

## PROCEEDING

# Approach to novel functional foods for stress control

## 4. Regulation of serotonin transporter by food factors

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**Abstract:** Serotonin transporters (SERTs) are pre-synaptic proteins specialized for the clearance of serotonin following vesicular release at central nervous system (CNS) and enteric nervous system synapses. SERTs are high affinity targets *in vivo* for antidepressants such as serotonin selective reuptake inhibitors (SSRIs). These include 'medical' psychopharmacological agents such as analgesics and antihistamines, a plant extract called St John's Wort (Hypericum). Osteoclasts are the primary cells responsible for bone resorption. They arise by the differentiation of osteoclast precursors of the monocyte/macrophage lineage. The expression of SERTs was increased in RANKL-induced osteoclast-like cells. Using RANKL stimulation of RAW264.7 cells as a model system for osteoclast differentiation, we studied the direct effects of food factor on serotonin uptake. The SSRIs (fluoxetine and fluvoxamine) inhibited markedly (~95%) in serotonin transport in differentiated osteoclast cells. The major components of St. John's Wort, hyperforin and hypericine were significantly decreased in serotonin transport activity. Thus, a new *in vitro* model using RANKL-induced osteoclast-like cells may be useful to analyze the regulation of SERT by food factors and SSRIs. *J. Med. Invest.* 52 Suppl. : 245-248, November, 2005

**Keywords :** serotonin transporter, serotonin, osteoclast, SSRI, St John's wort

### INTRODUCTION

Osteoclasts are the primary cells responsible for bone resorption. They arise by the differentiation of osteoclast precursors of the monocyte/macrophage lineage. These cells are required not only for the development of the skeleton but also for mineral homeostasis and normal remodeling of bone in adult animals (1, 2). Bone resorption depends on the ability

of the osteoclast to generate an acid extracellular compartment between itself and the bone surface (3). Increased osteoclastic resorption and subsequent bone loss are common features of many debilitating diseases including osteoporosis, bone metastases, Paget's disease, and rheumatoid arthritis. Local factors produced by stromal cells or osteoblastic cells in the bone microenvironment play an important role in osteoclast differentiation. Recent evidence supports a central role for tumor necrosis factor gene family members receptor activator of nuclear factor  $\kappa$ B (RANK) ligand and RANK receptor in osteoclast differentiation (4).

Serotonin transporters (SERTs) are pre-synaptic proteins specialized for the clearance of serotonin following vesicular release at central nervous system

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(CNS) and enteric nervous system synapses (5). SERT are also expressed on a number of specialized, non-neural cells, including platelets, and placental (5). SERT are high affinity targets *in vivo* for anti-depressants such as serotonin selective reuptake inhibitors (SSRIs) and Hypericum perforatum L (St John's wort) (6). The functions of SSRIs are believed to exert at least a portion of their physiologic actions by elevating extracellular serotonin concentrations and extending the duration, and spatial spread of serotonin signals (7). The inhibitory effects of anti-depressants on SERT activity are considered to be linked with the phosphorylation of SERT (8). SSRIs may facilitate protein kinase C-mediated phosphorylation and sequential internalization of SERT by stopping the transport of substrate through SERT. However, these studies are made difficult by the lack of an appropriate *in vitro* model. In this study, we investigated the regulation of serotonin reuptake by food factors in osteoclasts system.

## MATERIALS AND METHODS

Three constituents (hyperforin, hypericin and amentoflavone) were obtained from EMD Biosciences, Inc. (CA, USA), BIOMOL Research Laboratories Inc. (PA, USA) and Extrasynthese (Genay, France). Osteoclasts were generated from mouse monocyte/macrophage cell line RAW 264.7 cells and mouse bone marrow cells as described previously (9). The medium was refreshed on day 3, and osteoclast formation was assayed by counting the number of TRAP-positive cells per well between days 7 and 10. RT-PCR was generated using the following primer pairs : SERT, sense 5'-TTTGGAGGATCTGCTGGGTGG-3', antisense 5'-CTTTTCAGAGCTGAGGAGTCG-3. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used as control for success of the RT-PCR reaction (9).

