piscitelli et al., 2000; Ruschitzka et al., 2000), information regarding the effect of SJW on the activity of CYP3A is vital. Therefore, the aim of this study was to determine the minimum duration of SJW administration required to alter the catalytic activity and polypeptide levels of hepatic CYP3A in male Swiss Webster mice. Since the func-
tion and regulation of CYP3A is highly conserved among mammalian species (Maurel, 1996), a reasonable comparison will be able to be made between the results obtained in rodents and the existing human data.

A potential interaction between SJW and theophylline, a substrate metabolized by both CYP3A and CYP1A, has also been reported (Nebel et al., 1999) and thus determining the effect of SJW on other isoenforms of hepatic CYP450 is also important. Therefore, CYP1A and CYP2E1 were also examined for alterations in catalytic activity and polypeptide levels following SJW administration. Additionally, previous reports have demonstrated that changes in drug conjugation can result in biologically significant drug interactions (Bray and Rosen- gren, 2001; Douidar and Ahmed, 1987). Therefore, it is possible that the drug interactions reported with indinavir, cyclo- sporine A, and oral contraceptives following SJW ingestion (Ernst, 1999; Fugh-Berman, 2000; Piscitelli et al., 2000; Ruschitzka et al., 2000) could have resulted from an increase in the rate of drug conjugation. Accordingly, the ability of SJW to alter the catalytic activity of UDP-glucuronosyltransferase (UDPGT), a major conjugation enzyme, was also measured.

MATERIALS AND METHODS

Chemicals. Alanine aminotransferase (ALT) kit (#59-UV), b-naphthoflavone, dexamethasone, acetone, uridine diphasophogluconic acid (UDPGA), p-nitrophenol, 4 nitrocatechol, Tris-HCl and secondary antibodies were purchased from Sigma Chemical Co. (St. Louis, MO). Nitroblue tetrazolium chloride (NBT), 5-bromo-4-chloro-3-indoyl phosphate-p-toluidine salt (BCIP), avidin-alkaline phosphatase conjugate, biotinylated SDS-PAGE standards broad range, acrylamide, nitrocellulose, and glycine were purchased from Bio-Rad (Hercules, CA). Primary antibodies antirat CYP2E1, antirat CYP3A2, and antirat CYP1A2 were purchased from Amersham (Auckland, NZ). All other chemicals were of the highest purity commercially available.

St. John’s wort extraction and dosing. St. John’s wort extract HypericFin® containing 0.3% total hypericins (ratio pseudohypericin:hypericin 2.5:1) and 2.3% hyperforin (3.6% hyperforin homologues and adhyperforin) was supplied by Finzelberg GmbH & Co. KG (Andernach, Germany). This SJW preparation was chosen as it has been well characterized (Kurth and Spreeemann, 1998). The manufacturer’s claims for composition were confirmed by HPLC analyses using a Waters HPLC (Milford, MA) system, and a Merck (Darmstadt, Germany) Lichrospher 100 RP-18 column (250 × 10 mm). SJW was extracted from the silica carrier by dissolution with methanol (9 ml, 10 min ultrasonication) and filtering. The SJW was then dried and dissolved in a corn oil vehicle. Samples were stored at −20°C and thoroughly vortexed before administration. SJW was administered at 280 mg/kg as this dose contains 10 mg/kg of hyperforin, which was previously shown to exhibit antidepressant effects in rats (Chatterjee et al., 1998; Kaehler et al., 1999). SJW was also administered at a lower dose (140 mg/kg), which is approximately 10-fold greater than that routinely used in clinical investigations (300 mg, tid).

Animals. Male Swiss Webster mice (7 weeks of age, 22 g) were purchased from AgResearch (Hamilton, NZ). Mice were housed in plastic cages on shredded paper bedding and had constant access to rodent diet and water. They were maintained at 21–24°C on a 12 h light/dark cycle in an approved animal care facility. Mice were dosed with St. John’s wort extract (140 mg/kg or 280 mg/kg, po), or corn oil vehicle (5 ml/kg, po) for 1, 2, or 3 weeks. Necropsies were performed 24 h following the end of the treatment period. Positive control groups consisted of dexamethasone (75 mg/kg, ip) administered for 4 days, b-naphthoflavone (80 mg/kg, ip) administered for 3 days, and acetone (4.8 mg/kg, po) administered 16 h prior to necropsy. There were 8 mice in each of the treatment groups.

