A biolabel research based on metabonomics reveals the therapeutic potentials of Herba Lysimachiae in synovial diseases

Xu-zhao LI
Guizhou University of Traditional Chinese Medicine
Shuai-nan ZHANG (laurahoney@163.com)
Guizhou University of Traditional Chinese Medicine https://orcid.org/0000-0002-5530-9238
Qi LIU
Qiqihar Medical University
Yu-mei WANG
Qiqihar Medical University

Research

Keywords: Herba Lysimachiae; synovial diseases; biolabel; prostaglandin E1/E2; metabonomics analysis

Posted Date: July 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-41279/v1

Abstract

Background: Previous research has demonstrated that Herba Lysimachiae (HL) exerts the dual effects on platelet aggregation in the synovium, which may contribute to its protection against synovial lesions under different situations. However, the mechanism is unclear. In the present experiment, a biolabel research based on metabonomics was used to mine more information about the intervention of HL on synovium at the metabolite level, which may help to analyze the regulation of HL on synovial platelet aggregation and its possible treatment in synovial diseases.

Methods: Synovial metabolic profiling was analyzed using a Shimadzu Nexera UHPLC LC-30A system and an AB SCIEX Triple TOF 4600 mass spectrometer. Enzyme-linked immunosorbent assay was used to verify the biolabels analysis results.

Results: Totally, thirteen common metabolites were differentially expressed after treating with HL, and implicated in 2 key pathways (arachidonic acid metabolism and glycerophospholipid metabolism). ELISA showed that HL up-regulated the expression of prostaglandins E1 and E2 in synovial tissues.

Conclusions: This study reveals that HL may regulate the synovial platelet aggregation through prostaglandin E1/E2. Additionally, HL is suitable for treating synovial diseases, especially osteoarthritis, which may be associated with the platelet aggregation, apoptosis, inflammation, angiogenesis, and carcinogenesis processes.

Background

Synovial lesions are evident in a variety of diseases, such as rheumatoid arthritis [1], osteoarthritis [2], and gouty arthritis [3], etc. Synovial membrane supplies nutrients to cartilage and protects the joint structures [4], whose dysfunction is also one of the important inducers of cartilage damage in joint [5]. Additionally, synovial diseases have a high prevalence rate around the world (eg. 0.5-1% in rheumatoid arthritis [1] and 2–6% in osteoarthritis [6]). In traditional Chinese medicine theory, most synovial diseases belong to the category of rheumatism arthralgia syndrome, such as rheumatoid arthritis, osteoarthritis, and gouty arthritis, etc.

Herba Lysimachiae (HL), also called “zhuifengsan” in Chinese, is the herb applied in traditional medicinal systems of China, and has a history of application in treating rheumatic arthralgia [7]. Modern pharmacological studies have found that HL can play a certain protective role in synovial injury [8, 9]. Our previous proteomics research has also shown that [2], in a model of osteoarthritis, HL may produce the therapeutic effects similar to the positive drug (diclofenac sodium salt) by inhibiting synovial platelet aggregation. However, in physiological situations, HL may play a role similar to platelet-rich plasma to increase platelet count in the synovium, which may display a preventive role in the potential synovial damage. The dual effects of HL on synovial platelet aggregation may contribute to its protection against synovial injury under different conditions. However, the mechanism is unclear. The research information obtained from the analysis of protein data alone is restricted and inadequate, which requires the use of another level of research data to supplement the relevant content, in order to obtain more valuable information resources.

Metabonomics, the major components of systems biology, can be applied to explore the potential biolabels of drugs at the level of metabolic profiling [10, 11]. Biolabels are unique information groups composed of the drug-sensitive targets, which can be used to characterize the effects and mechanisms of drugs [2, 12]. Biolabel-led research pattern is an innovative research pattern to position the therapeutic potentials of Chinese material medicas (CMMs) in the physiological state [2]. This pattern can effectively explore the therapeutic scopes and advantages of CMMs, and make the direction and purpose of the follow-up researches clearer, so as to improve work efficiency and shorten the research cycle [12]. In the present experiment, based on biolabel-led research pattern, we used metabonomics to mine more information about the intervention of HL on synovium at the metabolite level. Based on the information, we may analyze the regulation of HL on synovial platelet aggregation and its possible treatment in synovial diseases [2, 12].

Materials And Methods

Plant material and extraction

The crude drug (the dried entire plant of *Lysimachia paridiformis* Franch. var. *stenophylla* Franch. (Primulaceae)) was obtained from Guanling County of Guizhou Province, PR China. The voucher specimen (GUCM-VS-2018-001) of the herb was authenticated by Prof. Xu-zhao LI, College of Pharmacy, Guizhou University of Traditional Chinese Medicine. The preparation of aqueous extract of HL was shown in our previous study [2]. The yield of the extract is 12.65% (w/w). The chromatographic profile of the same batch of extract is also shown in our previous study [2] and Supplementary Figure S1.

Animals and drug administration

All experimental treatments on the animals were performed in accordance with the Legislation on the European Council on Animal Care and Use of Experimental Animals (Directive 86/609/EEC) and permitted by the Ethics Committee of Institute of Radiation Medicine of Chinese Academy of Medical Sciences (Tianjin, PR China). All efforts were made to reduce animal suffering and the number of animals used. The health status of the experimental animals was monitored twice daily through the experiment period. Sprague Dawley rats (male, ~200 g) were purchased from Institute of Radiation Medicine of Chinese Academy of Medical Sciences (Tianjin, PR China). Twenty-four rats (n = 6) were randomly divided into control, HL low-dose, HL middle-dose, and HL high-dose groups. The low-, middle-, and high-dose groups were orally administrated with 0.11, 0.22, and 0.33 g/kg aqueous extract of HL, respectively, once daily for 7 days. The rats in control group were administrated with equal volume saline once a day for 7 days.

