Scelta dei principi attivi e delle migliori forme biofarmaceutiche

A. Fratter

Resp. Ricerca e Innovazione Tecnologica, Labomar Research, Istrana (Treviso)
Docente Corso di Perfezionamento in Farmacia e Farmacologia Cliniche, Università di Padova
**Biodisponibilità’**

Alcuni esempi:

- **Coenzima Q10**: assorbito dall’intestino solo se in forme solubilizzate con Polisorbato!!! *Scarso assorbimento da compresse, capsule ecc. in assenza di specifici promotori (Polisorbato 80)*;

- **EPA+DHA**: meglio assorbiti se in forme gastro-resistenti e se assunti da matrici emulsionate;

- **Quercetina diidrato**: flavonolo con ottime proprietà antistaminiche e antiflogistiche, antipertensive, ma buona biodisponibilità solo da *succo concentrato di aglio, oppure aglicone veicolato con dei MCT*;

- **Resveratrolo**: <1% in circolo immodificato se assunto come molecola isolata (Estratti di Poligonum 98-99%) tramite via intestinale (alternativa la via sublinguale oppure *circa 3 Litri di vino rosso*);

- **Berberina HCl**: quasi completamente espulsa dall’enterocita ad opera della PgP: ridotta biodisponibilità per OS;

- **Acidi boswellici**: terpeni totalmente liposolubili e insolubili in acqua; ridotto assorbimento enterico;
RESVERATROLO: <1% in circolo immodificato se assunto come molecola isolata (Estratti di Poligonum 98-99%) tramite via intestinale (alternativa la via sublinguale oppure circa 3 Litri di vino rosso);
Bioavailability of resveratrol.

Walle T.

Source
Department of Pharmacology, Medical University of South Carolina, Charleston, 29425, USA. wallet@musc.edu

Abstract
This paper reviews our current understanding of the absorption, bioavailability, and metabolism of resveratrol, with an emphasis on humans. The oral absorption of resveratrol in humans is about 75% and is thought to occur mainly by transepithelial diffusion. Extensive metabolism in the intestine and liver results in an oral bioavailability considerably less than 1%. Dose escalation and repeated dose administration of resveratrol does not appear to alter this significantly. Metabolic studies, both in plasma and in urine, have revealed major metabolites to be glucuronides and sulfates of resveratrol. However, reduced dihydroresveratrol conjugates, in addition to highly polar unknown products, may account for as much as 50% of an oral resveratrol dose. Although major sites of metabolism include the intestine and liver (as expected), colonic bacterial metabolism may be more important than previously thought. Deconjugation enzymes such as β-glucuronidase and sulfatase, as well as specific tissue accumulation of resveratrol, may enhance resveratrol efficacy at target sites. Resveratrol analogs, such as methylated derivatives with improved bioavailability, may be important in future research.
Meccanismi fisiologici che ostacolano l’assorbimento intestinale di principi attivi vegetali

- Lo spessore delle secrezioni mucose che possono intrappolare il principio attivo e renderlo indisponibile alla penetrazione (UWP);
- La metabolizzazione ad opera dei citocromi CYP3A (presenti nell’enterocita dei villi e nei microsomi epatici);
- L’estrusione da parte della P-glicoproteina (presente nell’enterocita dei villi);
- La natura peptidica delle sostanze (idrolisi acido-proteolitica gastrica);
- Ridotto flusso trans-murale da tight junctions enterica;
- Biotrasformazione da parte della flora saprofita enterica (flavonoidi);

**Fig. 1.** Modello di assorbimento enterico di sostanze assunte per OS.
Pharmacology of Intestinal Permeation II

Editor T.Z. Csáky

Springer-Verlag
Berlin Heidelberg New York Tokyo

Fig. 1. Location of hydrolysis of nutrients during different types of digestion. 1, extracellular fluid; 2, intracellular fluid; 3, intracellular vacuole; 4, lysosomes; 5, nucleus; 6, cell membrane; 7, enzymes; 8, substrates and their hydrolysis products. UGOLEV et al. (1979a)

Fig. 3. Schematic representation of the intestinal permeability barriers.

\[ J = \frac{(c_1 - c_2)D}{d} \]
Tight Junction

- Strutture proteiche con funzione di adesione cellulare, ancoraggio alle strutture del citoscheletro, polarizzazione cellulare e ostacolo al passaggio paracellulare di molecole esogene;

- Presenti nell’epidermide, cellule intestinali, endotelio capillare, tubuli renali, BEE;
Figure 2 Transmission electron micrographs of the tight junction region in ileal enterocytes. Specimens were fixed after exposure to vehicle or sodium caprate (C10) in Ussing chambers. (A) Cell membranes of adjacent cells in close apposition in a tight junction exposed to vehicle (arrows). (B) Tight junction with dilatation (arrows) after exposure to C10. Bars indicate 0.2 µm. C10 induced an increased frequency of dilatations within tight junctions (37%) versus vehicle experiments (5%).
P-gP

- Glicoproteina con funzione di pompa;
- Estrude principi attivi esogeni dall’enterocita;
- Chiamata anche Multi Drug Resistance Glycoprotein;
- Farmaci antiblastici, antibiotici, antiretrovirali, glucocorticoidi;
- Inibita da polifenoli da Grape Seed, Vitis Vinifera e Citrus Paradisi;

