Pharmacometabolomic Study of Novel Multitarget Drugs Based on Natural Prostaglandins in Terms of Therapeutic Effectiveness

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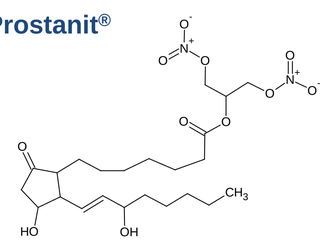
INTRODUCTION

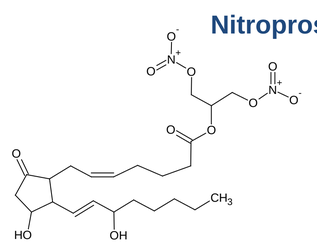
Prostanit®



In last few years multi-target drugs have gained high popularity at the drug development market. Novel biogenic molecules, prostanit® and nitroproston®, represent an interesting example of multi-target compounds. They are based on natural prostaglandins PGE1 (prostanit®) and PGE2 (nitroproston®) linked by a glycerol moiety to two nitric oxide (NO) – donating fragments. Despite of strong similarity in chemical structures of these drugs, their action is focused on different targets: Prostanint® was developed to serve from cardiovascular diseases, while Nitroproston® was described as an anti-asthmatic drug.

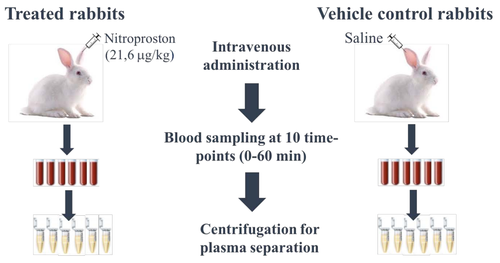
The biogenic nature of these drugs and rapid integration of their active compounds into biochemical cycles, makes difficult the identification of the metabolic pathways involved. Here we investigated the effects of Nitroproston® and Prostanit® administration on plasma metabolomic profiling *in vivo* [2]. To overcome these complexities, an untargeted and targeted metabolomic approaches were used, giving an opportunity not only to investigate metabolic pathways of the aforementioned substances, but also to understand mechanisms of their action and to discover clinical safety and efficacy. Thus, in this study we investigated the effects of nitroproston® and prostanit® administration on plasma metabolomic profiles of rabbits in time.

