γ-Mangostin increases serotonin2A/2C, muscarinic, histamine and bradykinin receptor mRNA expression

Monrudee Sukma a,∗, Michihisa Tohdab, Sunit Suksamran c, Boonyong Tantisirad d

a Department of Pharmacology and Toxicology, Faculty of Pharmacy, Silpakorn University, Muang, Nakhon Pathom 73000, Thailand
b Institute of Natural Medicine, University of Toyama, Japan
c Faculty of Science, Srinakharinwirot University, Bangkok, Thailand
d Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

A R T I C L E   I N F O

Article history:
Received 5 November 2010
Received in revised form 6 February 2011
Accepted 18 March 2011
Available online 2 April 2011

Keywords:
γ-Mangostin
5-HT2 receptor
Antagonist
Muscarinic
Histamine
Bradykinin

A B S T R A C T

Aim of the study: γ-Mangostin is a xanthone found in the fruit hulls of Garcinia mangostana L., which have long been used in Southeast Asia as a traditional medicine for the treatment of abdominal pain, dysentery, wound infections, fever and convulsions. Recent studies have revealed that γ-mangostin exhibits a variety of pharmacological activities, including serotonin 2 (5-HT2) receptor antagonism, anti-inflammatory effects and analgesic effects. To explore the mechanism of γ-mangostin responsible for these pharmacological activities, especially its effects on some related receptors, we investigated the effects of γ-mangostin on 5-HT2, histamine (H1) and bradykinin (BK2) receptor gene expression in neuroblastoma (NG 108-15) cells in vitro. Additionally, to extend the study of the pharmacological properties, we examined the effect of γ-mangostin on the muscarinic (M4) receptor.

Materials and methods: NG 108-15 cells were cultured in vitro and treated with γ-mangostin or a 5-HT2 receptor antagonist (either imipramine or ketanserin). Then, the levels of mRNA for 5-HT2A/C receptors were evaluated by semi-quantitative RT-PCR. The preventive effect of serotonin on the enhancement effects was also revealed. Additionally, the effects of γ-mangostin on the muscarinic, histamine and bradykinin receptors were determined.

Results: Chronic application of γ-mangostin at a concentration of 0.1 μM induced a significant increase in the level of 5-HT2A/C receptor mRNA. These effects were prevented by serotonin. Moreover, γ-mangostin up-regulated the M4, H1 and BK2 receptors.

Conclusion: The ability of γ-mangostin to enhance the expression of 5-HT2A/C, muscarinic, histamine and bradykinin receptor mRNA suggests that this compound has antagonistic effects. These pharmacological properties may partly account for the benefits of using mangosteen in the treatment of inflammation, pain and neuropsychiatric symptoms.

© 2011 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Mangosteen (Garcinia mangostana) is a tree that belongs to the family Guttiferae and is known for its medicinal properties. Traditional uses have included the treatment of inflammation, pain, fever and convulsions (Pedraza-Chaverri et al., 2008). Recent popularity has broadened these claims to include its use against dysentery, wound infections, fever and convulsions (Pedraza-Chaverri et al., 2008). The fruit hull of this plant has been reported to contain the major active components, α- and γ-mangostin and minor xanthones (Gopalakrishnan and Balaganesan, 2000; Suksamran et al., 2002). Here, we elected to study the effects of γ-mangostin. γ-Mangostin [1,3,6,7-tetrahydroxy-2,8-bis(3-methyl-2-butenyl)-9H-xanth-9-one] has been reported to have inhibitory effects on (1) 5-HT2A and 5-HT2C receptors in the peripheral nervous system and central nervous system (Chairungsri and others, 1996, 1998a,b; Furukawa et al., 1997; Sukma et al., 2006), (2) lipopolysaccharide-stimulated nitric oxide (NO) production, which has anti-inflammatory effects, and (3) cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) synthesis in C6 rat glioma cells (Nakatani et al., 2002, 2004; Chen et al., 2008). Moreover, γ-mangostin exhibited analgesic effects in the hot-plate and formalin tests. It was suggested that the analgesic effects of γ-mangostin may involve the histamine (H1), bradykinin and serotonin (5-HT2) receptors (Cui et al., 2010). Considering these confirmed effects of γ-mangostin and the traditional treatment with mangosteen, we hypothesized that γ-mangostin has...
Fig. 1. Effects of γ-mangostin, imipramine and ketanserin on the morphology of NG108-15 cells. (A) Control (19 days); (B) 0.1 μM γ-mangostin (19 days); (C) 0.3 μM (19 days); (D) 1 μM (12 days); (E) 3 μM (72 h); (F) 10 μM (48 h); (G) 30 μM (48 h); (H) 10 μM imipramine (23 days); and (l) 10 μM ketanserin (26 days).

antagonistic effects on the histamine, bradykinin (BK2) and 5-HT2 receptors. Because there is no current information regarding the effects of γ-mangostin on 5-HT2, H1 and BK2 receptor mRNA expression, we were interested in studying this topic. Additionally, to extend the study of the pharmacological properties, we examined the effect of γ-mangostin on muscarinic (M4) receptors, which are intrinsically expressed in NG108-15 cells.