Evaluation of hepatic injury. Immediately after euthanasia with carbon dioxide, blood was collected from the inferior vena cava and placed on ice. Plasma alanine aminotransferase (ALT) activity was measured as an indicator of hepatic injury and was determined kinetically with a commercially available kit. The results are expressed as international units per liter (IU/l).

CYP450 Assays

Hepatic microsomes were prepared by differential centrifugation (Gruenicher, 1989) and the protein was immediately determined by the bicin- choninic acid method (Smith et al., 1985). Microsomes were stored at −80°C until utilized in the following catalytic assays.

CYP1A catalytic activity. Ethoxyresorufin O-deethylation was used as a selective probe for changes in the catalytic activity of CYP1A (Ryan and Levin, 1990) and was performed as described previously (Bray et al., 2001). Results are expressed as nmol/mg/min.

CYP2E1 catalytic activity. p-Nitrophenol hydroxylation was used as a selective probe for changes in the catalytic activity of CYP2E1 (Koop et al., 1985) and was performed as described previously (Bray and Rosengren, 2001). Results are expressed as nmol/mg/min.

CYP3A catalytic activity. Erythromycin N-demethylation was used as a selective probe for changes in the catalytic activity of CYP3A (Wrighton et al., 1985) and was performed as described previously (Bray et al., 2001). Results are expressed as nmol/mg/min.

Conjugation Assays

p-Nitrophenol conjugation. The catalytic activity of UDPGT was determined by measuring p-nitrophenol glucuronidation (Fowler et al., 1994). The reaction mixture contained 1 mg microsomal protein, 0.25 M sodium phosphate buffer, pH 7.1, 3.5 mM UDPGA and 0.15 mM pNP in a final volume of 0.7 ml. The samples were preincubated in a shaking water bath for 2 min at 37°C and the reaction was initiated by the addition of UDPGA. After 10 min the reaction was terminated by the addition of 2 ml 10% TCA and the samples were centrifuged. After centrifugation, 0.05 ml 10 N NaOH was added to 1 ml of the resulting supernatant and the absorbance at 400 nm was read immediately. Results are expressed as nmol/mg/min.

Electrophoresis and Western blotting. Sodium dodecylsulfate-polyacryl- amide gel electrophoresis (SDS-PAGE) was performed as described (Laemmli, 1970). Briefly, 10 μg of microsomal protein was loaded onto wells of a 10% polyacrylamide gel. CYP1A, CYP2E1, and CYP3A polypeptide levels were quantified by Western immunoblotting as described (Towbin et al., 1979). Nitrocellulose membranes were incubated with rabbit-antirat primary antibody (either CYP1A, CYP2E1, or CYP3A), and the intensity of the bands was determined as described previously (Bray and Rosengren, 2001).

Statistical analysis. Individual groups were analyzed using a 2-way ANOVA coupled with the Student-Newman-Keuls post-hoc test with p < 0.05 as the minimum requirement for a statistically significant difference.

RESULTS

SJW was well tolerated by the mice as there was no difference in body weight gain throughout the treatment period (Table 1). To specifically test for hepatic necrosis induced by SJW, plasma ALT was measured. All of the treatments resulted in values for ALT activity in the normal range (45–55 IU/l; Table 1). In addition, at the time of dissection the livers were grossly normal and liver weight as a percent of body weight did not change following SJW treatment, which supports the finding that none of the treatments resulted in hepatotoxicity.
It has been proposed that SJW may cause induction of CYP450 isoforms, in particular CYP3A. Therefore, 3 CYP450 isoforms relevant to drug metabolism were characterized, namely CYP1A, CYP2E1, and CYP3A. The results indicated that CYP3A catalytic activity was increased 2-fold by SJW at both 140 mg/kg and 280 mg/kg but only after 3 weeks of oral gavage (0.71 ± 0.04, 0.69 ± 0.07, and 0.35 ± 0.03 nmol/mg/min for 140 mg/kg SJW, 280 mg/kg SJW, and vehicle control, respectively; Fig. 1). Similarly, SJW at both doses resulted in a 2-fold increase in CYP2E1 catalytic activity compared to vehicle treated mice (2.49 ± 0.30, 2.56 ± 0.44, and 1.27 ± 0.23 nmol/mg/min for 140 mg/kg SJW, 280 mg/kg SJW, and vehicle control, respectively), but only after 3 weeks of SJW administration (Fig. 2). However, CYP1A catalytic activity was not altered by any dose or duration of SJW treatment (Fig. 3).