Sample collection

All operations were performed under sterile conditions. Following the anesthesia of rats with an intraperitoneal injection of 400 mg/kg chloral hydrate, synovial tissue samples were obtained from the joints of rats at 24 h after the last administration. All samples were immediately frozen in liquid nitrogen

following accurate weighing, and then stored at -80 ºC for LC (liquid chromatography)-MS (mass spectrometry) and analysis and enzyme-linked immunosorbent assay (ELISA).

LC-MS analysis

The frozen samples were added to 10 volumes of ice-cold methanol and homogenized with a tissue homogenizer for 90 s in the iced bath. The homogenate was centrifuged at 13,000 g for 15 min at 4 ºC. The supernatant was transferred into the Eppendorf tube and stored at -80 ºC until used.

Synovial metabolic profiling was analyzed using a Shimadzu Nexera UHPLC LC-30A system (Shimadzu Corporation, Kyoto, Japan) and an AB SCIEX Triple TOF 4600 mass spectrometer (AB SCIEX, USA). LC-MS analysis was performed according to our previous study [2].

The quality control (QC) samples were injected at regular intervals (every 10 samples) throughout the analytical run to provide a set of data from which repeatability can be analyzed.

Multivariate data analysis

Metabonomics data were acquired using the Analyst® TF 1.7.1 software (AB SCIEX, USA). The mass data were introduced to EZinfo 2.0 software (Waters corp., Milford, USA) for the principle component analysis (PCA) and orthogonal partial least-squares-discriminant analysis (OPLS-DA). Hierarchical clustering analysis was performed by the heatmap tool implemented in MetaboAnalyst (http://www.metaboanalyst.ca/MetaboAnalyst/). PCA, OPLS-DA, and hierarchical clustering analysis can be used to investigate the synovial metabolic profiling of control and HL-treated groups. Variable importance in the projection (VIP) ranks the overall contribution of each variable to the OPLS-DA model, and the variables with VIP≥1 are considered relevant for group discrimination. The one-way analysis of variance was calculated with the R Programming Language (version 3.4.2). Differentially expressed metabolites were selected in accordance with their VIP value (VIP≥1) and predefined P-value thresholds (p≤0.05).

Differentially expressed metabolites identification

Differentially expressed metabolites were identified using MasterView 1.0 software (AB SCIEX, USA). The MS and MS/MS data were introduced to the software and analyzed by a standard database (AB SCIEX LibraryView, Version 1.0) and an in-house built database derived from HMDB (http://www.hmdb.ca/).

Metabolic pathway analysis and biochemical network construction

Pathway analysis tool implemented in MetaboAnalyst was applied for the metabolic pathway analysis of differentially expressed metabolites. Biochemical network was constructed by Cytoscape software (version 3.6.1).

The regulation of prostaglandins E1 and E2 levels by HL

Prostaglandin E1 (E-EL-0052c) and E2 (E-EL-0034c) ELISA kits were purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, PR China). The frozen samples were homogenized in ice-cold PBS. The homogenate was centrifuged at 5,000 × g for 10 min at 4 °C, and the supernatant was removed in a tube for the next assay. The levels of prostaglandins E1 and E2 in the tissue homogenate were analyzed with the ELISA kits according to the manufacturer's protocol. Statistical analysis data were presented as mean ± standard deviation. The one-way analysis of variance was calculated with the R Programming Language (version 3.5.2). Statistical significance was accepted if $P < 0.05$.

Results

Metabonomics analysis and Differentially expressed metabolites identification

Two thousand six hundred and seventy-one metabolite ions (1890 in positive ion mode and 781 in negative ion mode) were acquired through LC-MS analysis. QC samples in PCA score plots (Supplementary Figure S2) clustered well, indicating good reproducibility of the sample process procedure and the instrumental system. Both OPLS-DA score plots and hierarchical clustering analysis (Figs. 1 and 2) showed significant separation between control and HL-treated groups, suggesting that biochemical perturbation significantly occurred in synovial tissues.

With the help of MasterView software, thirteen differentially expressed metabolites were identified and considered as the potential biolabels responsible for the interventions of HL on synovial tissue (Table 1). Compared with the control group, the levels of 6 metabolites were up-regulated, and 7 metabolites were down-regulated in HL-treated groups.

Table 1
Potential biolabels identified of HL.

NO.	HMDB ID	Metabolites	RT (min)	m/z	Adduct	Delta (ppm)	Ion mode	Fold change (Low-dose/Control)	Fold change (Middle-dose/Control)	Fold (Hig dose
1	HMDB0011714	Vanilpyruvic acid	8.03	209.0458	M-H	1.21	NEG	2.08	2.08	2.03
2	HMDB0010386	LysoPC(18:2(9Z,12Z))	15.66	520.3385	M + H	2.44	POS	0.61	0.50	0.55
3	HMDB0002656	Prostaglandin A1	15.73	335.2229	M-H	0.35	NEG	6.23	5.72	4.06
4	HMDB0010382	LysoPC(16:0)	16.07	518.3229	M + Na	2.29	POS	0.63	0.60	0.68
5	HMDB0008558	PC(22:1(13Z)/14:0)	18.50	788.6165	M + H	0.15	POS	356.68	250.15	273.
6	HMDB0002925	8,11,14-Eicosatrienoic acid	18.52	329.2472	M + Na	6.37	POS	0.48	0.57	0.43
7	HMDB0001043	Arachidonic acid	18.68	305.2472	M + Na	1.01	POS	1.69	1.56	1.67
8	HMDB0013422	PC(o-18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	18.72	854.5869	M + Cl	5.16	NEG	0.16	0.53	0.81
9	HMDB0008590	PC(22:2(13Z,16Z)/14:0)	18.79	830.5926	M + HCOOH-H	2.45	NEG	0.41	0.62	0.84
10	HMDB0005047	9E,11E-Octadecadienoic acid	18.82	281.2473	M + H	0.74	POS	0.62	0.68	0.49
11	HMDB0007880	PC(14:0/20:2(11Z,14Z))	19.88	758.5673	M + H	2.81	POS	0.22	0.20	0.15
12	HMDB0013468	SM(d18:0/22:3(10Z,13Z,16Z))	19.97	803.5984	M + Na	6.66	POS	38.95	83.56	34.9
13	HMDB0007881	PC(14:0/20:3(5Z,8Z,11Z))	19.98	756.5530	M + H	1.04	POS	122.85	169.28	196.
Delta: ppm error between the observed mass and the theoretical mass.										
Fold change: the ratio of the average level of metabolites in HL-treated group to that in control group.										
The metabolites were detected in (POS) positive and (NEG) negative ESI mode.										

