In vitro Metabolism of Grandisin, a Lignan with Anti-chagasic Activity
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Abstract

Tetrahydrofuran lignans represent a well-known group of phenolic compounds capable of acting as antiparasitic agents. In the search for new medicines for the treatment of Chagas disease, one promising compound is grandisin which has shown significant activity on trypanocidal activity of tetrahydrofuran lignans showed a good scale [13, 14]. Previous investigations of the trypanocidal activity of tetrahydrofuran lignans showed several significant results [15–17] which were already registered as a patent [18]. Chagas disease affects more than 10 million people in the world and is concentrated in Latin America where it has an impact on the medical health system [19]. In this context, the aim of this work was to investigate the metabolism of grandisin in the pig cecum model by applying biomimetic reactions in order to improve information on its preclinical pharmacokinetic and also on the biological activity of the putative metabolite. Under the chosen experimental conditions, no metabolization of grandisin was observed in the pig cecum model. Even though there are some studies showing fungal grandisin metabolism [20] and metabolism of lignans from flax by intestinal bacteria [21], the yield was very low and apparently the bacteria from the pig intestinal tract were not able to metabolize this lignan. The high recovery value observed in addition to the control metabolism using quercetin eliminates any possibility of technical problems. This result indicates high intestinal stability of grandisin, which is one prerequisite for good absorption in an oral administration.

Recently, the biotransformation of grandisin by rat liver microsomes showed a dehydro metabolite [22] suggesting that a possible oxidized metabolite may be formed by liver phase I reactions. To confirm this proposition, we applied a biomimetic oxidation of grandisin catalyzed by Mn(salen). The results demonstrate the presence of two products, a minor signal resulting from the oxidative cleavage of grandisin, as previously observed for caterpillars’ metabolism [23]. A second product was isolated by TLC and characterized as dehydro-grandisin: 3,4-dimethyl-2,5-bis(3,4,5-trimethoxyphenyl)-2,3-dihydrofuran (2) (Fig. 1). The ESI-HRMS spectrum showed the [M + H]+ signal at m/z 431.2064, confirming the molecular formula as the same as observed for the product obtained after metabolization by liver microsomes [22]. The putative metabolite was submitted to 1H NMR analysis, and the data was compared to grandisin. Grandisin has trans,trans,trans relative stereochemistry, so that the oxygenazylic and methinic protons are equivalent. In contrast, the putative metabolite lost this symmetry and appeared as two sets of signals with similar chemical shifts at δH 4.80 (H-2/H-6) and δH 6.58 ppm for the aromatic protons H-2/H-6 and H-2/H-6’, respectively. The same effect was observed for the –OCH3 singlets confirming the symmetry loss and that no reaction occurred in any aromatic ring. The major difference was observed for the positions H-7/H-8 which were already registered as a patent [18].

Supporting information available online at http://www.thieme-connect.de/ejournals/toct/plantamedica

Fig. 1 Structures of grandisin (1) and its dehydro putative metabolite (2).

There are few studies on intestinal and phase I metabolites of natural products [1–3]. During the last years the “pig cecum model” was developed and further improved, showing good results for the metabolization of phenolic compounds [4–10]. For phase I metabolism, several in vitro biomimetic models were developed, while Mn(salen) appeared as an alternative to produce similar metabolites of CYP450 enzymes [11, 12] yielding a putative metabolite in a good scale [13, 14]. Previous investigations of the trypanocidal activity of tetrahydrofuran lignans showed several significant results [15–17] which were already registered as a patent [18]. Chagas disease affects more than 10 million people in the world and is concentrated in Latin America. In recent years, new medicines for the treatment of Chagas disease have been developed [19]. The aim of this work was to investigate the metabolism of grandisin in the pig cecum model by applying biomimetic reactions in order to improve information on its preclinical pharmacokinetic and also on the biological activity of the putative metabolite. Under the chosen experimental conditions, no metabolization of grandisin was observed in the pig cecum model. Even though there are some studies showing fungal grandisin metabolism [20] and metabolism of lignans from flax by intestinal bacteria [21], the yield was very low and apparently, the bacteria from the pig intestinal tract were not able to metabolize this lignan. The high recovery value observed in addition to the control metabolism using quercetin eliminates any possibility of technical problems. This result indicates high intestinal stability of grandisin, which is one prerequisite for good absorption in an oral administration.