To validate the findings of the catalytic studies, Western immunoblotting was conducted to determine changes in immunoreactive protein levels. The results from the immunoblotting studies supported the catalytic data. Both CYP3A and CYP2E1 protein levels were increased following 3 weeks of SJW treatment at both 140 mg/kg and 280 mg/kg. Specifically, CYP3A polypeptide levels were 606 ± 135 and 610 ± 55% of control following SJW at 140 and 280 mg/kg, respectively (Fig. 4). While CYP2E1 polypeptide levels were 250 ± 39 and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight (% body weight)</th>
<th>Body weight gain (g)</th>
<th>ALT Activity (IU/l)</th>
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<tbody>
<tr>
<td></td>
<td>1 Week</td>
<td>2 Weeks</td>
<td>3 Weeks</td>
</tr>
<tr>
<td>SJW 140 mg/kg</td>
<td>5.9 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>SJW 280 mg/kg</td>
<td>5.8 ± 0.1</td>
<td>5.7 ± 0.3</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6.0 ± 0.2</td>
<td>6.1 ± 0.3</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>Untreated</td>
<td>6.1 ± 0.2</td>
<td>5.9 ± 0.1</td>
<td>5.9 ± 0.2</td>
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Note. The values represent the mean ± SE from 8 animals. Significance was analyzed with an ANOVA coupled with the Student-Newman-Keuls post-hoc test in which \( p < 0.05 \) was required for a statistically significant difference. None were significantly different at this level.
245 ± 20% of control following SJW at 140 and 280 mg/kg, respectively (Fig. 5). Similar to the catalytic activity results, CYP1A immunoreactive protein levels were unchanged following SJW treatment. Therefore, the results demonstrate that SJW has the ability to increase CYP3A and CYP2E1, but not CYP1A, following 3 weeks of administration in the Swiss Webster mouse.

Since changes in drug conjugation can result in biologically significant drug interactions (Bray and Rosengren, 2001; Doudar and Ahmed, 1987), the effect of SJW on microsomal UDPGT activity was also examined. Results from p-nitrophenol conjugation studies demonstrated that UDPGT catalytic activity was unchanged following each of the various SJW treatments (Table 2), while phenobarbital (80 mg/kg/day, ip, 3 days) treatment resulted in a 2-fold increase in UDPGT activity.

**DISCUSSION**

There have been numerous clinical reports that have observed a decrease in the plasma concentration of various drugs metabolized by CYP3A following SJW ingestion (Ernst, 1999; Fugh-Berman, 2000; Piscitelli et al., 2000; Ruschitzka et al., 2000). These reports demonstrate the need to determine the effect of SJW on various drug-metabolizing enzymes. Therefore, SJW was examined for its ability to alter CYP450 isoforms and enzymes involved in drug conjugation, namely CYP3A, CYP2E1, CYP1A, and UDPGT. These parameters were chosen because a decrease in drug plasma concentration can occur not only through an increase in CYP450 activity but also through an increase in drug conjugation. Previously, we have shown that short-term administration of hyperforin (10 mg/kg), hypericin (1 mg/kg), and SJW (280 mg/kg) for 4 days did not alter CYP3A, CYP1A, or CYP2E1 catalytic activity (Bray et al., in press). This study concluded that treatment duration may need to be extended before induction of the various CYP450 isoforms occurred. Therefore, to determine the minimum dose and duration of SJW required to alter drug metabolizing enzyme activity, mice were administered SJW (140 and 280 mg/kg, po) or corn oil vehicle (5 ml/kg, po) for 1, 2, or 3 weeks. All treatments were well tolerated by the mice and did not alter liver integrity as there was no change in body
weight, liver weight, or plasma ALT activity compared to untreated and vehicle controls. Dose-response and time-course experiments demonstrated that SJW failed to alter the catalytic activity or polypeptide levels of any of the CYP450 isoforms examined when administered for up to 2 weeks. Additionally, there was no change in UDPGT catalytic activity following all doses of SJW. However, after 3 weeks of administration, SJW increased the catalytic activity and polypeptide levels of both CYP3A (a 2-fold increase in catalytic activity and a 6-fold increase in immunoreactive protein) and CYP2E1 (2-fold increase in catalytic activity and a 2.5-fold increase in immunoreactive protein), regardless of the dose administered.