Metabolic pathway analysis and biochemical network construction

Metabolic pathway analysis (Fig. 3) showed that the potential biolabels were involved in 6 pathways. Through an impact-value screen (impact-value 0.1), arachidonic acid metabolism and glycerophospholipid metabolism were selected as the significantly dysregulated pathways (Fig. 3). Biochemical network in Fig. 4 showed that the potential biolabels participated in 4 biochemical processes, that is, inflammation, apoptosis, platelet aggregation, and angiogenesis and carcinogenesis.

The levels of prostaglandins E1 and E2 in synovial tissues

The regulation of prostaglandins E1 and E2 levels by HL were tested to confirm the biolabels analysis results. Compared with the levels of prostaglandins E1 and E2 in control group (Fig. 5), those in low-dose group were increased by 157.4 and 206.4%, respectively; those in middle-dose group were increased by 243.8 and 290.6%, respectively; those in high-dose group were increased by 354.9 and 358.9%, respectively.

Discussion

To our knowledge, this is the first research using synovial metabonomic approach to explore the potential applications of HL in treating synovial diseases. According to the pathway analysis of MetaboAnalyst, HL mainly affected two key pathways, that is, arachidonic acid metabolism and glycerophospholipid metabolism. Previous studies have shown that both pathways are closely associated with some synovial diseases, such as osteoarthritis and rheumatoid arthritis, which are involved in the pathogenesis of both diseases [13–17]. Previous proteomics study shows that HL also affects 4 key protein pathways (ribosome, RNA transport, hematopoietic cell lineage, and mRNA surveillance pathway), which may participate in the degenerative synovial diseases [2]. Therefore, combined with the pathway analysis of both studies, we find that HL may have a therapeutic advantage for the treatment of osteoarthritis (one of the degenerative diseases in synovium). The speculation is consistent with the validation test results of previous research [2].

Combined with the previous proteomics and current metabonomics studies, we find that both protein and metabolite biolabels of HL may affect the platelet aggregation and apoptosis regulation processes in the synovium [2]. In addition, the present experiment also showed that HL can regulate the expression of some endogenous metabolites, which are involved in inflammation, angiogenesis, and carcinogenesis processes (Fig. 4). In the following sections, we briefly discuss the biochemical processes of these potential biolabels, which may be helpful to explore the therapeutic potentials of HL for synovial diseases.

The aggregation of platelets in the synovium is significant for the treatment of synovial diseases, which can release the growth factors and promote synovial tissue repair and regeneration [18, 19]. Our previous proteomics study has demonstrated that HL can significantly increase the levels of Gp1bb, Itga2b, and Itgb3 in physiological situation, which indicates that HL may promote platelet aggregation in the synovium [2]. In this respect, the data from the current experiment complemented our previous research. Arachidonic acid is the precursor of prostaglandin E2, an increase in whose level may facilitate prostaglandin E2 production. As shown in Fig. 5b, the levels of prostaglandin E2 are increased significantly in HL-treated groups, which can potentiate platelet

aggregation [20]. However, prostaglandin E1 may produce an opposite effect on prostaglandin E2 in this process, which inhibits platelet aggregation [21]. HL reduced the content of 8,11,14-eicosatrienoic acid in synovial tissue. The metabolite can be converted into prostaglandin E1, whose levels were increased significantly in HL-treated groups (Fig. 5a). From these, we known that HL may promote the conversion of 8,11,14-eicosatrienoic acid to prostaglandin E1. In addition, the up-regulation of arachidonic acid by HL can also induce prostaglandin E1 production [22]. Therefore, we speculate that the up-regulation of prostaglandin E1 may be one of the important molecular mechanisms by which HL inhibits platelet aggregation in synovial membrane of osteoarthritis model [2]. Combining the above description, through its regulation of prostaglandin E1/ prostaglandin E2, HL may selectively promote or inhibit platelet aggregation in synovial membrane under different situations. This may also explain our previous research results showing the dual effects of HL on synovial platelet aggregation in the joints of healthy rats and osteoarthritis rats [2].

Abundant mononuclear cells infiltration and overexpression of inflammatory factors are seen in synovial diseases including osteoarthritis and rheumatoid arthritis [23, 24]. The changes in the levels of vanilpyruvic acid, 8,11,14-eicosatrienoic acid, arachidonic acid, and prostaglandin A1 may participate in the mechanism of HL against synovial inflammation. Vanilpyruvic acid is a catecholamine metabolite, an increase in whose level may mean the accumulation of catecholamine in synovial tissue. One previous study has shown that catecholamine-producing cells are present in inflamed synovial tissue of osteoarthritis and rheumatoid arthritis, and produced catecholamines has strong anti-inflammatory activities [25]. Prostaglandin E1, converted from 8,11,14-eicosatrienoic acid, may modify the inflammatory response and suppress arthritis [26, 27]. However, prostaglandin E2 (one of the metabolites of arachidonic acid) may antagonize the effects of prostaglandin E1, which is an inflammatory mediator and triggers the inflammatory response in the synovium [28]. Additionally, synovial NF-κB plays a crucial role in synovial inflammation [29]. Prostaglandin A1, a potent inhibitor of NF-κB, also has anti-inflammatory effects [30]. From these data, HL may also have a bidirectional effect on synovial inflammation.