2. Materials and methods

2.1. Plant material

At 7 weeks of maturity after anthesis, the young fruit of Garcinia mangostana were collected from the Ra-ngae district, Narathiwat province, Thailand, in April 2009, and a voucher specimen (RU 0038) was deposited at the Faculty of Science, Ramkhamhaeng University, Thailand.

2.2. Extraction and isolation

Air-dried and powdered fruit (2.06 kg) of Garcinia mangostana was successively extracted with EtOAc and MeOH at 50 °C in a water bath for 48 h each, and the solvents were evaporated to yield the EtOAc (295 g) and MeOH (251 g) extracts. The EtOAc-soluble fraction (42 g) was subjected to quick column chromatography over silica gel using a gradient of hexane–CH2Cl2, CH2Cl2–CH2Cl2–EtOAc, EtOAc and then EtOAc–MeOH (5% increment of polar solvent for 500 ml of each proportion) and were combined into nine main fractions by TLC examination. Fraction 7 (3.39 g) was crystallized with CHCl3 to furnish γ-mangostin (0.89 g).

2.3. Cell cultures

The neuroblastoma cell line (NG 108-15) was cultured as described previously (Sukma et al., 2003).

2.4. Morphological observation

After starting the cell culture, the appropriate drugs were added to the culture medium. Morphological observations were made every day.

2.5. Semi-quantitative RT-PCR

The cells were treated with repeated administration of various concentrations of the appropriate drugs. The culture medium was changed every 2 days. After the cells reached confluence in the 3rd subculture (after 19 days or more), the total RNA was isolated as described in a previous report (Tohda et al., 2008). The
5-HT$_{2A/2C}$ receptor mRNA expression was semi-quantified by the reverse transcription polymerase chain reaction (RT-PCR).

One microgram of total RNA was used to generate cDNA by reverse transcription using ReverScript III (Wako, Tokyo, Japan). The levels of mRNA expression were quantified by RT-PCR using an endogenous internal standard, β-actin, and different primer pairs for each mRNA (Table 1), as previously described (Sukma et al., 2003). The RT-PCR products were resolved by gel electrophoresis on a 6% polyacrylamide gel and stained by ethidium bromide. The ratios of the band intensity of the targeted gene to β-actin were calculated. Data are presented as the mean ± S.E.M. of the 3 independent cultures.

2.6. Drugs

γ-Mangostin isolated from Garcinia mangostana was dissolved in DMSO and then diluted to the desired concentration with serum-free culture medium. The final concentration of DMSO in the culture medium was less than 0.1%. The following drugs were obtained from Sigma (Sigma Chem., St. Louis, MO): imipramine, ketanserin, tartrate and serotonin. Drug solutions were prepared just before the start of the experiments. The cell culture medium and fetal bovine serum were from Invitrogen®, USA.

2.7. Data analysis

Data were analyzed by the t-test versus the vehicle group. Values are expressed as the mean ± S.E.M.

3. Results and discussion

3.1. Morphological changes

The morphologies of NG108-15 cells treated with 0.1–30 μM γ-mangostin and 10-μM imipramine and ketanserin are shown in Fig. 1. Chronic application of 1–30 μM γ-mangostin showed cytotoxic effects within various times of the treatment period. The cells became more round and smaller. Additionally, the number of cell death in a concentration-dependent manner within 48 h. At the concentrations of 3 and 1 μM, γ-mangostin induced cell death at the 3rd and 12th day of culture, respectively. The concentration of 0.3 μM showed a growth inhibition effect. The cells could not reach the confluent state of the 3rd cycle culture. No cytotoxic effect was observed in the group treated with 0.1–30 μM ketanserin (KET) and with 10 μM serotonin (5-HT) for more than 19 days. A semi-quantitative RT-PCR for 5-HT$_{2A/2C}$ receptors was performed. The PCR products shown represent 3 independent experiments. The ratios of the band intensity of the targeted gene to β-actin were calculated. Data are shown as the mean ± S.E.M. of the 3 determinations. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05 versus the vehicle control group.

