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Novel neurological and immunological targets for salicylate-based phytopharmaceuticals and for the anti-depressant imipramine

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ABSTRACT

Inflammatory processes are increasingly recognised to contribute to neurological and neuropsychatric disorders such as depression. Thus we investigated whether a standardized willow bark preparation (WB) which contains among other constituents salicin, the forerunner of non-steroidal antiphlogistic drugs, would have an effect in a standard model of depression, the forced swimming test (FST), compared to the antidepressant imipramine. Studies were accompanied by gene expression analyses. In order to allocate potential effects to the different constituents of WB, fractions of the extract with different compositions of salicyl alcohol derivative and polyphenols were also investigated.

Male Sprague Dawley rats ($n = 12$/group) were treated for 14 days (p.o.) with the WB preparation STW 33-I (group A) and its fractions (FR) (groups FR-B to E) in concentrations of 30 mg/kg. The FRs were characterized by a high content of flavone and chalcone glycosides (FR-B), flavonoid glycosides and salicyl alcohol derivatives (FR-C), salicin and related salicyl alcohol derivatives (FR-D) and proanthocyanidines (FR-E). The tricyclic antidepressant imipramine (20 mg/kg) (F) was used as positive control. The FST was performed on day 15. The cumulative immobility time was significantly ($p < 0.05$) reduced in group A (36%), group FR-D (44%) and by imipramine (16%) compared to untreated controls. RNA was isolated from peripheral blood. RNA samples (group A, group FR-D, and imipramine) were further analysed by rat whole genome microarray (Agilent) in comparison to untreated controls. Quantitative PCR for selected genes was performed.

Genes (>2 fold, $p < 0.01$), affected by WB and/or FR-D and imipramine, included both inflammatory (e.g. IL-3, IL-10) and neurologically relevant targets. Common genes regulated by WB, FR-D and imipramine were GRIA 2 ↓, SRP54 ↓, CYP26B ↓, DNM1L ↑ and KITLG ↓. In addition, the hippocampus of rats treated (27 d) with WB (15–60 mg/kg WB) or imipramine (15 mg/kg bw) showed a slower serotonin turnover (5-hydroxyindol acetic acid/serotonin ($p < 0.05$)) depending on the dosage. Thus WB (30 mg/kg), its ethanolic fraction rich in salicyl alcohol derivatives (FR-D) (30 mg/kg) and imipramine, by being effective in the FST, modulated known and new targets relevant for neuro- and immunofunctions in rats. These findings contribute to our understanding of the link between inflammation and neurological functions and may also support the scope for the development of co-medications from salicylate-containing phytopharmaceuticals as multicomponent mixtures with single component synthetic drugs.

Abbreviations: 5-HT, serotonin; 5-HIAA, 5-hydroxyindol acetic acid; AD, Alzheimer's disease; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASA, acetylsalicylic acid; CAT, catalase; CD, Sprague-Dawley; CNS, central nervous system; COX, cyclooxygenases; CRH, corticotrophin-releasing hormone; CSF, cerebral spinal fluid; CYP26B1, cytochrome P450 protein 26B1; DNM1L, dynamin like protein 1; EDNRB, endothelin B receptor gene; ER, endoplasmatic reticulum; EtOH-FR, ethanol fraction; FR, fraction; FST, Porsolt-Swimming Test; GR, glutathione reductase; GST, glutathione S-transferase; GTPase, guanosine triphosphatase; HGF, haematopoietic growth factor; HPA-axis, hypothalamic-pituitary-adrenocortical axis; MS, multiple sclerosis; NMDA, N-methyl-D-aspartate; RA, retinoic acid; SCF, stem cell factor; SNP, single nucleotide polymorphism; SNRIs, serotonin and noradrenalin-reuptake inhibitors; SOD, superoxidedismutase; SRP, signal recognition protein; SSRIs, selective serotonin-reuptake inhibitors; TNFRSF1A, TNF-receptor superfamily member 1 A; WB, willow bark.

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Introduction

Willow bark (WB) was already used by Hippocrates as an anti-inflammatory agent against fever and pain. It contains salicin and other salicylic acid derivatives which were designated as active principles since 1831. These compounds are prodrugs which are metabolised in the gut and the liver via salicylic alcohol to salicylic acid – the active compound. To improve tolerability compared to isolated salicyclic acid, Felix Hoffmann synthesised acetylsalicylic acid (ASA) in 1897, today one of the mostly consumed analgetic and anti-inflammatory drugs worldwide.

Salicylic acid is known to inhibit the cyclooxygenases (COX) 1 and 2 (Aronoff et al. 2003). Therefore, COX-inhibition was first regarded the main mechanism of the anti-inflammatory activity of WB. In the meanwhile it was shown that WB can also modulate relevant pro- and anti-inflammatory cytokines like TNF-α, IL-6, IL-1, IL-10, and IL-8 (Bonaterra et al. 2009; Fiebich et al. 2005; Fiebich and Chrubasik 2004) and that not only salicyl alcohol derivatives, but also the polyphenols of WB contribute to this modulation (Khayyal et al. 2005; Nahrstedt et al. 2007). In addition, polyphenols are known to possess antioxidant and neuroprotective effects which can also interfere with inflammatory events (Kannappan et al. 2011). Very recently also a contribution of catechol to an antiinflammatory effect of WB was discussed (Freischmidt et al. 2012; Knuth et al. 2011).