Apoptosis is one of the inducing factors of synovial diseases [31, 32]. The regulation of prostaglandin A1, 9E,11E-octadecadienoic acid, PC(o-18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)), PC(22:2(13Z,16Z)/14:0), and PC(14:0/20:2(11Z,14Z)) by HL may show the possibility of inhibiting synovial apoptosis. In addition to its anti-inflammatory activity, prostaglandin A1 also exerts anti-apoptotic effects via inhibiting the activity of NF-κB [33, 34]. 9E,11E-Octadecadienoic acid is a conjugated linoleic acid isomer, which can induce apoptosis [35]. Additionally, the phosphatidylcholines have pro-apoptotic potentials as well [36], the dysregulation of which in synovial tissue is associated with osteoarthritis [37]. Suppression of these four metabolites by HL might enhance the inhibition of prostaglandin A1on apoptosis. However, on the contrary, HL can significantly increase the contents of two phosphatidylcholines (PC(22:1(13Z)/14:0) and PC(14:0/20:3(5Z,8Z,11Z))), which might lead to apoptosis [36]. In addition, phosphatidylcholine can donate its phosphocholine to ceramide for the synthesis of sphingomyelin, abnormal metabolism of which may affect the level of sphingomyelin to some extent [36]. SM(d18:0/22:3(10Z,13Z,16Z)) is a sphingomyelin, the activation of whose pathway is also involved in the apoptosis process [38]. From these point of view, HL may produce the bidirectional effects on apoptosis, which was in line with the results of previous proteomics study [2]. Besides protein biolabels [2], endogenous metabolites may also be used to characterize the apoptosis regulation potentials of HL.

LysoPC(18:2(9Z,12Z)) and LysoPC(16:0) are the lysophospholipids, which have a role in lipid signaling by acting on lysophospholipid receptors. Lysophospholipids can promote angiogenesis [39]. Synovial angiogenesis is involved in the pathogenesis of various synovial diseases, such as osteoarthritis and rheumatoid arthritis [40]. Antiangiogenic drugs used in synovial diseases are available [41]. Additionally, lysophospholipids also have stimulatory roles in cancer progression and stimulate the proliferation/survival of cancer cells [39]. Therefore, the inhibitory effects of HL on these two lysophospholipids might be conducive to the inhibition of synovial angiogenesis and carcinogenesis.

In summary, through the regulation of prostaglandin E1 and E2, HL exerts the dual effects on platelet aggregation in the synovium. This may be helpful for the protection of HL from synovial lesions under different situations. Additionally, from the analysis at the level of metabolite, HL is suitable for treating synovial diseases, especially osteoarthritis. The treatment may be associated with the platelet aggregation, apoptosis, inflammation, angiogenesis, and carcinogenesis processes, which may be applied to update the information characterizing the treatment of HL on synovial diseases.

Abbreviations

CMM, Chinese material medica; **ELISA**, enzyme-linked immunosorbent assay; **HL**, Herba Lysimachiae; **LC**, liquid chromatography; **MS**, mass spectrometry; **OPLS-DA**, orthogonal partial least-squares-discriminant analysis; **PCA**, principle component analysis; **QC**, quality control; **VIP**, variable importance in the projection;

Declarations

Ethics approval and consent to participate

All experimental treatments on the animals were performed in accordance with the Legislation on the European Council on Animal Care and Use of Experimental Animals (Directive 86/609/EEC) and permitted by the Ethics Committee of Institute of Radiation Medicine of Chinese Academy of Medical Sciences (Tianjin, PR China).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This article is supported by the National Natural Science Foundation of China (81760784).