Previous studies in humans, which examined the effect of SJW on CYP3A activity, have shown no change in this parameter when 7 healthy volunteers were administered SJW (300 mg, tid) for less than 7 days (Markowitz et al., 2000). However, when SJW (300 mg) was taken by 13 human subjects 3 times a day for 14 days, CYP3A4 was increased as measured by urinary 6-β-hydroxycortisol/cortisol ratios (Roby et al., 2000). The results showed that SJW increased this ratio from a baseline of 7.1 ± 4.5 to 13 ± 4.9 (Roby et al., 2000). This increase was seen in all but 1 subject and the increase in 6-β-hydroxycortisol/cortisol ratios ranged from 0.14 to 2.59-fold (Roby et al., 2000). Additionally, Durr et al. (2000) reported a 1.4-fold increase in the functional activity of CYP3A and a 1.4-fold increase in intestinal P-glycoprotein expression following SJW (300 mg, tid) administration for 2 weeks in 8 healthy male volunteers. Therefore, it appears from these 2 clinical investigations and our results that repeated dosing for extended periods is required before changes in CYP450 activity are observed. However, the clinical studies performed to date are limited as they analyzed sample sizes of less than 15 individuals.

One rodent study has demonstrated that SJW (1 g/kg, po), when administered to rats for 14 days, resulted in a 2.5-fold increase in the expression of hepatic CYP3A and a 3.8-fold increase in the expression of intestinal P-glycoprotein, as determined by Western immunoblotting (Durr et al., 2000). Interestingly, these results were tissue-specific as SJW had no effect on the expression of intestinal CYP3A or hepatic P-glycoprotein. This study is limited by the fact that it was necessary to house the rats in the dark throughout the experiment.

### Table 2

Effect of SJW on UDPGT Catalytic Activity

<table>
<thead>
<tr>
<th>Treatment duration (dose)</th>
<th>p-Nitrophenol conjugation</th>
</tr>
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<tbody>
<tr>
<td>1 Week</td>
<td></td>
</tr>
<tr>
<td>SJW (140 mg/kg)</td>
<td>1.96 ± 0.03</td>
</tr>
<tr>
<td>SJW (280 mg/kg)</td>
<td>1.93 ± 0.07</td>
</tr>
<tr>
<td>Corn oil (5 ml/kg)</td>
<td>2.12 ± 0.18</td>
</tr>
<tr>
<td>2 Weeks</td>
<td></td>
</tr>
<tr>
<td>SJW (140 mg/kg)</td>
<td>1.97 ± 0.13</td>
</tr>
<tr>
<td>SJW (280 mg/kg)</td>
<td>2.27 ± 0.24</td>
</tr>
<tr>
<td>Corn oil (5 ml/kg)</td>
<td>2.10 ± 0.14</td>
</tr>
<tr>
<td>3 Weeks</td>
<td></td>
</tr>
<tr>
<td>SJW (140 mg/kg)</td>
<td>2.64 ± 0.35</td>
</tr>
<tr>
<td>SJW (280 mg/kg)</td>
<td>2.23 ± 0.30</td>
</tr>
<tr>
<td>Corn oil (5 ml/kg)</td>
<td>2.15 ± 0.12</td>
</tr>
<tr>
<td>3 Days</td>
<td></td>
</tr>
<tr>
<td>Phenobarbital (80 mg/kg)</td>
<td>3.98 ± 0.06*</td>
</tr>
</tbody>
</table>

Note. p-Nitrophenol conjugation is given in nmol/mg/min. Each value represents the mean ± SEM from 8 independent determinations. Significance was analyzed with an ANOVA coupled with the Student-Newman-Keuls post-hoc test in which p < 0.05 was required for a statistically significant difference.
*Significantly increased from all other treatments p < 0.04.
ment to prevent phototoxicity. Therefore, the extremely high dose of SJW has tempered the significance of these results.