Simplified cartoon of P-glycoprotein structure and function: The P-glycoprotein molecule spans the cell membrane and in this way is in contact not only with the membrane but also the inside and the outside of the cell. The central portion of the molecule is a channel or pore through which toxic chemicals are pumped back out into the environment. The toxic chemicals can enter the transport pore either from the interior of the cell or from its membrane as shown. Molecules of ATP power the pumping action. Edwards Filaria Journal 2003 2(Suppl 1):S8 doi:10.1186/1475-2883-2-S1-S8
Sostanze che modulano i meccanismi di passaggio intestinale

- Chitosano e chitosano modificato (interazione con TJ, aumento del flusso apicale-latero basale);
- N-AcetilCisteina, agisce da mucolitico;
- Polifenoli da pompelmo e Vitis Vinifera (interazione con P-gp, CYP 3A);
- Pepe nero (Piperina, aumento della permeabilità dell’enterocita);
- Tensioattivi (Polisorbato 80, sali degli acidi biliari);
Effetti di altre sostanze sul tadalafil

*Inibitori del citocromo P450*

Il tadalafil è metabolizzato principalmente dal CYP3A4. Un inibitore selettivo del CYP3A4, il ketoconazolo (200 mg al giorno), ha aumentato di 2 volte l'esposizione (AUC) e del 15 % la $C_{\text{max}}$ del tadalafil (10 mg) rispetto ai valori dell'AUC e della $C_{\text{max}}$ del tadalafil da solo. Il ketoconazolo (400 mg al giorno) ha aumentato di 4 volte l'esposizione (AUC) e del 22 % la $C_{\text{max}}$ del tadalafil (20 mg). Un inibitore delle proteasi, il ritonavir (200 mg due volte al giorno) che è un inibitore del CYP3A4, CYP2C9, CYP2C19 e CYP2D6, ha aumentato di 2 volte l'esposizione (AUC) e non ha modificato la $C_{\text{max}}$ del tadalafil (20 mg). Sebbene le interazioni specifiche non siano state studiate, altri inibitori delle proteasi, come il saquinavir, e altri inibitori del CYP3A4, come l’eritromicina, la claritromicina, l’itraconazolo e il succo di pompelmo devono essere somministrati insieme con cautela poiché è prevedibile che aumentino le concentrazioni plasmatiche del tadalafil (vedere paragrafo 4.4). Di conseguenza, l’incidenza delle reazioni avverse elencate nel paragrafo 4.8 potrebbe aumentare.
Berberina
Principali proprietà salutistiche (terapeutiche?) della berberina

-Alcaloide estratto da piante del genere Berberis (Berberis aistata);

-Azione antinfiammatoria;

-Aumento della tolleranza e riduzione della neurotossicità del glucosio;

-Azione epato-protettiva;

-Azione cardio-protettiva;

-Riduzione LDL plasmatiche;

*queste attività sono state a vario livello confermate da studi scientifici pubblicati su riviste indicizzate e impattate su PUBMED, SCOPUS, EMBASE
Efficacy of Berberine in Patients with Type 2 Diabetes

Jun Yin\textsuperscript{a,b,*}, Huili Xing\textsuperscript{a}, and Jianping Ye\textsuperscript{b}

\textsuperscript{a}Department of Endocrinology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai 200092, China

\textsuperscript{b}Pennington Biomedical Research Center, Baton Rouge, Louisiana 70808, U.S.A.

Abstract

Berberine has been shown to regulate glucose and lipid metabolism in vitro and in vivo. This pilot study was to determine the efficacy and safety of berberine in the treatment of type 2 diabetic patients. In study A, 36 adults with newly diagnosed type 2 diabetes were randomly assigned to treatment with berberine or metformin (0.5 g t.i.d.) in a 3-month trial. The hypoglycemic effect of berberine was similar to that of metformin. Significant decreases in hemoglobin A1c (HbA\textsubscript{1c}; from 9.5% ± 0.5% to 7.5% ± 0.4%, \(P<0.01\)), fasting blood glucose (FBG; from 10.6 ± 0.9 mmol/L to 6.9 ± 0.5 mmol/L, \(P<0.01\)), postprandial blood glucose (PBG; from 19.8 ± 1.7 to 11.1 ± 0.9 mmol/L, \(P<0.01\)) and plasma triglycerides (from 1.13 ± 0.13 mmol/L to 0.89 ± 0.03 mmol/L, \(P<0.05\)) were observed in the berberine group. In study B, 48 adults with poorly controlled type 2 diabetes were treated supplemented with berberine in a 3-month trial. Berberine acted by lowering FBG and PBG from one week to the end of the trial. HbA\textsubscript{1c} decreased from 8.1% ± 0.2% to 7.3% ± 0.3% (\(P<0.001\)). Fasting plasma insulin and HOMA-IR were reduced by 28.1% and 44.7% (\(P<0.001\), respectively. Total cholesterol and low-density lipoprotein cholesterol (LDL-C) were decreased significantly as well. During the trial, 20 (34.5%) patients suffered from transient gastrointestinal adverse effects. Functional liver or kidney damages were not observed for all patients. In conclusion, this pilot study indicates that berberine is a potent oral hypoglycemic agent with beneficial effects on lipid metabolism.
REVIEW

Effects and mechanisms of berberine in diabetes treatment

Jun Yin\textsuperscript{a}, Jianping Ye\textsuperscript{b}, Weiping Jia\textsuperscript{a,*}