Declaration of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Experiment design, innovation of the experiments, and embellishment of the article by SZ and XL; drug administration by YW; high-throughput sequencing and content determination by QL; statistics by QL; data proofreading by XL; drafting of the manuscript by SZ and XL. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Li XZ, Zhang SN. Herbal compounds for rheumatoid arthritis: Literatures review and cheminformatics prediction. Phytother Res. 2020;34: 51-66.
2. Li XZ, Huang HJ, Zhang SN, Liu Q, Wang YM. Label-free quantitative proteomics positions the effects and mechanisms of Herba Lysimachiae on synovial diseases based on biolabel-led research pattern. J Chromatogr B Analyt Technol Biomed Life Sci. 2020;1138: 121969.
3. Lu F, Liu L, Yu DH, Li XZ, Zhou Q, Liu SM. Therapeutic effect of Rhizoma Dioscoreae Nipponicae on gouty arthritis based on the SDF-1/CXCR 4 and p38 MAPK pathway: an in vivo and in vitro study. Phytother Res. 2014;28: 280-8.
4. Pan L, Zhang Y, Chen N, Yang L. Icariin Regulates Cellular Functions and Gene Expression of Osteoarthritis Patient-Derived Human Fibroblast-Like Synoviocytes. Int J Mol Sci. 2017;18: E2656.
5. Fell HB, Jubb RW. The effect of synovial tissue on the breakdown of articular cartilage in organ culture. Arthritis Rheum. 1977;20: 1359-71.
6. Hsu H, Siwiec RM. Knee Osteoarthritis StatPearls [Internet]ed. Treasure Island (FL): StatPearls Publishing, 2019.
7. FDA of Guizhou Province. Herba Lysimachiae (Zhuifengsan) In:Quality standards for Chinese and ethnic material medicas of guizhou provinceed. Guiyang, China: GUIZHOU SCIENCE AND TECHNOLOGY PRESS, 2003: 290.
8. Xie HQ, Zhao B, Deng LL, Huang ZY, Zhang YH. Study on the anti-inflammatory activity of Lysimachia paridiformis Franch. var. stenophylla Franch. Lishizhen Med Mater Med Res. 2013;24: 1847-9.
9. Qi LY, Yu JP, Tian J, Sun CL, Zheng QX. Studies on Anti-rheumatism Activity of Total Flavonoids from Lysimachia paridiformis Franch var. stenophylla Franch. Trad Chin Drug Res Clin Pharm. 2010;21: 369-72.
10. Zhang SN, Li XZ, Liu SM, Lu F. Metabonomic Study of the Effects of Acanthopanax senticosus on Peripheral System of Rats. Planta Med. 2015;81: 722-32.
11. Zhang SN, Li XZ, wang Y, zhang N, Yang ZM, Liu SM, et al. Neuroprotection or neurotoxicity? new insights into the effects of Acanthopanax senticosus harms on nervous system through cerebral metabolomics analysis. J Ethnopharmacol. 2014;156: 290-300.
12. Li XZ, Zhu KY, Zhang SN. Exploration of Biolabel: A New Pattern For The Study On The Nature Of Chinese Materia Medica. World Sci Tech-Modernization Trad Chin Med Mater Med. 2019;21: 14-8.
13. Carlson AK, Rawle RA, Adams E, Greenwood MC, Bothner B, June RK. Application of global metabolomic profiling of synovial fluid for osteoarthritis biomarkers. Biochem Biophys Res Commun. 2018;499: 182-8.
14. Kim S, Hwang J, Kim J, Ahn JK, Cha HS, Kim KH. Metabolite profiles of synovial fluid change with the radiographic severity of knee osteoarthritis. Joint Bone Spine. 2017;84: 605-10.
15. Sowers MR, McConnell D, Jannausch M, Buyuktur AG, Hochberg M, Jamadar DA. Estradiol and its metabolites and their association with knee osteoarthritis. Arthritis Rheum. 2006;54: 2481-7.
16. Langholz E, Nielsen OH, Ahnfelt-Ronne I, Elmgreen J. Arachidonic acid metabolism in neutrophil granulocytes obtained from synovial fluid in rheumatoid arthritis. Scand J Rheumatol. 1990;19: 387-91.
17. Smith DM, Johnson JA, Turner RA. Alterations in arachidonic acid metabolism and chemotactic response in polymorphonuclear leukocytes from patients with rheumatoid arthritis. Clin Exp Rheumatol. 1989;7: 471-7.
18. Tong S, Liu J, Zhang C. Platelet-rich plasma inhibits inflammatory factors and represses rheumatoid fibroblast-like synoviocytes in rheumatoid arthritis. Clin Exp Med. 2017;17: 441-9.
19. Andia I, Maffulli N. Platelet-rich plasma for managing pain and inflammation in osteoarthritis. Nat Rev Rheumatol. 2013;9: 721-30.
20. Vezza R, Roberti R, Nenci GG, Gresele P. Prostaglandin E2 potentiates platelet aggregation by priming protein kinase C. Blood. 1993;82: 2704-13.

21. Sinha AK, Colman RW. Prostaglandin E1 inhibitis platelet aggregation by a pathway independent of adenosine 3',5'-monophosphate. Science. 1978;200: 202-3.
22. Athanassakis I, Dionyssopoulou E, Papanikou S, Evangeliou A, Vassiliadis S. Early events of the exogenously provided L-Carnitine in murine macrophages, T- and B-lymphocytes: modulation of prostaglandin E1 and E2 production in response to arachidonic acid. J Nutr Biochem. 2003;14: 350-7.
23. Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis. 2005;64: 1263-7.
24. Sweeney SE, Firestein GS. Rheumatoid arthritis: regulation of synovial inflammation. Int J Biochem Cell Biol. 2004;36: 372-8.
25. Capellino S, Cosentino M, Wolff C, Schmidt M, Grifka J, Straub RH. Catecholamine-producing cells in the synovial tissue during arthritis: modulation of sympathetic neurotransmitters as new therapeutic target. Ann Rheum Dis. 2010;69: 1853-60.
26. Moriuchi-Murakami E, Yamada H, Ishii O, Kikukawa T, Igarashi R. Treatment of established collagen induced arthritis with prostaglandin E1 incorporated in lipid microspheres. J Rheumatol. 2000;27: 2389-96.
27. Zurier RB, Quagliata F. Effect of prostaglandin E 1 on adjuvant arthritis. Nature. 1971;234: 304-5.
28. Wang H, Dong BW, Zheng ZH, Wu ZB, Li W, Ding J. Metastasis-associated protein 1 (MTA1) signaling in rheumatoid synovium: Regulation of inflammatory response and cytokine-mediated production of prostaglandin E2 (PGE2). Biochem Biophys Res Commun. 2016;473: 442-8.
29. Muller-Ladner U, Gay RE, Gay S. Role of nuclear factor kappaB in synovial inflammation. Curr Rheumatol Rep. 2002;4: 201-7.
30. Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, et al. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature. 2000;403: 103-8.
31. Aigner T, Kim HA, Roach HI. Apoptosis in osteoarthritis. Rheum Dis Clin North Am. 2004;30: 639-53, xi.
32. Nishioka K, Hasunuma T, Kato T, Sumida T, Kobata T. Apoptosis in rheumatoid arthritis: a novel pathway in the regulation of synovial tissue. Arthritis Rheum. 1998;41: 1-9.
33. Wang X, Qin ZH, Leng Y, Wang Y, Jin X, Chase TN, et al. Prostaglandin A1 inhibits rotenone-induced apoptosis in SH-SY5Y cells. J Neurochem. 2002;83: 1094-102.
34. He JK, Gu ZL, Fan PS. Inhibitory effects of prostaglandin A1 on apoptosis of rat cardiac microvascular endothelial cells was mediated by NF-kappaB. Acta Pharmacol Sin. 2002;23: 331-5.
35. Miner JL, Cederberg CA, Nielsen MK, Chen X, Baile CA. Conjugated linoleic acid (CLA), body fat, and apoptosis. Obes Res. 2001;9: 129-34.
36. Wright MM, Howe AG, Zaremberg V. Cell membranes and apoptosis: role of cardiolipin, phosphatidylcholine, and anticancer lipid analogues. Biochem Cell Biol. 2004;82: 18-26.
37. Zhang WD, Sun G, Likhodii S, Aref-Eshghi E, Harper PE, Randell E, et al. Metabolomic analysis of human synovial fluid and plasma reveals that phosphatidylcholine metabolism is associated with both osteoarthritis and diabetes mellitus. Metabolomics. 2016;12: 24.
38. Andrieu-Abadie N, Levade T. Sphingomyelin hydrolysis during apoptosis. Biochim Biophys Acta. 2002;1585: 126-34.
39. Ishii I, Fukushima N, Ye X, Chun J. Lysophospholipid receptors: signaling and biology. Annu Rev Biochem. 2004;73: 321-54.
40. Brown RA, Tomlinson IW, Hill CR, Weiss JB, Phillips P, Kumar S. Relationship of angiogenesis factor in synovial fluid to various joint diseases. Ann Rheum Dis. 1983;42: 301-7.
41. Szekanecz Z, Besenyei T, Paragh G, Koch AE. New insights in synovial angiogenesis. Joint Bone Spine. 2010;77: 13-9.

