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Nanoemulsion formulation of fisetin improves bioavailability and antitumour activity in mice

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ABSTRACT

The natural flavonoid fisetin (3,3′,4′,7-tetrahydroxyflavone) has shown antitumour activity but its administration is complicated by its low water solubility. Our aim was to incorporate fisetin into a nanoemulsion to improve its pharmacokinetics and therapeutic efficacy. Solubility and emulsification tests allowed to develop an optimal nanoemulsion composed of Miglyol® 812N/Labrasol®/Tween® 80/Lipoid E80®/water (10%/10%/2.5%/1.2%/76.3%). The nanoemulsion had an oil droplet diameter of 153 ± 2 nm, a negative zeta potential (−28.4 ± 0.6 mV) and a polydispersity index of 0.129. The nanoemulsion was stable at 4 °C for 30 days, but phase separation occurred at 20 °C. Pharmacokinetic studies in mice revealed that the fisetin nanoemulsion injected intravenously (13 mg/kg) showed no significant difference in systemic exposure compared to free fisetin. However, when the fisetin nanoemulsion was administered intraperitoneally, a 24-fold increase in fisetin relative bioavailability was noted, compared to free fisetin. Additionally, the antitumour activity of the fisetin nanoemulsion in Lewis lung carcinoma bearing mice occurred at lower doses (36.6 mg/kg) compared to free fisetin (223 mg/kg). In conclusion, we have developed a stable nanoemulsion of fisetin and have shown that it could improve its relative bioavailability and antitumour activity.

1. Introduction

Among the plant-derived compounds that have been linked to the chemoprevention and treatment of cancer, the flavonoids occupy a special place due to their abundance in human food and their relative non toxicity (Havsteen, 2002; Lopez-Lazaro, 2002; Middleton et al., 2000; Surh, 2003).

In a program aimed at finding new antiangiogenic agents in the flavonoid family, we have recently identified the natural flavonoid fisetin (3,3′,4′,7-tetrahydroxyflavone) as an interesting lead that can stabilize endothelial cells in vitro at non cytotoxic concentrations (Touil et al., 2009). Fisetin is found in several fruits, vegetables, nuts and wine (Arai et al., 2000; Kimira et al., 1998) and displays a variety of biological effects including antioxidant, anti-inflammatory (Park et al., 2007; Woodman and Chan, 2004), anti-carcinogenic and in vitro anti-angiogenesis (Fotsis et al., 1997). Fisetin has been shown to inhibit several molecular targets, including cyclin-dependent kinases (Lu et al., 2005a,b; Sung et al., 2007), DNA topoisomerases I and II (Constantinou et al., 1995; Olaharski et al., 2005), urokinase (Jankun et al., 2006), actin polymerization (Böhl et al., 2007), and androgen receptor signalling (Khan et al., 2008).

In vivo, fisetin has recently been shown to possess interesting anticancer activity in mice bearing lung carcinoma (Touil et al., 2011), prostate tumours (Khan et al., 2008), and human embryonal carcinoma (Tripathi et al., 2011). Its in vivo mechanism of action appears rather complex and includes antiangiogenic, antiandrogenic and anti-metastatic activities (Chien et al., 2010; Khan et al., 2008; Touil et al., 2011; Tripathi et al., 2011).

Despite its highly interesting properties for cancer therapy, fisetin administration in vivo remains problematic partly due to its poor water solubility (Guzzo et al., 2006; Mignet et al., 2012). Fisetin bioavailability must therefore be significantly improved in order to optimize its delivery to tumours after in vivo administration. Although the design of suitable molecular carriers for flavonoids is an area of intense research, solutions are still far from being developed for therapy, and suitable molecular carriers for flavonoids have yet to be designed and tested (Leonarduzzi et al., 2010). To

Abbreviations: Fisetin, 3,3′,4′,7-tetrahydroxyflavone; EAhy 926, immortalized human umbilical vein endothelial cells; HLB, hydrophilic–lipophilic balance; HPLC, high performance liquid chromatography; PDI, polydispersity index.

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Table 1
Fisetin solubility in various solvents.

Solvents	Solubility (mg/g)
Water	<1
Ethanol	<14
Long chain triglycerides	
Soybean oil	<1
Carthame oil	<1
Medium chain mono- di-or triglycerides	
Miglyol® 812 N (capric and caprylic acid triglycerides)	<1
Captex® 355 (capric and caprylic acid triglycerides)	<1
Labrafac Lipophile WL 1349® (capric and caprylic acid triglycerides)	<1
Imwitor® 742 (capric and caprylic mono- di- and triglycerides)	<4
Short chain triglycerides: Triacetin (triester of glycerol and acetic acid)	<6
Fatty acid esters: Ethyl oleate	<1
Capmul® PG8 (propylene glycol monocaprylate)	<5
Capric acid	<1

Table 2
Fisetin solubility in various surfactants.