\textsuperscript{a}Shanghai Clinical Center for Diabetes, Department of Endocrinology and Metabolism, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Diabetes Institute, Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, Shanghai 200233, China
\textsuperscript{b}Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA 70808, USA

Received 1 April 2012; revised 26 May 2012; accepted 8 June 2012

KEY WORDS
Berberine;
Diabetes mellitus;
Complications;
Traditional Chinese medicine

Abstract Berberine from Rhizoma Coptidis is an oral hypoglycemic agent with anti-dyslipidemia and anti-obesity activities. Its metabolic activity of regulating blood glucose and lipids has been widely studied and evidenced in patients and various animal models. Berberine is known as an AMP-activated protein kinase (AMPK) activator. Its insulin-independent hypoglycemic effect is related to inhibition of mitochondrial function, stimulation of glycolysis and activation of AMPK pathway. Additionally, berberine may also act as an α-glucosidase inhibitor. In the newly-diagnosed type 2 diabetic patients, berberine is able to lower blood insulin level via enhancing insulin sensitivity. However, in patients with poor β-cell function, berberine may improve insulin secretion via resuscitating exhausted islets. Furthermore, berberine may have extra beneficial effects on diabetic cardiovascular complications due to its cholesterol-lowering, anti-arrhythmias and nitric oxide (NO) inducing properties. The antioxidant and aldose reductase inhibitory activities of berberine may be useful in alleviating diabetic nephropathy. Although evidence from animal and human studies consistently supports the therapeutic activities of berberine, large-scale multicenter trials are still necessary to evaluate the efficacy of berberine on diabetes and its related complications.
Berberine moderates glucose metabolism through the GnRH-GLP-1 and MAPK pathways in the intestine

Qian Zhang, Xinhua Xiao*, Ming Li, Wenhui Li, Miao Yu, Huabing Zhang, Fan Ping, Zhixin Wang and Jia Zheng

Abstract

Background: Berberine is known to improve glucose and lipid metabolism disorders, but it poorly absorbed into the blood stream from the gut. Therefore, the exact underlying mechanism for berberine is still unknown. In this study, we investigated the effect of berberine on glucose metabolism in diabetic rats and tested the hypothesis that berberine acts directly in the terminal ileums.

Methods: Rats were divided into a control group, diabetic group (DM), low dose of berberine group (BerL) and high dose of berberine group (BerH). Ileum samples were analyzed using a Roche NimbleGen mRNA array, qPCR and immunohistochemistry.

Results: We found that 8 weeks of treatment with berberine significantly decreased fasting blood glucose levels. An oral glucose tolerance test (OGTT) showed that blood glucose was significantly reduced in the BerL and BerH groups before and at 30 min, 60 min and 120 min after oral glucose administration. Plasma postprandial glucagon-like peptide-1 (GLP-1) levels were increased in the berberine-treated groups. The ileum from the BerH group had 2112 genes with significantly changed expression (780 increased, 1332 decreased). KEGG pathway analyses indicated that all differentially expressed genes included 9 KEGG pathways. The top two pathways were the MAPK signaling pathway and the GnRH signaling pathway. qRT-PCR and immunohistochemistry verified that glucagon-like peptide 1 receptor (Glp1r) and mitogen activated protein kinase 10 (Mapk10) were significantly up-regulated, in contrast, gonadotropin releasing hormone receptor (Gnrhr) and gonadotropin-releasing hormone 1 (Gnrh1) were down-regulated in the BerH group.

Conclusion: Our data suggest that berberine can improve blood glucose levels in diabetic rats. The mechanisms involved may be in the MAPK and GnRH-GLP-1 pathways in the ileum.

Keywords: Diabetes, Digestive tract, Gene expression, GnRH
Retrospective analysis of the effects of a highly standardized mixture of *Berberis aristata*, *Silybum marianum*, and monacolins K and KA in patients with dyslipidemia

**Background:** *Berberis aristata*, because of its berberine content, and *Monascus purpureus* fermented rice, because of the presence of monacolins (naturally derived statins), are widely investigated food-grade ingredients used to formulate cholesterol-lowering supplements. Although they are extensively used, berberine is poorly absorbed and monacolins are poorly chemically characterized, not standardized, and possibly contaminated with toxic compounds. Silymarin is reported to enhance berberine absorption, while Monakopure™-K20 (MK-20) is a highly standardized red yeast rice containing monacolins K and KA in the ratio of 1:1 but not secondary monacolins, dehydromonacolins, or citrinin.

**Aim:** The effects of a cholesterol-lowering supplement (Berberol™K) containing berberine, silymarin, and MK-20 (BSM) in patients with dyslipidemia were clinically analyzed.

**Methods:** The clinical role of BSM in naïve and in statin-intolerant patients was retrospectively evaluated and the effects observed were compared with those obtained in patients without treatment or treated with lovastatin.

**Results:** Total cholesterol, low density lipoprotein, and triglyceride levels were approximately 4%, 6%, and 11% lower, respectively, and the creatine phosphokinase increase was reduced in patients treated with BSM compared to those treated with lovastatin. Similar results were also obtained in statin-intolerant subjects where BSM was administered as add-on therapy to ezetimibe or fenofibrate.

**Conclusion:** BSM is a food supplement potentially useful 1) as a primary intervention in low-cardiovascular-risk subjects with dyslipidemia; 2) as add-on therapy in mildly statin-intolerant patients; and 3) in dyslipidemic patients with a negative perception of statins who prefer a treatment seen as natural.