Figures

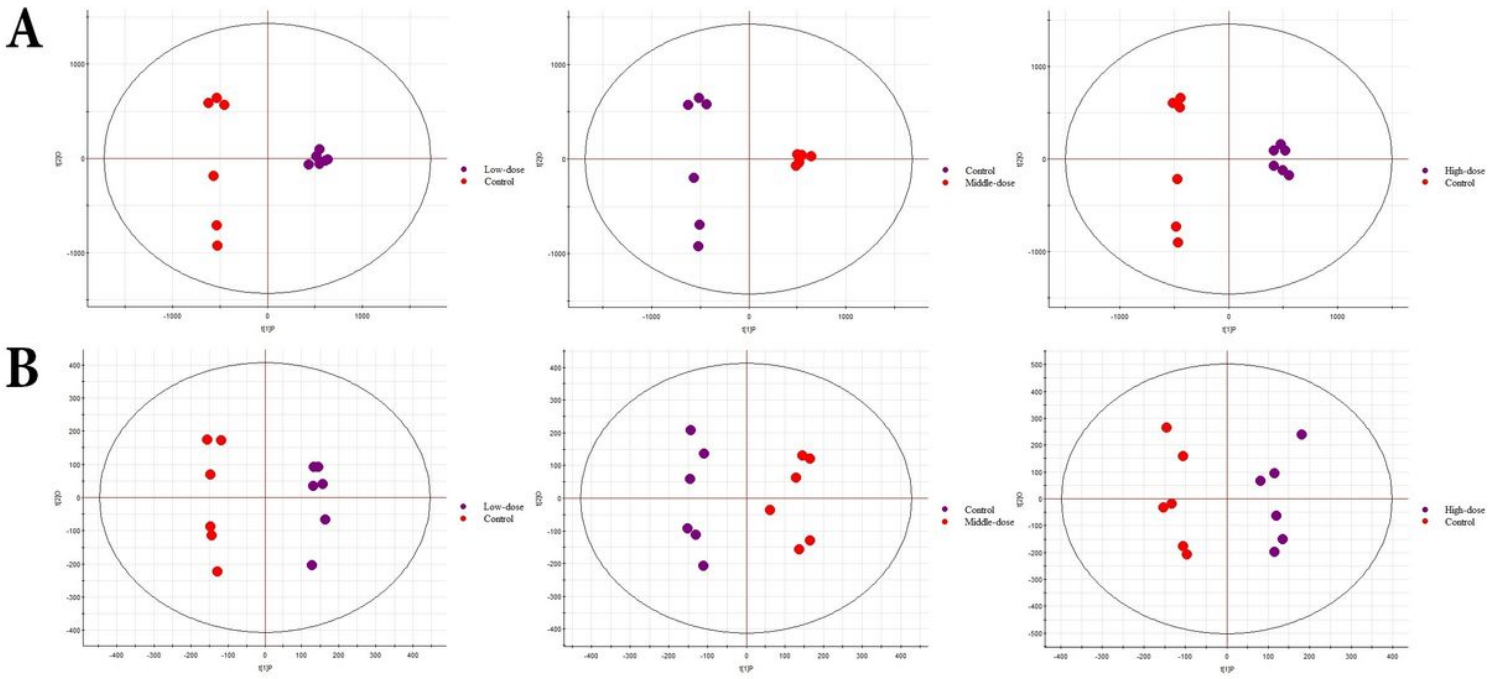

Figure 1

OPLS-DA score plots based on the synovial metabolic profiling of control and HL-treated groups in (A) positive and (B) negative ion modes.