3.2. Effect of γ-mangostin on 5-HT$_{2A/2C}$ receptor mRNA expression

To determine the effect of γ-mangostin on the expression of 5-HT$_{2A/2C}$ receptors, RT-PCR measurements were performed in neuroblastoma cells. The ratio of the amount of PCR product of interest to that of β-actin provided a relative gene expression level. We found that not only imipramine and ketanserin, but also γ-mangostin, enhanced the expression of the 5-HT$_{2A/2C}$ receptor mRNA (Fig. 2) without producing cytotoxicity. In the second experiment, the effects of the co-administration of target drugs, 0.1-μM γ-mangostin or 10-μM ketanserin and 10-μM serotonin, were determined. It was demonstrated that serotonin could attenuate the inhibitory effects of γ-mangostin and ketanserin on 5-HT$_{2A/2C}$ receptor mRNA expression (Fig. 3).

**Table 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward</th>
<th>Backward</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>GTGACAGATGCTCTTCTGC</td>
<td>AAC GGT CTC AGCTCAGTG TA</td>
<td>222</td>
</tr>
<tr>
<td>5-HT$_{2A}$R</td>
<td>TGACCTCAATTCACACTCC</td>
<td>CACTGC CAT CAT CAC CAG TA</td>
<td>266</td>
</tr>
<tr>
<td>5-HT$_{2C}$R</td>
<td>GTTCCTACTCTGGTAGTCG</td>
<td>CAC AAA GAATACATG CAA</td>
<td>402</td>
</tr>
<tr>
<td>M$_{2A}$R</td>
<td>ACAGTAACGACGAGACTCC</td>
<td>CTG TCTGCGCA CAT AGT CA</td>
<td>428</td>
</tr>
<tr>
<td>H$_{1}$R</td>
<td>CTGCTCTTCCTCAACGGATCT</td>
<td>CAACATCATGACATG TA</td>
<td>549</td>
</tr>
<tr>
<td>BK$_{2}$R</td>
<td>GTGAACAGTCACACAGACAAG</td>
<td>CTG TAT TCC CTC ATGGTC CT</td>
<td>528</td>
</tr>
</tbody>
</table>

Size indicates the estimated PCR product size calculated from the sequence.
Fig. 4. Effects of γ-mangostin, imipramine and ketanserin on the expression of the muscarinic (M4), histamine (H1) and bradykinin (BK2) receptors. Cells were cultured with 0.1 μM γ-mangostin (GM) for more than 19 days. Semi-quantitative RT-PCRs for the M4, H1 and BK2 receptors were performed. The PCR products shown represent 3 independent experiments. The ratios of the band intensity of the targeted gene to β-actin were calculated. Data are shown as the mean ± S.E.M. of 3 determinations. *p ≤ 0.05 versus the vehicle control group.

The data from the present study are in agreement with previous findings, which indicate that γ-mangostin is a 5-HT2 receptor antagonist. For example, it has been shown that γ-mangostin is a competitive antagonist for 5-HT2A receptors in vascular smooth muscles and platelets (Furukawa et al., 1997; Chairungsrilerd et al., 1998b). γ-Mangostin attenuated the 5-fluoro-α-methyltryptamine (5-FMT)-induced head-twitch response (Chairungsrilerd et al., 1998a). γ-Mangostin also reduced inositol accumulation in brain slices stimulated by 5-HT2A receptor activation. The specific binding of [3H] spiperone (a selective radioligand for 5-HT2A receptors) to mouse brain membranes was competitively inhibited by γ-mangostin (Chairungsrilerd et al., 1998b). For 5-HT2C receptors, γ-mangostin showed inhibitory effects on meta-chlorophenylpiperazine (mCPP)-induced hypolocomotion, a 5-HT2C receptor-mediated behavioral response, without alteration of spontaneous locomotor activity (Sukma et al., 2006). In this study, γ-mangostin produced an increase in the 5-HT2A/2C receptor mRNA levels, which was completely blocked by serotonin, suggesting that (1) γ-mangostin has an antagonistic effect on the 5-HT2A/2C receptor and (2) the actions of γ-mangostin on 5-HT2A/2C receptor mRNA expression are mediated by serotonin transmission.