Nitroproston®



METHODS

1. Treatment and preparation of samples

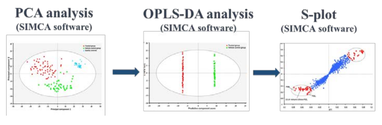


2. Untargeted metabolomics

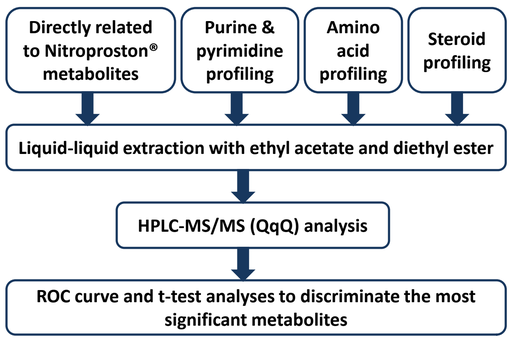
1. Protein precipitation

2. UPLC-IT-TOF/MS Analysis and preprocessing

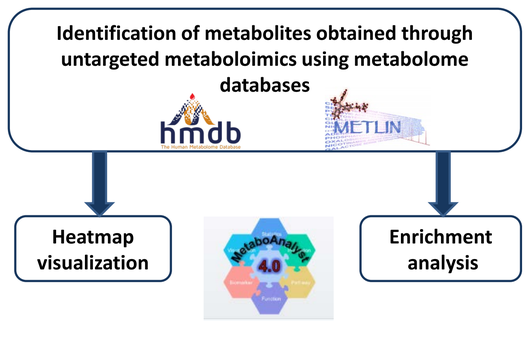
3. Statistical analysis



3. Targeted metabolomics



4. Data analysis



RESULTS & DISCUSSION

Prostanit®

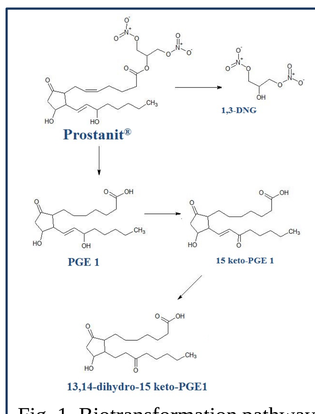


Fig. 1. Biotransformation pathway of Prostanit ®

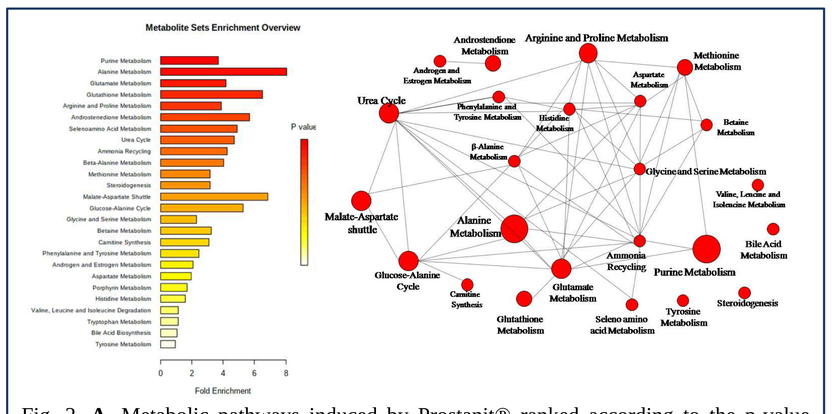


Fig. 2. **A.** Metabolic pathways induced by Prostanit® ranked according to the p-value corresponded the number of metabolites involved in each pathway. At higher color red higher level of significance. **b** Network metabolomics visualization. Lines represent connections between pathways based on available evidence.

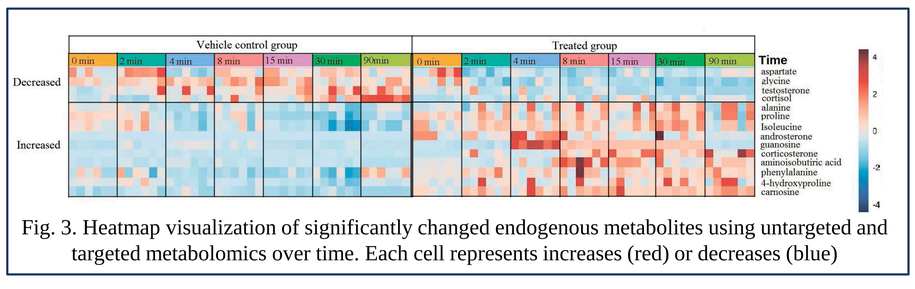


Fig. 3. Heatmap visualization of significantly changed endogenous metabolites using untargeted and targeted metabolomics over time. Each cell represents increases (red) or decreases (blue)

Nitroproston®

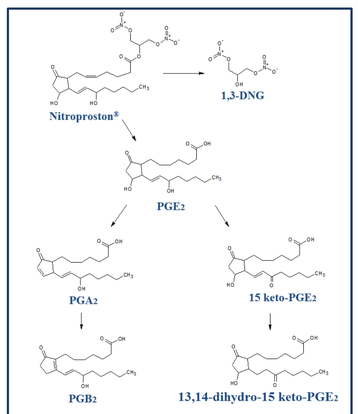


Fig. 4. Biotransformation pathway of Nuitroproston®

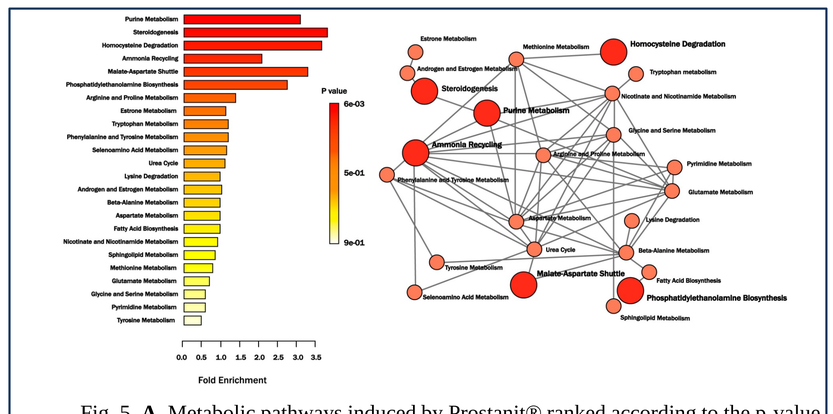
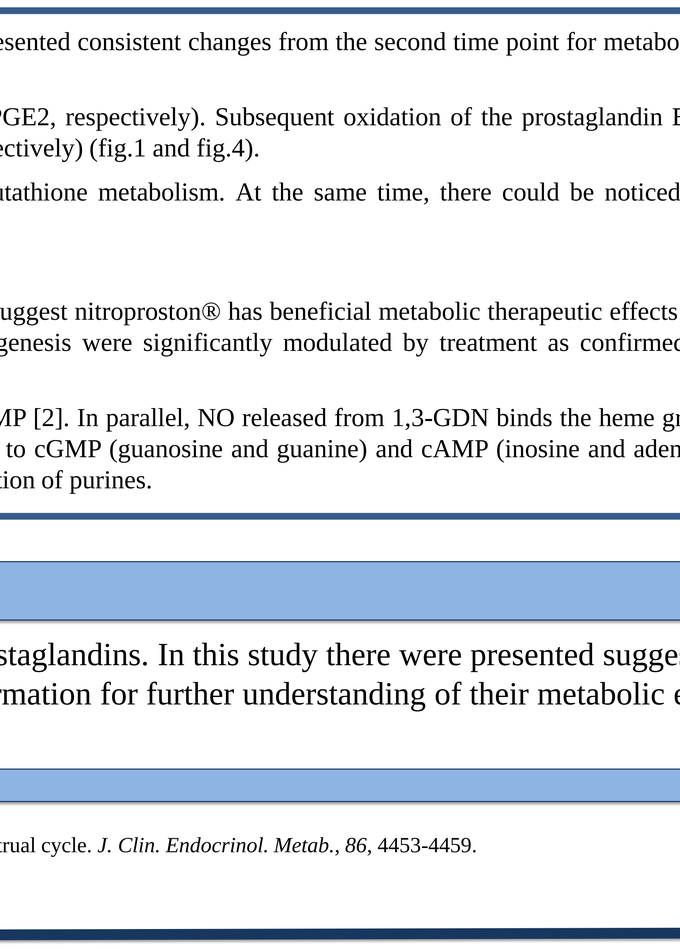