Recent clinical data propose that proinflammatory cytokines also influence the pathogenesis of depression. It was shown that the IL-1RA (interleukin-1 receptor antagonist) is increased in the plasma of middle aged and elderly patients (>65 years) suffering from depression (Milaneschi et al. 2009; Ovaskainen et al. 2009). Lindqvist et al. (2009) demonstrated increased IL-6 concentrations in the CSF of patients with depression. A recent meta-analysis summarising med-line studies on depression between 1967 and 2008 concluded that major markers of inflammation like CRP, IL-6 and IL-1 are significantly associated with depression (Howren et al. 2009). Even though the causal relationship is presently not clear, the link between the immune system via cytokines and the pathogenesis of psychiatric disorders led to the understanding that immunomodulatory drugs may also be beneficial for the treatment of psychiatric disorders (Berthold-Losleben et al. 2009).

In addition, it was observed that in clinical studies with willow bark extract the treatment groups had a lower number of adverse events (AEs) than in the placebo group (e.g. Schmid et al. 2000). Thus, it had been hypothesised by Winterhoff that willow bark might have an anxiolytic effect and could thereby be useful in depression. This was first evidenced by her working group (Hegger et al. 2005; Winterhoff et al. 2008) using the forced swimming test (FST) as an animal model of depression.

We now queried which molecular targets are affected by WB compared to the antidepressant imipramine and which components of the WB extract are active, especially considering that plant constituents have been repeatedly proposed for a possible future application in co-medications (Wagner 2011).

Materials and methods

Willow bark extract

The dried willow bark preparation STW 33-I (WB) was obtained from Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. The extract was prepared from willow bark according to PhEur. 6.1, with a DEV_{nativ} of 16–23:1, total salicin content 23–26% (m/m) Europe C (2008). Imipramine hydrochloride was obtained from Sigma (Deisenhofen, Germany).

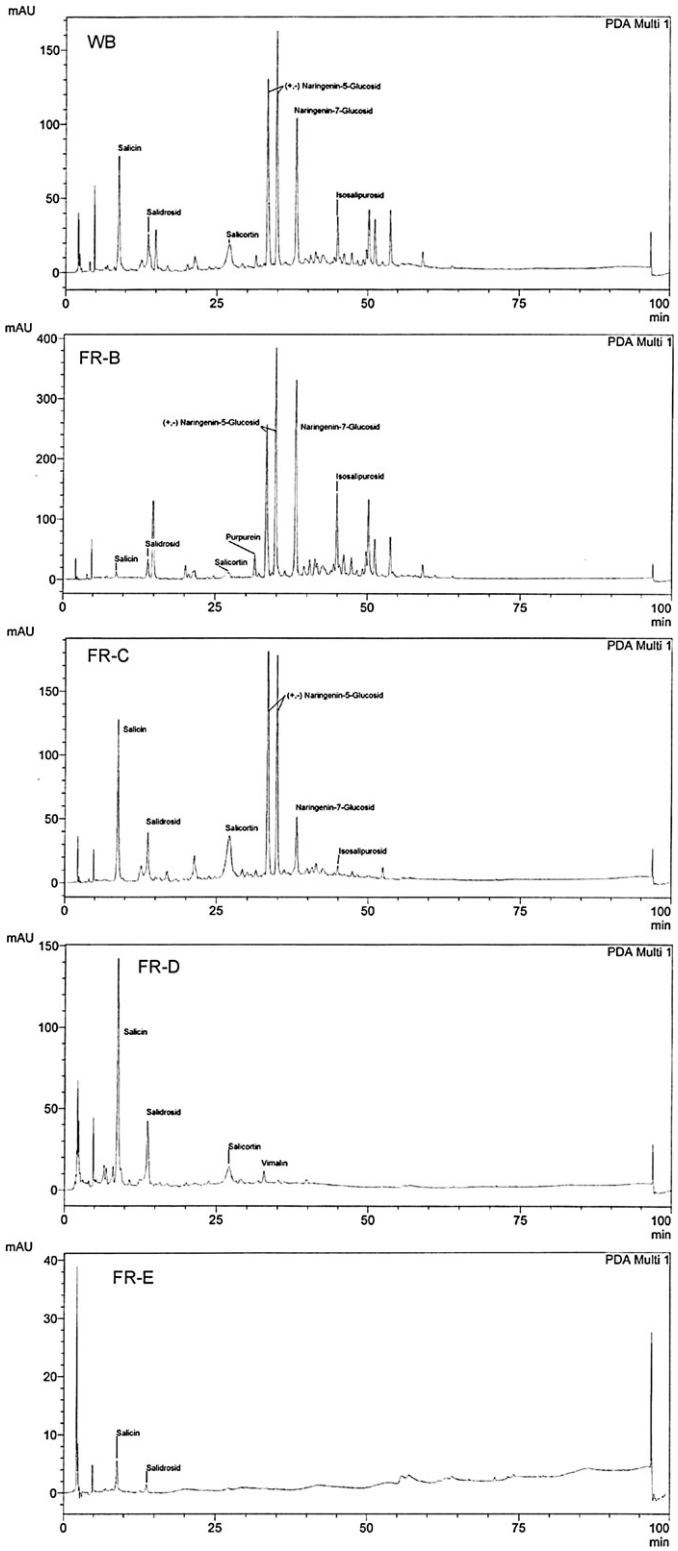

Fig. 1. HPLC-fingerprints of willow bark (WB) and the different isolated fractions (FR).

Preparation and characterization of the tested fractions

The investigated fractions were prepared as described in Freischmidt (2011) by application of partition and precipitation processes with ethyl acetate (Fr-B), butanol (Fr-C), ethanol (Fr-D) and water (FR-E) (Fig. 1).