**Keywords:** berberine, Berberol™K, silymarin, P-glycoprotein, cholesterol, triglycerides, *Monascus purpureus*, Monakopure™-K20
Farmacocinetica

- Scarsa biodisponibilità orale;

- In seguito ad assunzione orale la BerberinaHCl si scioglie nelle secrezioni duodenali (pH 6,8-7,5);

- Substrato della Pg-P;

- Scarsa internalizzazione enterocitaria a causa dell’estrusione da parte della Pg-P;
The involvement of P-glycoprotein in berberine absorption.

Pan GY¹, Wang GJ, Liu XD, Fawcett JP, Xie YY.

Abstract

Berberine is an important ingredient in a number of traditional Chinese medicines but has been shown to have poor bioavailability in the dog. The aim of this study was to use the P-glycoprotein (P-glycoprotein) inhibitors cyclosporin A, verapamil and the monoclonal antibody C219 in in vivo and in vitro models of intestinal absorption to determine the role of P-glycoprotein in berberine absorption. In the rat recirculating perfusion model, berberine absorption was improved 6-times by P-glycoprotein inhibitors. In the rat everted intestinal sac model, berberine serosal-to-mucosal transport was significantly decreased by cyclosporin A. In Ussing-type chambers, the rate of serosal-to-mucosal transport across rat ileum was 3-times greater than in the reverse direction and was significantly decreased by cyclosporin A. In Caco-2 cells, berberine uptake was significantly increased by P-glycoprotein inhibitors and by monoclonal antibody C219. P-glycoprotein appears to contribute to the poor intestinal absorption of berberine which suggests P-glycoprotein inhibitors could be of therapeutic value by improving its bioavailability.

Schmitz T¹, Hombach J, Bernkop-Schnürch A.

Author information

Abstract

This study evaluated three chitosan-N-acetyl cysteine (CAC) conjugates of increasing molecular mass as a valuable tool to improve the absorption of drugs by assessing its permeation enhancing effect regarding the active P-gp substrate rhodamine-123 in comparison to the trans- and paracellular marker FD 4 both in rat intestine and Caco 2 monolayers. Additional LDH and MTT cytotoxicity tests have attested a non-toxic profile to CAC, which can consequently be seen as a safe and promising novel drug carrier with the ability to enhance drug absorption and to inhibit P-gp efflux transporters.
**Principali proprietà salutistiche (terapeutiche?) della Boswellia Serrata**

*Nome botanico:* Boswellia Serrata;

*Origine:* Asia

*Droga:* rizoma

*Principali principi attivi:* Terpenoidi steroidici (Acidi β ChetoBoswellici);

Gli acidi boswellici contenuti agiscono come energici inibitori della biosintesi dei Leucotrieni (inibizione della 5-LA);

Sono molecole ad attività antinfiammatoria e anti-proliferativa;

Buoni dati clinici nel trattamento delle patologie infiammatorie intestinali (inibizione del TNF-α);

Grosso problema sulla reperibilità di estratti standardizzati in acidi beta-chetoboswellici!

– Molti estratti raggiungono titolazioni improbabili >70% perché titolano la frazione carbossilica totale dell'estratto senza indicare in modo preciso e con metodologia analitica univoca e standardizzata la frazione beta;
The resin of *Boswellia* species has been used as incense in religious and cultural ceremonies and in medicines since time immemorial. *Boswellia serrata* (*Salai/Salai guggul*), is a moderate to large sized branching tree of family Burseraceae (Genus Boswellia), grows in dry mountainous regions of India, Northern Africa and Middle East. Oleo gum-resin is tapped from the incision made on the trunk of the tree and is then stored in specially made bamboo basket for removal of oil content and getting the resin solidified. After processing, the gum-resin is then graded according to its flavour, colour, shape and size. In India, the States of Andhra Pradesh, Gujarat, Madhya Pradesh, Jharkhand and Chhattisgarh are the main source of *Boswellia serrata*. Regionally, it is also known by different names. The oleo gum-resins contain 30-60% resin, 5-10% essential oils, which are soluble in the organic solvents, and the rest is made up of polysaccharides. Gum-resin extracts of *Boswellia serrata* have been traditionally used in folk medicine for centuries to treat various chronic inflammatory diseases. The resinous part of *Boswellia serrata* possesses monoterpenes, diterpenes, triterpenes, tetracyclic triterpenic acids and four major pentacyclic triterpenic acids i.e. β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid, responsible for inhibition of pro-inflammatory enzymes. Out of these four *boswellic acids*, acetyl-11-keto-β-boswellic acid is the most potent inhibitor of 5-lipoxygenase, an enzyme responsible for inflammation.
Biodisponibilita’

• Gli studi di farmacocinetica condotti sugli Acidi Boswellici hanno evidenziato uno scarso assorbimento enterico a causa della scarsa solubilità in acqua e difficoltoso passaggio in circolo ostacolato;

• Necessaria tecnologia che ne aumenti il passaggio enterico verso la circolazione generale;
Boswellia serrata: an overall assessment of in vitro, preclinical, pharmacokinetic and clinical data.

Abdel-Tawab M¹, Werz O, Schubert-Zsilavecz M.