Several lines of evidence indicate that the 5-HT2 receptors in the CNS are involved in psychiatric disorders, such as depression, anxiety, schizophrenia, sleep disorders, and hallucinations. The impact of this plethora of roles of 5-HT2 receptors has led to the development of many compounds of therapeutic value. One of the modern benefits of 5-HT2 receptor antagonists is their use as antidepressants. It has been suggested that 5-HT2A may be involved in the pathogenesis of depression and could mediate the effects of some antidepressant treatments. Correspondingly, the selective knock-down of 5-HT2A receptor expression with an injection of antisense oligonucleotides evokes antidepressant-like effects in the mouse forced-swim test. Indeed, it has been suggested that levels of 5-HT2A receptor expression are altered in the depressed brain. It was found that there were reductions in the 5-HT2A receptor levels within the frontal, occipital, temporal and cingulated cortices of drug-naïve depressed patients relative to control subjects. The 5-HT2C receptor has been shown to be blunted in depressed patients (Lajtha, 2008). Blockade of the 5-HT2A/2C receptor subtype has been hypothesized to result in antidepressant activity based on the actions of nefazodone and mirtazapine (combined 5-HT2A/2C receptors antagonists), ritanserin (a serotonin 5-HT2A and 5-HT2C antagonist) and agomelatine (a selective 5-HT2C antagonist). Available antidepressants have 5-HT2 receptor antagonistic effects, such as imipramine, which up-regulates 5-HT2 receptors in vitro and in vivo (Tohda and Watanabe, 1996; Sukma et al., 2003). Although further study
is desired, the antagonistic effects of γ-mangostin provide the opportunity to develop novel antidepressants and/or drugs based on γ-mangostin for use in the treatment of neuropsychiatric disorders.

3.3. Effect of γ-mangostin on mRNA levels of the muscarinic, histamine and bradykinin receptors

We found that γ-mangostin up-regulated the expression of the M4, H1 and BK2 receptors to statistically significant levels (Fig. 4). These findings suggest that γ-mangostin has an antagonistic effect on these receptors. The M4 receptors are highly and almost exclusively expressed in neurons. A possible therapeutic implication of the receptor interaction is that the selective M4 receptor blockade may result in an enhancement of dopamine D2 receptor signaling and may improve striatal dopamine transmission in pathological situations, such as Parkinson’s disease, and motor side effects induced by antipsychotics, which are associated with a dopamine/acyetylcholine imbalance (Olanans and Onali, 1999; Fink-Jensen et al., 2011).

Several lines of evidence indicate that γ-mangostin also has anti-inflammatory and analgesic effects (Chen et al., 2008; Pedraza-Chaverri et al., 2008; Tewtrakul et al., 2009; Cui et al., 2010). Additionally, the pericarp (peel, rind and hull) of mangosteen has been used traditionally for the treatment of pain and ulcers. In the present study, we found that γ-mangostin up-regulated the expression levels of the H2 and BK2 receptors, and it is well known that these receptors play an important role in inflammation and pain perception. Numerous studies have demonstrated the role of H2 receptors in physiological and pathological pain perception. For example, previous studies have reported that both peripheral pain perception and central sensitization could be attenuated in H1 receptor knockout mice (Cui et al., 2010).

The BK2 receptor is a constitutive receptor for kinins, which are among the most potent autacoids involved in inflammatory, vascular and pain processes. The activation of BK2 receptors promotes polymodal nociceptor activation and hyperalgesia through the production of diacylglycerol and the activation of protein kinase C. Moreover, in addition to its interaction with mast cell mediators, bradykinin can sensitize nociceptors following the release of prostaglandins, cytokines and nitric oxide either from sensory neurons, endothelial and immune cells or fibroblasts. It was found that the blockade of BK2 receptors with selective antagonists induces analgesia in acute inflammatory hyperalgesia models. Additionally, there are alterations of the nociceptive responses in BK2 receptor knockout mice (Couture et al., 2001). The antagonist effects of γ-mangostin on H1 and BK2 receptors presented in this study support the traditional use of mangosteen for the treatment of inflammation and pain.

In summary, we found that γ-mangostin is a 5-HT2A/2C receptor antagonist at the transcriptional level. Although many additional pharmacological studies are still required, information from this study provides the opportunity to develop γ-mangostin from Garcinia mangostana, a plant that is abundantly available in Thailand, as a novel antidepressant and/or drug used to treat neuropsychiatric disorders. Additionally, we found that γ-mangostin up-regulated M4, H1 and BK2 receptor expression, suggesting the antagonistic effects. These findings support the traditional uses of Garcinia mangostana.

Acknowledgments

This work was supported by research grants from the Thailand Research Fund (MRG4880110) and the Japan Society for the Promotion of Science (JSPS). The authors thank Miss Aree Wansuntornwong and Miss Areeat Sripattanaporn for their help with the experimental techniques.

References


Olanans, M.C., Onali, P., 1999. PD 102807, a novel muscarinic M4 receptor antagonist, discriminates between striatal and cortical muscarinic receptors coupled to cyclic AMP. Life Sciences 65, 2233–2240.