One study in primary human hepatocytes has demonstrated that both SJW and hyperforin induced CYP3A4 mRNA via activation of the orphan nuclear pregnane X receptor (PXR; Moore et al., 2000). Activation of PXR was achieved in CV-1 cells transfected with expression plasmids for human PXR. Three commercial SJW extracts (7–75 μg/ml) and hyperforin (1 μM) were tested in this system. All 4 displayed a 6 to 7-fold increase in the activation of PXR (Moore et al., 2000). Further studies revealed that the 3 SJW preparations and hyperforin induced CYP3A4 mRNA expression in primary human hepatocytes. The results showed that all 4 treatments produced an increase in CYP3A4 mRNA levels following a 30 h incubation period (Moore et al., 2000). Since pure hyperforin was as effective as SJW in the activation of PXR and CYP3A4 mRNA, the authors concluded that hyperforin was responsible for much, if not all, of this activity. However, no indication was given as to whether the level of hyperforin in the 3 SJW extracts was equivalent to the dose of isolated hyperforin tested.

As a whole, the in vivo results demonstrate that SJW when administered for 2–3 weeks increases CYP3A (in mice, rats and humans) and intestinal P-glycoprotein/Mdr1 (in rats and humans). However, the increases in the catalytic activity of CYP3A are quite modest in both the rodent and human investigations and range from 0.14 to 2.5-fold. These studies all administered SJW preparations that contained 0.3% hyperforin. Therefore, a comparison of the results can be made despite the lack of specific data with regard to the CYP3A induction potential of isolated hyperforin.

CYP1A catalytic activity and polypeptide levels were unchanged following 3 weeks of SJW administration in mice. Since the results obtained for CYP3A catalytic activity in the mouse closely resembles those reported from clinical investigations, it is possible that CYP1A activity following SJW will be similar in both species. If so, the interactions between SJW and theophylline reported by Nebel et al. (1999) would be mediated through CYP3A induction only. However, clinical investigations have not examined CYP1A activity following SJW and this must be conducted before conclusions are made regarding SJW’s effect on this enzyme in humans.

There have been no previous reports of SJW altering CYP2E1 activity, but this study has demonstrated that the catalytic activity and polypeptide levels of CYP2E1 were increased in the mouse following 3 weeks of SJW administration. While the clinical significance of drug interactions with CYP2E1 is less important than with CYP3A, it is relevant to characterize changes in CYP2E1 as overexpression of this isoform has been shown to increase reactive oxygen species and elicit toxicity in the absence of added oxidant (Nieto et al., 1999; Wu and Cedarbaum, 2001). Moreover, CYP2E1 activity can also be induced by changes in normal physiological parameters such as fasting and diabetes (Hong et al., 1987; Ioannides et al., 1996). Therefore, it is possible that SJW may interact with both disease states and substrates for CYP2E1 such as acetaminophen, chloroform, and vinyl chloride (Brady et al., 1989; Raucy et al., 1989).

In conclusion, this is the first in vivo study to demonstrate changes in CYP450 isoforms following moderate doses of SJW. While no single preclinical rodent study can predict with certainty clinical drug interactions, our results in conjunction with clinical studies (Durr et al., 2000; Roby et al., 2000) demonstrate that SJW use for 2–3 weeks increases CYP3A catalytic activity and polypeptide levels. Additionally, SJW did not alter CYP1A or UDPGT activity in the mouse, while CYP2E1 catalytic activity and polypeptide levels was increased 2-fold. No other rodent or human study has examined these parameters. Therefore, these results provide a framework for elucidating the species-specific response of CYP3A, CYP2E1, and CYP1A to SJW. Since SJW has increased CYP3A in multiple species, future studies should focus on determining the specific constituent(s) responsible for this effect.

ACKNOWLEDGMENTS

The authors would like to thank H. Kurth for the SJW extract and N. Brennan and L. Glennie for SJW analysis and extraction. This project was funded by a grant from the Deans’ Bequest Fund at the University of Otago.

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