Abstract

Non-steroidal anti-inflammatory drug (NSAID) intake is associated with high prevalence of gastrointestinal or cardiovascular adverse effects. All efforts to develop NSAIDs that spare the gastrointestinal tract and the cardiovascular are still far from achieving a breakthrough. In the last two decades, preparations of the gum resin of Boswellia serrata (a traditional ayurvedic medicine) and of other Boswellia species have experienced increasing popularity in Western countries. Animal studies and pilot clinical trials support the potential of B. serrata gum resin extract (BSE) for the treatment of a variety of inflammatory diseases like inflammatory bowel disease, rheumatoid arthritis, osteoarthritis and asthma. Moreover, in 2002 the European Medicines Agency classified BSE as an 'orphan drug' for the treatment of peritumoral brain oedema. Compared to NSAIDs, it is expected that the administration of BSE is associated with better tolerability, which needs to be confirmed in further clinical trials. Until recently, the pharmacological effects of BSE were mainly attributed to suppression of leukotriene formation via inhibition of 5-lipoxygenase (5-LO) by two boswellic acids, 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA). These two boswellic acids have also been chosen in the monograph of Indian frankincense in European Pharmacopoeia 6.0 as markers to ensure the quality of the air-dried gum resin exudate of B. serrata. Furthermore, several dietary supplements advertise the enriched content of KBA and AKBA. However, boswellic acids failed to inhibit leukotriene formation in human whole blood, and pharmacokinetic data revealed very low concentrations of AKBA and KBA in plasma, being far below the effective concentrations for bioactivity in vitro. Moreover, permeability studies suggest poor absorption of AKBA following oral administration. In view of these results, the previously assumed mode of action - that is, 5-LO inhibition - is questionable. On the other hand, 100-fold higher plasma concentrations have been determined for β-boswellic acid, which inhibits microsomal prostaglandin E synthase-1 and the serine protease cathepsin G. Thus, these two enzymes might be reasonable molecular targets related to the anti-inflammatory properties of BSE. In view of the results of clinical trials and the experimental data from in vitro studies of BSE, and the available pharmacokinetic and metabolic data on boswellic acids, this review presents different perspectives and gives a differentiated insight into the possible mechanisms of action of BSE in humans. It underlines BSE as a promising alternative to NSAIDs, which warrants investigation in further pharmacological studies and clinical trials.
Tecnología «Enterosomi»
Labomar Research
European Patent Granted
Figure 1. Chitosan cationized by NAC. Carboxylic group of NAC protonates aminic group of Glucosamine so producing a polycation.
7. CONCLUSIONE

Su richiesta di LABOMAR è stato effettuato uno studio di passaggio intestinale in Vitro su cellule Caco-2 dei seguenti prodotti:
BERBERINA CLORURO “ENTEROSOMA” (BRA 1283) E BERBERINA CLORURO "ENTEROSOMA” PLACEBO testati alla concentrazione 1mM definita sulla base dei valori di citotossicità preliminare.

L'obiettivo era quello di evidenziare le differenze di assorbimento tra i due prodotti evidenziando come l'utilizzo del chitosano fosse fondamentale per aumentare la biodisponibilità della Berberina Cloruro.

Sia i dati del passaggio paracellulare con lucifer yellow che la misura di TEER hanno mostrato che la berberina non induce tossicità a livello delle giunzioni intercellulari.

La letteratura contrariamente a quanto sembrerebbe lecito aspettarsi in relazione ai buoni risultati clinici ottenuti, ci conferma che la berberina dimostra una non completa biodisponibilità orale che, evidentemente, ne limita l'efficacia potenziale.

La glicoproteina P (Gp–P) è particolarmente abbondante sulla membrana, soprattutto sul versante apicale, degli enterociti (cellule dell’epitelio intestinale). Qui la sua attività conduce all’espulsione, direttamente nel lume intestinale, di alcuni composti, tra cui la berberina, penetrati, dopo somministrazione orale, per diffusione passiva all’interno delle cellule della mucosa.

Quindi la ridotta presenza plasmatica della berberina non è tanto dovuta ad un suo scarso assorbimento diretto, quanto ad una sua massiva ri-estrusione nel lume intestinale (Pan GY et al. 2002).

Dopo aver effettuato il passaggio A–B e B–A è stata calcolato il coefficiente di permeabilità apparente della Berberina e il rapporto di efflusso relativo ai due prodotti testati come mostrato nella seguente tabella:

<table>
<thead>
<tr>
<th>Prodotti</th>
<th>Trasporto</th>
<th>ER (PAPP B–A/PAPP A–B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRA 1283</td>
<td>PASSIVO</td>
<td>0,216</td>
</tr>
<tr>
<td>BRA 1284 PLACEBO</td>
<td>ATTIVO</td>
<td>4,108</td>
</tr>
</tbody>
</table>
TITOLO: ASSORBIMENTO INTESTINALE IN VITRO DI BERBERINA CLORURO “ENTEROSOMA” SU CACO-2

L’assorbimento intestinale del prodotto BRA 1283 Berberina Cloruro contenente Chitosano, che blocca il trasportatore gp–P, ha evidenziato un rapporto di efflusso (ER) pari a 0,216. L’aggiunta di tale componente risulta essere una strategia utile per bloccare la berberina a livello plasmatico evitando una sua estrusione a livello luminale.

L’estrusione a livello apicale è invece osservata con il prodotto BRA 1284 PLACEBO in cui è presente solamente Berberina Cloruro che attraverso la pg–P viene espulsa nel lume: il suo rapporto di efflusso (ER) è 4,108, dimostrando come il passaggio è esclusivamente di tipo attivo.
La Berberina Cloruro contenuta nel prodotto BRA 1283 contenente chitosano è 19 volte più trattenuta a livello plasmatico rispetto alla Berberina Cloruro Placebo.