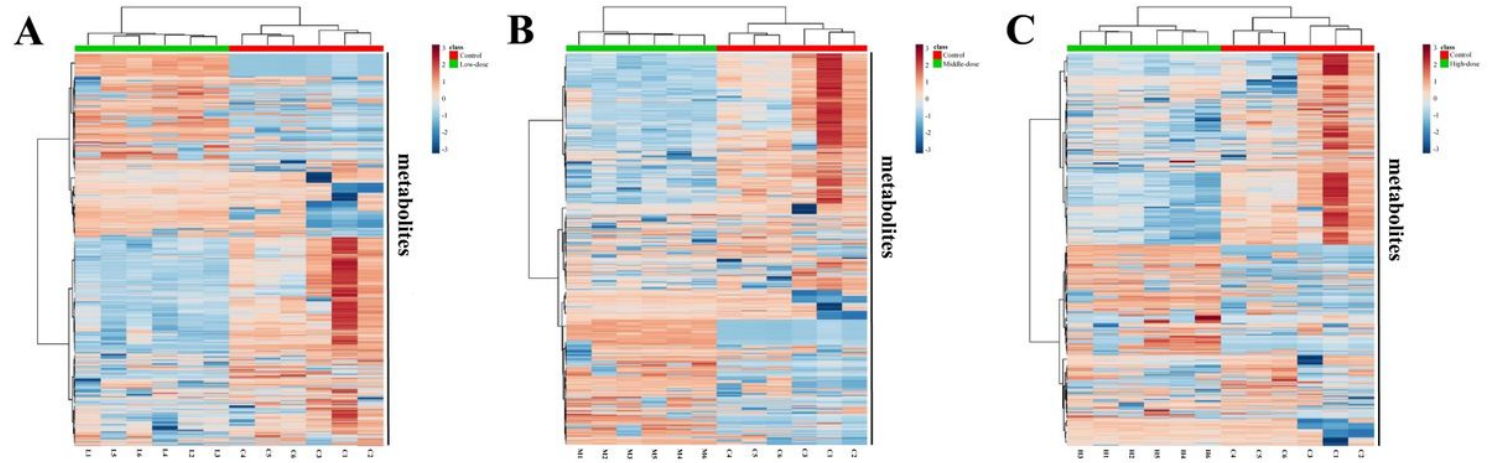

Figure 2

Hierarchical clustering analysis for endogenous metabolites expression profile. Heat maps show endogenous metabolites profiles that differentiate (A) low-dose, (B) middle-dose, or (C) high-dose groups from control group. Rows: metabolites; Columns: samples; Color key indicates metabolite expression value, blue: lowest; red: highest.

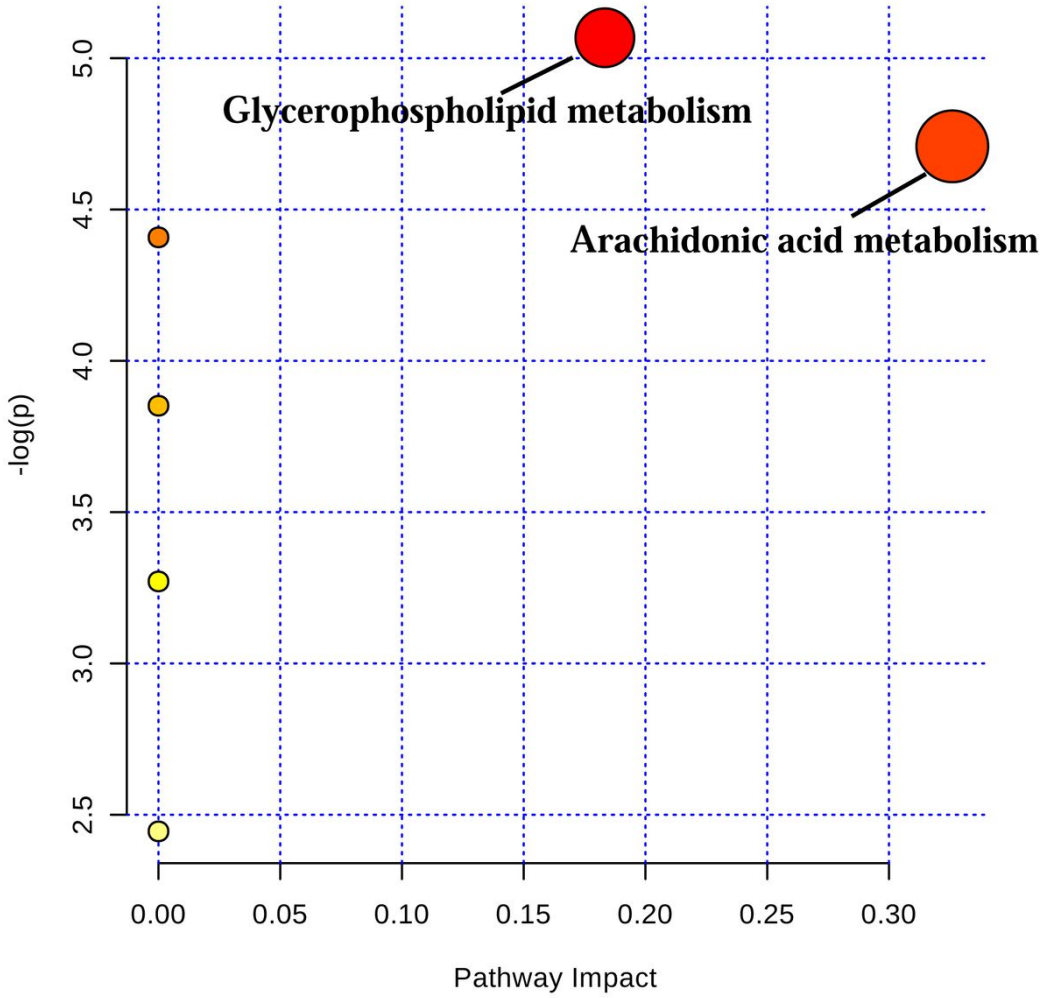

Figure 3

Metabolic pathway analysis by MetaboAnalyst. Through an impact-value screen (impact-value≥0.1), arachidonic acid metabolism and glycerophospholipid metabolism were selected as the significantly dysregulated pathways.