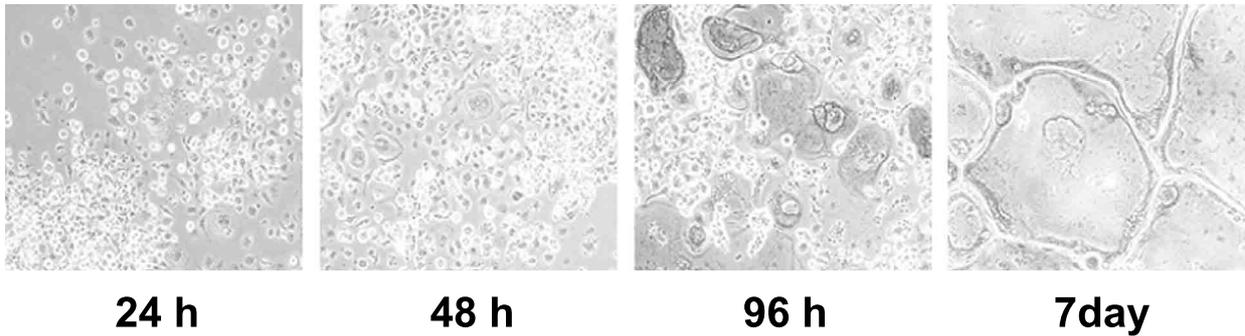
Serotonin transport was studied in monolayers of RANKL-differentiated RAW 264.7 cells (9). The measurement of serotonin transport was performed using a modification of a previously described procedure (10, 11). Briefly, RAW 264.7 cells were plated in 12-well plates at a density of  $5 \times 10^3/\text{cm}^2$  and cultured for 7 days with or without recombinant RANKL (320 ng/ml) in  $\alpha$ -MEM without phenol-red supplemented with 10% charcoal-stripped fetal bovine serum. Cells were washed twice with KRH (120 mM

NaCl, 4.7 mM KCl, 2.2 mM  $\text{CaCl}_2$ , 10 mM HEPES, 1.2 mM  $\text{KH}_2\text{PO}_4$ , 1.2 mM  $\text{MgSO}_4$ ) and incubated in KRH with 1.8g/l D-glucose for 10 min. Inhibitors were added in the preincubation steps. Cells were incubated with the indicated concentrations of 10 nM [ $^3\text{H}$ ] serotonin ( $\sim 100$  Ci/mmol; Amersham Pharmacia Biotech, Piscataway, NJ, USA) containing 100  $\mu\text{M}$  pargyline and 100  $\mu\text{M}$  L-ascorbic acid with or without inhibitors for 10 minutes at 37°C. Incubation was terminated with three washes of ice-cold KRH. The accumulated [ $^3\text{H}$ ] serotonin was determined by solubilizing the cells with 250  $\mu\text{l}$  0.1 N NaOH and liquid scintillation spectrometry. Serotonin transport activity was calculated as nanomoles of per mg protein taken up in 10 min or plotted as a percentage of specific serotonin uptake obtained in the absence of inhibitors.

## RESULTS AND DISCUSSION

To characterize the osteoclast-like cells, we first performed a histochemical analysis of RANKL-treated RAW 264.7 cells. After seven days of treatment of RANKL, the multinucleated cells were markedly increased compared with cells treated for 24h (Fig. 1A). Using gene microarray, we found that mouse SERT was strongly expressed in RANKL-induced osteoclast-like cells (data not shown). The expression was also indicated by RT-PCR in osteoclast derived from RAW 264.7 or mouse bone marrow cells (Fig. 1B). In RANKL stimulation of RAW 264.7 cells, the serotonin uptake was strongly upregulated in RANKL-induced cells (Fig. 2A). The SSRIs, fluoxetine and fluvoxamine markedly inhibits the serotonin transport activity at concentrations of  $\sim 1\mu\text{M}$  (Fig. 2A). To further investigate the effects of extracts of St John's wort on SERT activity, [ $^3\text{H}$ ] -serotonin uptake was measured in this system (Fig. 2B). Hyperforin and hypericine that inhibited brain synaptosomal serotonin uptake were also significantly decreased in transport activity. Thus, a new *in vitro* model using RANKL-induced osteoclast-like cells may be useful to analyze the regulation of SERT by food factors and SSRIs.