Surfactants	HLB[a]	Solubility (mg/g)
Span® 85 (sorbitan trioleate)	1.8	<2
Labrafil M 1944 CS® (glycerides and PEG 300 ester mixture)	4	<3
Capmul® MCM (mono diglyceride of capric and caprylic acids)	5	<7
Vitamin E TPGS (α tocopheryl acid succinate ester/PEG 1000)	13	<10
Cremophor EL® (polyethoxylated ricin oil)	13	<26
Myrj® 52 (polyoxyethylene glycol 2000 monostearate)	16.9	<30
Tween® 80 (polysorbate 80)	15	<30
Labrasol® (caprylocaproyl polyoxyl-8 glycerides)	14	<54

[a] HLB, hydrophilic–lipophilic balance (a value <10 indicates a majority of lipophilic fractions and a value >10 indicates a majority of hydrophilic fractions).

do so, we therefore chose to formulate fisetin into nanoemulsion in order to hopefully achieve a better bioavailability.

Nanoemulsions represent good vehicles to formulate hydrophobic active molecules (Sarker, 2005). For example, nanoemulsions are widely used for parenteral administration of lipids, and have also been employed for intravenous administration of anticancer drugs like paclitaxel (Kan et al., 1999) and chlorambucil (Ganta et al., 2008). Also noteworthy, nanoemulsion has also been recently reported to contribute to the in vivo increase in efficacy of anticancer drugs, e.g., dacarbazine (Tagne et al., 2008) and camptothecin (Han et al., 2009).

The aim of the present study was therefore to design and characterize a nanoemulsion of fisetin that could be suitable for parenteral administration. We also evaluated the fisetin nanoemulsion pharmacokinetics after intravenous (i.v.) or intraperitoneal (i.p.) administration in mice, and determined its relative i.p. bioavailability compared to the i.p. administration of the free fisetin. Finally, the antitumour activity of the fisetin nanoemulsion was compared to the administration of free fisetin in Lewis lung carcinoma bearing mice.

2. Materials and methods

2.1. Materials

Fisetin (98% purity) was purchased from Shanghai FWD Chemicals Limited (Shanghai, China). The various purified oil phases were provided by the following companies: soybean oil (Société Industrielle des Oléagineux, Saint Laurent Blangy, France); carthame oil, ethyl oleate and n-capric acid (Sigma–Aldrich, Saint Quentin Fallavier, France); Miglyol® 812N and Imwitor® 742 (Sasol Witten, Germany); Captex® 355 and Capmul® PG-8 (Abitec, Janesville, WI, USA); Labrafac lipophile WL 1349® (Gattefossé, Saint Priest, France); triacetin (VWR Fontenay-sous-Bois, France).

The surfactants were purchased from the following companies: egg lecithin containing 82.3% phosphatidylcholine (Lipoid E80®, Lipoid GmbH Ludwigshafen, Germany); polysorbate 80 (Tween® 80), sorbitan trioleate (Span 85®), polyoxyethylene glycol 2000 monostearate (Myrj® 52) (Uniquema, Everberg, Belgium); polyoxyethylenated ricin oil (Cremophor EL®, BASF, Ludwigshafen, Germany); vitamin E TPGS (Eastman Chemical B.V., Paris, France); a mixture of glycerides and esters of PEG-8 (Labrasol®), a mixture of glycerides and esters of PEG 300 (LabrafilM 1944 CS®) (Gattefossé Saint Priest, France); glycerol monocaprylocaprate (Capmul MCM®, Abitec, Janesville, WI, USA). Glycerol was purchased from Labosi (Paris, France). Sterile water for injection was from Fresenius-Kabi (Sèvres, France). Sodium hydroxide 0.1 N was from Carlo Erba Reactif SDS (Peypin, France).

The other chemicals used for drug dissolution, plasma preparation and HPLC analysis were the following: methanol, acetonitrile, perchloric acid (Carlo Erba Reactif SDS, Peypin, France); DMSO, PEG 200, morin, phosphate buffer (pH 7.4) and mouse serum (Sigma–Aldrich, Saint Quentin Fallavier, France). All other

Table 3
Fisetin solubility in various mixtures.

Mixtures (in percent)	Solubility (mg/g)
Ethanol/Tween® 80 (2%)	<21
Miglyol® 812N/Tween® 80 (2%)	<1
Soybean oil/Tween® 80 (15%)	<3
Soybean oil/Span 85® (10%)	<1
Miglyol® 812N/Span 85® (7.5%)	<3
Soybean oil/Labrasol®(50/50)	ND[a]
Miglyol® 812N/Labrasol®(50/50)	ND[a]
Capmul MCM®/Labrasol® (50/50)	<21
Tween® 80/Labrasol®(20/80)	<45
Tween® 80/Labrasol®/soybean oil (12/44/44)	ND[a]
Tween® 80/Labrasol®/Miglyol® 812 N (12/44/44)	<30
Mirj 52®/Solutol HS 15®/Capmul MCM® (57/29/14)	<24
Tween® 80/Labrasol®/Capmul MCM® (17/69/14)	<40

[a] ND, not determined because the phases were not miscible in these proportions.