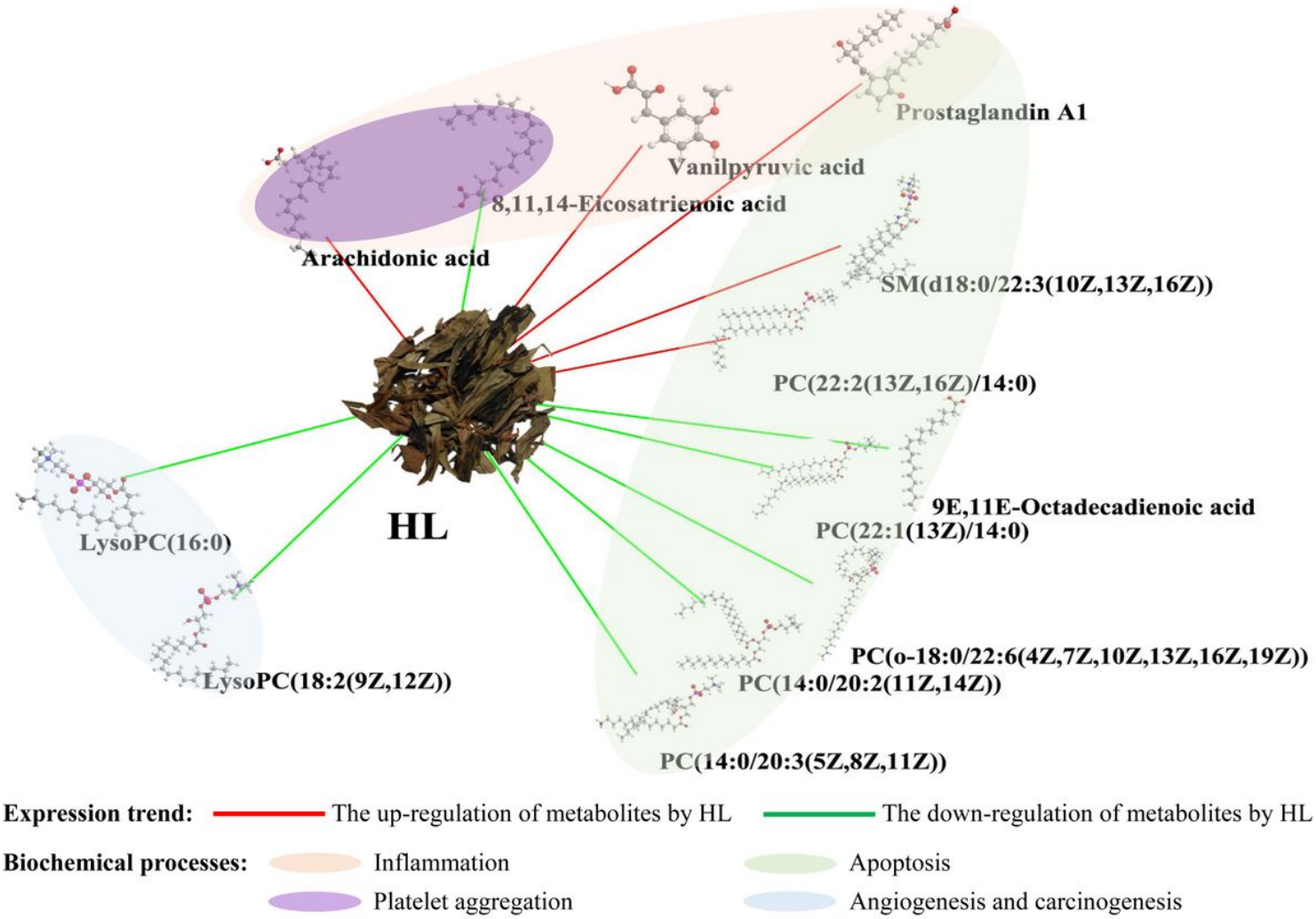

Figure 4

The network of potential biolabels in response to HL treatment.

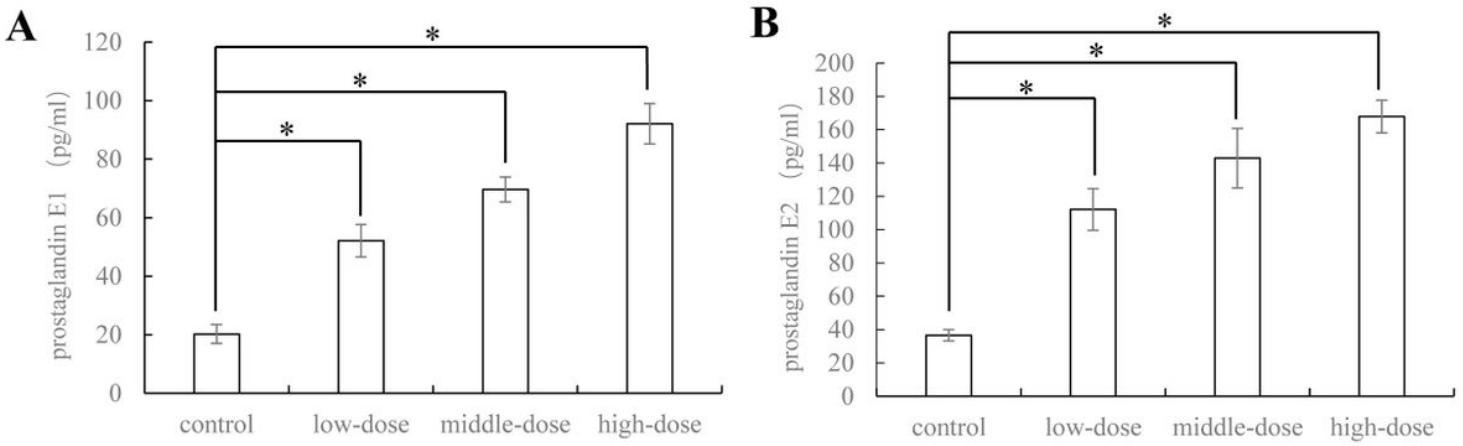

Figure 5

The levels of prostaglandins E1 and E2 in synovial tissues. Data in histograms are mean ± standard deviation (n = 6 rats/group). Values are from three independent experiments. *P < 0.05 vs control group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigureS2.jpg
- SupplementaryFigureS2.jpg
- SupplementaryFigureS2.jpg
- SupplementaryFigureS1.tif
- SupplementaryFigureS1.tif
- SupplementaryFigureS1.tif
- Supplementaryfigurelegends.doc
- Supplementaryfigurelegends.doc
- Supplementaryfigurelegends.doc