Fig. 5. **A.** Metabolic pathways induced by Prostanit® ranked according to the p-value corresponded the number of metabolites involved in each pathway. At higher color red higher level of significance. **b** Network metabolomics visualization. Lines represent connections between pathways based on available evidence.

Fig. 6. Heatmap visualization of significantly changed endogenous metabolites using untargeted and targeted metabolomics over time. Each cell represents increases (red) or decreases (blue)



- The heatmap visualization (Fig.3 and 6) showed clear discrimination between the vehicle control and treated groups for all metabolites; the treated group presented consistent changes from the second time point for metabolites that increased and decreased after treatment, demonstrating the effect of the administration drugs over time.
- Prostanit® and Nitroproston® undergo rapid hydrolysis resulted in formation of there two main components: 1,3-GDN and Prostglandin E (PGE1 and PGE2, respectively). Subsequent oxidation of the prostaglandin E by NAD+-dependent 15-hydroxy prostaglandin dehydrogenase leads to its conversion to 15-keto-PGE and 13,14-dehydro-15-keto-PGE (PGE1 and PGE2, respectively) (fig.1 and fig.4).
- We identified that the most significantly changed metabolic pathways, induced after Prostanit® administration were: purine, alanine, glutamate and glutathione metabolism. At the same time, there could be noticed the activation of oxidation processes (e.g. transformation of proline to hydroxyproline) (Fig.2).
- Nitroproston ® impacted steroidogenesis, purine metabolism, malate-aspartate shuttle and ammonia recycling (fig. 5).
- Multiple metabolites identified in our study connected to Nitroproston® (Fig. 6) have been previously considered as having anti-asthma properties [4]. We suggest nitroproston® has beneficial metabolic therapeutic effects and serves as an effective anti-asthmatic drug acting on several targets related to asthma and bronchopulmonary diseases. Purine metabolism and steroidogenesis were significantly modulated by treatment as confirmed by significant ($p < 0.05$) influence on enrichment analysis (Fig. 5).
- The mechanism of action of Nitroproston® can be explained by specific binding of released PGE2 to EP2 and EP4 receptors that induce the activity of cAMP [2]. In parallel, NO released from 1,3-GDN binds the heme group of soluble guanylate cyclase that leads to subsequent elevation in the concentration of cyclic guanosine monophosphate (cGMP) [5]. Intermediates related to cGMP (guanosine and guanine) and cAMP (inosine and adenine) pathways were significantly decreased in the treated group, while the final products xanthine and uric acid increased, what can be explained by higher utilization of purines.

CONCLUSIONS

To the best of our knowledge this study is the first that describes the metabolic profiles of novel drugs based on natural prostaglandins. In this study there were presented suggested mechanisms of action and metabolic pathway interactions of novel drugs, Prostanit® and Nitroproston®, as well as useful information for further understanding of their metabolic effects and therapeutic effectiveness.

References:

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[3] Horgan R.P. & Kenny L.C. (2011) 'Omic'technologies: genomics, transcriptomics, proteomics and metabolomics. *The Obstetrician & Gynaecologist. 13*,189–195.
[4] Comhair S.A.A., Jonathan McDunn J., Bennett C., Fettig J., Erzurum S.C. & Kalhan S.C. (2015) Metabolomic Endotype of Asthma. J. Immunol, 195, 643-650.
[5] Jerca, L., Jerca, O., Mancas, G., Constantinescu, I. & Lupusoru, R. (2002) Mechanism of action and biochemical effects of nitric oxide. *The journal of preventive medicine.* 2002, *10*, 35-45.

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