Le evidenze sperimentali e cliniche ad oggi disponibili in letteratura mostrano interessanti prospettive di impiego della berberina nel trattamento dell’ipercolesterolemia e del diabete nonostante la sua biodisponibilità orale sia piuttosto bassa. Essendo quest’ultimo parametro legato al ruolo ‘farmaco-estrusore’ della gp–P presente sul lato apicale degli enterociti della mucosa intestinale, il concomitante impiego di un inibitore della gp–P, come il chitosano (Werle and Hoffer 2006), consente il miglioramento cinetico della berberina e il conseguente miglioramento della sua resa farmacoclinica.

Fratici1, B. De Servi2

1Laboratory Research, Nutraceutical R&D Laboratory-Innovation Technology, Etriana (TV), Italy
2IVT Screen, Milano, Italy

ABSTRACT
Berberiscin Chloride (BC) is an isoquinolimine alkaloid that is extracted from plants of genus Berberis. During the last decade, researchers and clinicians have paid increasing attention to BC, because of its impressive hypoglycemic and blood lipids lowering properties. Several clinical studies gave proof of evidence regarding BC efficacy in humans and pharmacological mechanism of action, related to the previously mentioned activities, have been proposed and substantially confirmed. On the other side, BC shows a very poor oral bioavailability mainly because of the interaction with P-Glycoprotein (P-G-P) pump, which extrudes BC from inside to outside of the enteric cell. This paper describes a novel oral delivery system containing a Chitosan-N-Acetylcysteine salt capable to interact with P-G-P, partially inhibiting BC extruding process. Preliminary data confirming the aforementioned postulated mechanism on Caco-2 in vitro model have been hereafter reported and discussed.

Key words: Berberine chloride, Chitosan, N-Acetylcysteine, P-G-P, Tight Junctions, Bioavailability

INTRODUCTION
Berberine is an isoquinolimine alkaloid that is extracted from several plants of genus Berberis such as Berberis vulgaris and officinalis. Traditionally, the use of Berberis extracts to treat several diseases, especially in traditional Chinese medicine, can’t be traced back. Currently Berberis extract and especially BC, the main active component identified, are widely employed to approach several diseases and clinical conditions characterized by inflammation and immune-based inflammatory pattern. Nevertheless, BC has been receiving increasing attention by the scientific community as blood lipids lowering and glucose tolerance increasing molecule12. Despite the numerous further clinical indications mentioned for BC and reported in more or less serious papers, these latter are the most scientifically proved and substantiated by impressive pharmacological and clinical data.

Lipid lowering activity of BC
Several papers published during the last years postulated plausible pharmacological mechanisms of action for lipid lowering activity and glucose tolerance enhancing activity of BC. Concerning lipids lowering action, BC is believed to enhance Low Density Lipoprotein Receptors (LDLR) mRNA expression post-transcriptionally. This mechanism, almost partially, could explain the synergistic effect of BC with statins13 and with other vegetal derived actives such as polyunsaturated vegetal steroids and red rice yeast extract in lowering blood lipids as some clinical investigations reported13. The mechanism involved in BC lipids lowering action, indeed, does not regard the partial inhibition of HMG Co-A reductase as statins and red yeast extract do, neither concern to the competition mechanism of cholesterol enteric absorption like vegetal sterols show14. According to this plausible mechanism in blood lipids lowering, BC can potentially represent an interesting weapon to reduce blood LDL in patients who experienced muscular side effects during statins based therapy or showing different pharmacological contraindications. More investigations are however needed to clarify the real mechanism of action involved in lipid lowering activity of BC and most likely more than one will be identified confirming a synergy of biochemical events. Glucose tolerance enhancing activity of BC
Some papers recently published report that BC is effective in animals and in vitro models to approach diabetes mellitus15,16 and convincingly clinical evidences on humans begin to be collected17,18,19. In a recent clinical investigation, human adults with a recent diagnosis for type 2 diabetes received daily and randomly BC and Metformin for a period of 90 days and the authors’ conclusions were that both the molecules are similarly effective to reduce blood glucose. Particularly, authors registered and emphasized remarkable reduction in Hemoglobin A1c, fasting blood glucose, post-prandial blood glucose and plasma triglycerides in patients who received BC18. The most plausible mechanisms of action involved in the hypoglycemic activity of BC seem concern aldose reductase inhibition,20 glycosis induction20 and insulin resistance prevention through increasing gene expression of insulin receptors21,22. Another convincing hypothesis...
Fig 1: Structure of Barbitone Chloride

Fig 2: Chitosan cationized by NAC. Carboxylic group of NAC protonates amine group of Glucosamine so producing a polycation.

Fig 3: Postulated mechanism of action of Extrosomal™ in modulating Pgp activity and TJ cohesion loosening.

Fig 4: Scheme of the device employed to assess Caco-2 intestinal absorption.