**A**



**B**

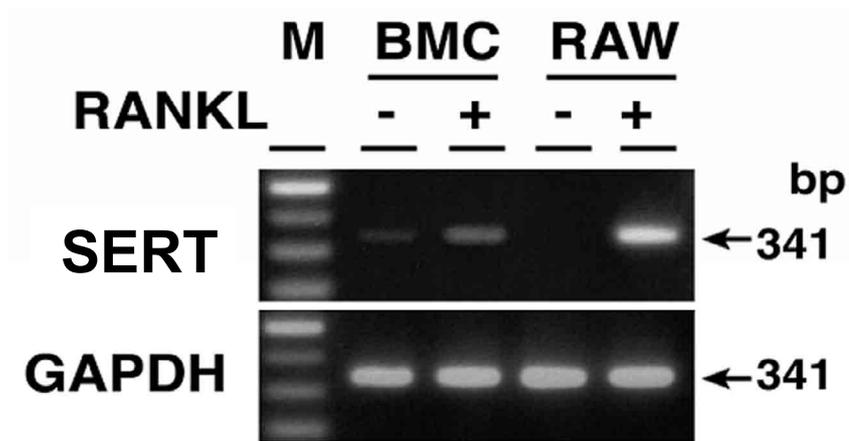
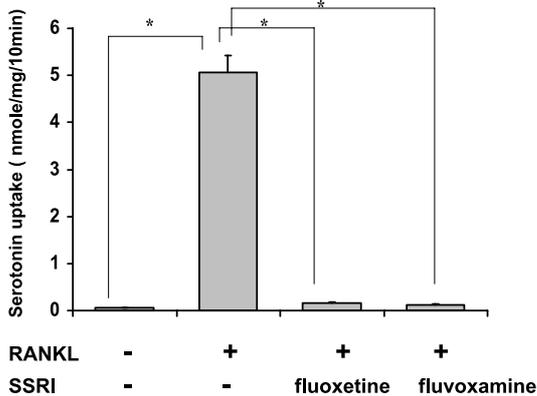


Figure 1. RANKL-induced osteoclastogenesis of macrophage-derived RAW 264.7 cells. (A) Differentiation of osteoclast-like cells (24 h, 48 h, 96 h, and 7 days after treated of RANKL). (B) On day 7, the expression of serotonin transporter (SERT) was detected by RT-PCR in RAW 264.7(RAW) or bone marrow cells (BMC) which is cultured in the presence or absence of RANKL.

**A**



**B**

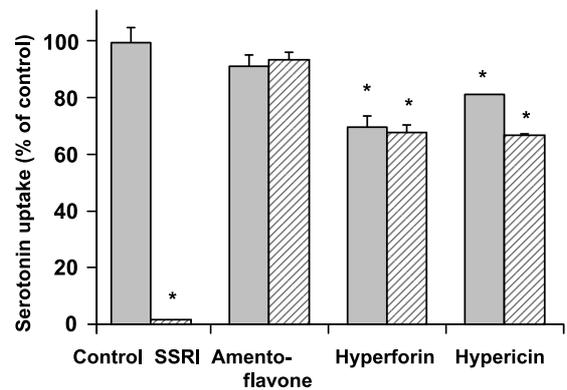


Figure 2. Effects of SSRIs and extracts of St John's wort in osteoclast. (A) RAW 264.7 cells were cultured in the presence or absence of RANKL. The cells were preincubated for 15 min, and then the uptake was assayed with 10 nM [<sup>3</sup>H]-serotonin. The inhibitors were added in preincubation and uptake steps. \**p*<0.05. Values are reported as means±S.E. (B) Effects of extracts of St John's wort. The grey bar is indicated 1 μM and stripe bar is 10 μM.

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