Recently, the biotransformation of grandisin by rat liver microsomes showed a dehydro metabolite [22] suggesting that a possible oxidized metabolite may be formed by liver phase I reactions. To confirm this proposition, we applied a biomimetic oxidation of grandisin catalyzed by Mn(salen). The results demonstrate the presence of two products, a minor signal resulting from the oxidative cleavage of grandisin, as previously observed for caterpillars’ metabolism [23]. A second product was isolated by TLC and characterized as dehydro-grandisin: 3,4-dimethyl-2,5-bis(3,4,5-trimethoxyphenyl)-2,3-dihydrofuran (2) (Fig. 1). The ESI-HRMS spectrum showed the [M + H]+ signal at m/z 431.2064, confirming the molecular formula as the same as observed for the product obtained after metabolization by liver microsomes [22]. The putative metabolite was submitted to 1H NMR analysis, and the data was compared to grandisin. Grandisin has trans,trans,trans relative stereochemistry, so that the oxygenazylic and methinic protons are equivalent. In contrast, the putative metabolite lost this symmetry and appeared as two sets of signals with similar chemical shifts at δH 4.80 (H-2/H-6) and δH 6.58 ppm for the aromatic protons H-2/H-6 and H-2/H-6’, respectively. The same effect was observed for the –OCH3 singlets confirming the symmetry loss and that no reaction occurred in any aromatic ring. The major difference was observed for the positions H-7/H-8 which were already registered as a patent [18].
hydro-grandisin, the CD spectra were recorded for the metabolite and grandisin. Both spectra showed two similar bands, one centered at about 208 (positive) and other at 243 nm (negative) for grandisin and the metabolite, proving that they have the same configuration (R,R) [24].

The final step was to define if the putative metabolite still had biological activity as observed with grandisin against the trypanastigote form of T. cruzi [2]. After isolation, dehydro-grandisin was evaluated against this parasite. The absence of activity indicates that the opposite spacial geometry of rings and methyl groups is important in the grandisin molecule to show an effect against this parasite.

Materials and Methods

Piper solmsianum C. DC, was collected in Ubatuba (São Paulo, Brazil) and identified by Dr. Elci Franklin Guimarães (Jardim Botânico do Rio de Janeiro, Brazil) where a voucher specimen (329676) is deposited. This work was carried out with the CGEN license no 005/2009. Dried and pulverized leaves were extracted as previously described, and (−)-grandisin (98%, determined by GC–MS) was obtained [25].

(−)-Grandisin was assayed in the pig cecum model and the Mn (salen) oxidation procedure as reported before. The putative metabolite obtained using Mn(salen) was assayed against T. cruzi in a specific and sensitive test [26]. For a description of these methods, see Supporting Information.

Supporting information

3,4R-Dimethyl-2,5R-bis(3,4,5-trimethoxyphenyl)-2,3-dihydrofuran (2): white powder; [α]D25 = −69.1 (0.09 g/100 mL MeCN); UV (CH3CN) λmax 239, 270 nm; CD (CH3CN) 307 + 4.8, 317 + 5.2, 324 + 4.4, 329 − 1.1, 318 − 1.3 mdeg (4.4 ± 10−4 mol/L); 'H NMR (CDCl3, 300 MHz) δH 6.75 (s, H-2,6'), 6.58 (s, H-2',6'); 4.80 (d, J = 8.6, H-7'), 3.82 (s, OCH3 at 4), 3.81 (s, OCH3 at 3.5), 3.80 (s, OCH3 at 4.3',5'); 2.91 (m, H-8'), 1.84 (d, J = 1.4, H-9'), 1.03 (d, J = 6.5, H-9'); ESI MS (pos. ion mode) m/z 431.2062 [M + H]− (calcld. for C24H30O7 431.2064); EI MS m/z (% rel int): 430 ([M]+, 45), 348 (21), 347 (100), 247 (21), 235 (17), 219 (16), 204 (19), 195 (61).

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Conflict of Interest

Each author of this article has read this manuscript. We declare that there is no conflict of interest.

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