Substantiated by sciuride proof of evidence is that BC may prevent insulin resistance via modulating the release of key molecules involved in insulin signaling pathway leading to increased glucose uptake in insulin-resistant cells. A further mechanism behind the hypoglycemic activity of BC might be the up-regulation of Hepatocyte Nuclear Factor 4 Alpha (HNF-4α) expression, which probably acts modulating gluconeogenesis and glucose reaction. For last, a recent biochemical investigation based on advanced analytical method and performed on plasma of 10 type 2 diabetes, evidenced a...
CONCLUSION
To conclude, even though this must be considered a preliminary scientific evaluation to assess a new technology intended to improve oral absorption of BC, it seems suggestive that an association of CH cationized with a mucolytic agent such as NAC and with a solubilizing agent such as PS 80, could potentially represent an interesting new insight to achieve this goal. The experimental and clinical evidences available in published literature, show interesting perspectives on use of BC in the treatment of high blood LDL cholesterol and diabetes even though the poor oral bioavailability. Since this poor bioavailability is mainly due to the role of the P-gP-MDRP, placed on the apical side of the enteric cells, the use of an effective P-gP inhibitor like CH or its quaternized or cationized derivatives, could improve BC enteric kinetic after oral administration and consequently its clinical efficacy performances. Further experimental tests on animals and above all clinical evaluation on humans should be carried out to assess if the preliminary encouraging data collected in this work can be confirmed, contextually opening the status of art to a new pharmaceutical technology capable to enhance clinical expectation regarding BC.
<table>
<thead>
<tr>
<th></th>
<th>Dosaggio 200 mg</th>
<th>Dosaggio 400 mg</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.166</td>
<td>0.3825</td>
<td>0.101</td>
</tr>
<tr>
<td>1</td>
<td>0.466</td>
<td>0.5125</td>
<td>0.156</td>
</tr>
<tr>
<td>2</td>
<td>0.298</td>
<td>0.5335</td>
<td>0.366</td>
</tr>
<tr>
<td>3</td>
<td>0.736</td>
<td>0.5335</td>
<td>0.201</td>
</tr>
<tr>
<td>4</td>
<td>0.341</td>
<td>0.366</td>
<td>0.025</td>
</tr>
<tr>
<td>6</td>
<td>0.025</td>
<td>2.1105</td>
<td>1.105</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**AUC**

<table>
<thead>
<tr>
<th></th>
<th>Dosaggio 200 mg</th>
<th>Dosaggio 400 mg</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.315</td>
<td>0.332</td>
<td>0.101</td>
</tr>
<tr>
<td>1</td>
<td>0.349</td>
<td>0.303</td>
<td>0.156</td>
</tr>
<tr>
<td>2</td>
<td>0.257</td>
<td>0.804</td>
<td>0.366</td>
</tr>
<tr>
<td>3</td>
<td>1.351</td>
<td>0.835</td>
<td>0.201</td>
</tr>
<tr>
<td>4</td>
<td>0.319</td>
<td>0.812</td>
<td>0.025</td>
</tr>
<tr>
<td>6</td>
<td>0.493</td>
<td>3.086</td>
<td>1.096</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**AUC**

<table>
<thead>
<tr>
<th></th>
<th>Dosaggio 200 mg</th>
<th>Dosaggio 400 mg</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.38</td>
<td>0.248</td>
<td>0.101</td>
</tr>
<tr>
<td>1</td>
<td>0.116</td>
<td>0.138</td>
<td>0.156</td>
</tr>
<tr>
<td>2</td>
<td>0.16</td>
<td>0.171</td>
<td>0.366</td>
</tr>
<tr>
<td>3</td>
<td>0.182</td>
<td>0.1635</td>
<td>0.201</td>
</tr>
<tr>
<td>4</td>
<td>0.145</td>
<td>0.176</td>
<td>0.025</td>
</tr>
<tr>
<td>6</td>
<td>0.031</td>
<td>0.8965</td>
<td>1.096</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**AUC**

---

**Dosaggio 200 mg**

**Dosaggio 400 mg**

**Placebo**
Anti-inflammatory effects exerted by Killox®: an innovative formulation of food supplement with curcumin, in urology

V. Cosentino1, A. Fratter2, M. Cosentino3

1Private Hospital ‘Regina Pacis’, San Gavino (CL), Italy
2Research and Innovation Technology, LABOMAR Research, Istrana (TV), Italy
3Andrology Department, Fundació Puigvert, Barcelona, Spain

Abstract. — OBJECTIVE: In this Open Controlled Trial we administered an innovative formulation of food supplement with curcumin (Killox®) to test its efficacy, safety and compatibility with other drugs, in the therapy of post-surgery complications of transurethral resection of prostate (TURP®) and transurethral resection of bladder (TURB), and in the prevention of late complications. Furthermore, Killox® effects were verified in subjects with benign prostatic hyperplasia (BPH).

PATIENTS AND METHODS: Killox® was administered to 40 TURP patients for 20 days, to 10 TURB patients for 10 days and to 30 BPH patients for 90 days. The study was an open controlled trial, approved by the internal Review Board, with a completely independent set of retrospective observations.

RESULTS: In the subjects who underwent surgery the treatment warded off postoperative and late complications, whereas among controls, without anti-inflammatory therapy after surgery until one week later, 21 (52.5%) out of 40 TURP subjects and 4 (40%) out of 10 TURB subjects were still found with symptoms of inflammation and urinary burning, and they had to be treated with non-steroidal anti-inflammatory drugs (NSAIDs) for seven days. Moreover among controls 2 in TURP group presented an urethral stricture, and no one in TURB group. Killox® patients did not report any adverse effect and the therapy was well tolerated, instead among 21 control subjects, who were treated with NSAIDs, 7 reported nausea and epigastric pain. Also in BPH patients the product was effective in a satisfying manner, shortening the duration of irritation symptoms. Noteworthy, Killox® administration did not modify the efficacy of the other treatments. The effect of Killox® was found statistically significant vs controls.

CONCLUSIONS: The therapeutic activity and safety of Killox® in urology allow physicians to administer a new efficient product in substitution of NSAIDs.

Introduction

Benign prostatic hyperplasia (BPH) is a highly frequent diagnosis among the elderly, related to the presence of prostatic enlargement connected with an inflammatory state. BPH is a major cause of lower urinary tract symptoms (LUTS)1. BPH can be treated only pharmacologically or also surgically, using the transurethral resection of the prostate (TURP). This procedure still represents the best choice to reduce surgically BPH. TURP consists in the elimination of a part of the gland, by means of an instrument (the resectoscope) inserted through the urethra. In this way it is possible to excise only the excess of prostate tissue, while the outer capsule is left whole.2 Many other techniques are described and in use for BPH treatment, like laser and plasma vaporization or laser and plasma excision, but, compared with them, TURP actually represents the gold standard for benign prostatic hyperplasia treatment. Evidences indicate that prostatic inflammation may contribute to prostatic growth, in terms of hyperplastic changes3. Thus, any pharmacological treatment of BPH, even without surgery, has always involved the administration of NSAIDs. On the other hand, transurethral resection of bladder (TURB) is a surgical procedure with a lighted tube inserted through the urethra into the bladder. TURB is the first form of treatment in bladder cancers to eradicate and examine bladder tissue and/or tumor, and also to remove lesions. It provides diagnostic, therapeutic and prognostic
Tecnologia Lipomatrix
Labomar Research
Patent pending
Complet emulsification of 250 mg Boswellia Serrata tit. 75% Boswellic acids in SIF medium at 37°C after 1 hour disaggregating simulation of the Lipotablet.

Enteric SIF \( (pH=7.8 \ NaHCO_3) \)

**Composition:**
- Physiologic solution (NaCl 0,9%)
- Sodium Glycocholate (4.5 mmoles)
- Lecithin (3.75 mmoles)
- Cholesterol (0,2 mmoles)
- Porcine lipase 200 mg/ml (300 UI/mg);

250 mg of Boswellia Serrata tit. 75% Boswellic acids after «classic tablet» disaggregation at 37°C in enteric SIF: surface floating and precipitation at the bottom. No uniform dispersion is achieved.

Enteric lipase coadjuvates emulsification process cleaving up glycerides and boosting up phenomenon of micellar formation.
Boswellic acids are lipophilic terpenes completely insoluble in water and consequently in the intestinal water secretions. Boswellic acids float on the surface of the enteric SIF at 37°C. This phenomenon makes poor the BA bioavailability along the epithelium facing the first intestinal tract.
Albino and diglycerides of fatty acids.
These are esters of glycerol with fatty acids and are completely non ionic and not ionizable fats.
They melt at low temperature and are widely used in foods as emulsifiers.
These fats are completely not sensitive to pH variation and completely unaffected to add even at very low pH.
Lipotabs and powders in which are made of remain a melted core of these fats and ascorbyl palmate.
This core guarantees the complete gastro-resisting profile and at the same time doesn’t permit the active ingredients delivery in the stomach.

Ascorbyl palmate
Ascorbyl radical is ionizable at pH 7.5 and for this reason this molecule behaves as a ionic hydrophilic surfactant.
The consequence is that once in the first enteric tract the tablet or powder begins to disaggregate and emulsify.
The fatty core of the tablet/powder takes place to a thin emulsion containing active lipophilic compound. This phenomenon boost up enteric permeation of active agent in synergy with bile salts.

Dodecyl acetic acid
Lipophilic terpenes with poor water solubility and poor enteric bioavailability
75% of disaggregation occurring in 1 h in SIF. Complete disaggregation in 90 minutes in SIF.

Complete and uniform emulsification of lipotablet in SIF.
Simulazione della dispersione in liquido intestinale (SIF) di Olio di Zenzero (tit. in Gingeroli 40%) da Lipomatrix vs Softgel
Simulazione della dispersione in liquido intestinale (SIF) di Olio di Zenzero (tit. in Gingeroli 40%) da Lipomatrix vs Softgel
Simulazione della dispersione in liquido intestinale (SIF) di Olio di Serenoa Repens (tit. in Ac. Grassi 80%) da Lipomatrix vs Softgel
Main nutraceutical applications

- **Boswellia Serrata and Vitamin D3 (tablets or capsules);**
  - Inflammatory based diseases;
  - Psoriatic arthritis and psoriasis;
  - Crohn disease;

- **Omega-3 fatty acids** (capsules containing lipo-powder up to 200 mg DHA 70% oil/CPS);
  - EPA+DHA and association with Vit. D3;
  - Cardiovascular prevention;
  - Psoriasis;
  - Acne;

- **Lipophilic vitamins (tablets or capsules)**
  - A, D, E, K;

- **Quercetine (tablets or capsules)**
  - Quercetine is much better absorbed by intestine if mixed with fats;

- **Vegetal oils (tablets or capsules)**
  - Serenoa Repens;
  - Zingiber Officinalis;
«Bello come il fortuito incontro tra un ombrello e una macchina da cucire su di un tavolo anatomico»

Conte di Lautréamont
Racconti di Molderor
Manifesto del movimento surrealista