Research in Veterinary Science 117 (2018) 255–259

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Quercetin decrease somatic cells count in mastitis of dairy cows

Artur Burmańczuk[a,*], Piotr Hola[b], Andrzej Milczak[c], Tomasz Piech[d], Cezary Kowalski[a], Beata Wojciechowska[e], Tomasz Grabowski[f]

[a] Department of Pharmacology, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland
[b] Agromarina Sp. z o. o., Kulczyn Kolonia 48, 22-235 Hańsk Pierwszy, Poland
[c] Department and Clinic of Animal Internal Diseases, Sub-Department of Companion Animal Internal Medicine, Faculty of Veterinary Medicine, University of Life Sciences, Głęboka 30, 20-612 Lublin, Poland
[d] Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine, University of Life Science, Głęboka 30, 20-612 Lublin, Poland
[e] Labconsulting, ul. Duńska 9, 54-427 Wrocław, Poland
[f] Polpharma Biologics, Trzy Lipy 3, 80-172 Gdańsk, Poland

ARTICLE INFO

Keywords:
Mastitis
Quercetin
Flavonoids
Immunomodulator
Inflammation

ABSTRACT

Quercetin is a dietary flavonoid which has an effect on inflammation, angiogenesis and vascular inflammation. In several other flavonoids (*e.g.* kaempferol, astragalin, alpinetin, baicalein, indirubin), anti-inflammatory mechanism was proven by using mice mastitis model. The aim of the current study was pilot analysis of quercetin tolerability and its impact on somatic cells count (SCC) after multiple intramammary treatment on dairy cows with clinical mastitis. Based on SCC and clinical investigation, 9 dairy cows with clinical mastitis of one quarter were selected for the pilot study. Baseline analysis (hematology, TNFα, SCC) was performed every 24 h among all cows three days before the first dose (B1–B3). After the baseline monitoring (B1–B3) eight days treatment (D1–D8) was performed with a high and low dose. Selected blood parameters were analyzed. Starting from D1 to D8, a decrease of SCC in relation to baseline was characterized by declining trend. The presented results allowed the confirmation of the significant influence of quercetin on the reduction of SCC in mastitis in dairy cows after 8 days of therapy.

1. Introduction

Quercetin is a dietary flavonoid which has an effect on inflammation, angiogenesis, vascular inflammation and reverse cholesterol transport (Bhaskar and Helen, 2016; Caglayan Sozmen et al., 2016; Cui et al., 2017; Maurya and Vinayak, 2017). Quercetin decreases Th1 lymphocytes differentiation, decreasing the levels of numerous cytokines and factors like IL-1β, IL-4, IL-6, IL-10, IL-12, IL-25, IL-33, MCP-1, NF-κB, VEGF-A, COX-2, 5-LOX, iNOS, NO, CRP, TNFα, TNFγ, IgE and reducing the expression of VCAM-1, ICAM-1, MIP-2 as was shown in different models (Bhaskar et al., 2016; Caglayan Sozmen et al., 2016; Chen et al., 2016; Maurya and Vinayak, 2017; Meng et al., 2016; Muthian and Bright, 2004). Current studies suggest that quercetin prevents from chronic histopathological changes of thickness in lung tissue (Caglayan Sozmen et al., 2016). There was an attempt to utilize quercetin in different diseases in humans, for example in destructive pulmonary tuberculosis, diabetes, rheumatoid arthritis or dry eye syndrome (Butov et al., 2016a,b; Javadi et al., 2016).

Currently, > 10 different clinical studies with human subjects in phase II and one in phase III cover an impact of quercetin on cancer associated with thrombosis (ClinicalTrials, 2017). It was proven that inhibitory effect of quercetin on interleukin production is dose dependent (Chen et al., 2016). Human daily intake of quercetin with food varies 5–80 mg daily (0.07–1.14 mg/kg) in different countries. *In vitro* effects of quercetin were shown in doses and varied between 10 and 200 μM (Chen et al., 2016). In human subjects 150 mg of quercetin daily (2.14 mg/kg) was utilized to show an impact on TNF levels (Egert et al., 2009). In human subjects quercetin was used typically in 2 mg/cm^2 doses (Maramaldi et al., 2016). In woman's polycystic ovary syndrome 500 mg (7.14 mg/kg) was used twice daily (Rezvan et al., 2016).

Pharmacodynamic effect of quercetin after intramammary administration in dairy cows mastitis was not analyzed. Single papers have proven that products rich in natural quercetin could have therapeutic potential in mastitis (Wang et al., 2016). In several other flavonoids (kaempferol, astragalin, alpinetin, baicalein, indirubin), using mice mastitis model, anti-inflammatory mechanism was proven as well (Cao et al., 2014; Chen et al., 2013; He et al., 2015; Lai et al., 2017; Li et al., 2013). Many studies showed that IL-6 could be a marker for bovine

* Corresponding author at: Veterinary Department, University of Life Science, Akademicka 12, Lublin 20-033, Poland.
E-mail addresses: artur.burmanczuk@up.lublin.pl (A. Burmańczuk), tomasz.piech@up.lublin.pl (T. Piech), cezary.kowalski@up.lublin.pl (C. Kowalski), beata.wojciechowska@aol.pl (B. Wojciechowska), tomasz.grabowski@polpharma.com (T. Grabowski).

https://doi.org/10.1016/j.rvsc.2018.01.006
Received 19 May 2017; Received in revised form 1 December 2017; Accepted 8 January 2018

mastitis (Usman et al., 2017). TNFα, NF-κB, IL-1β, and IL-8 released from bovine mammary epithelial cells, has an important role in mastitis (Brenaut et al., 2014; Fitzgerald et al., 2007). Based on that findings and quercetin mechanism of action, the aim of the current study was the pilot analysis of quercetin tolerability and impact on somatic cells count (SCC) after multiple intramammary treatment on dairy cows with clinical mastitis.

2. Material and methods

2.1. Dosage and drug preparation

Solution of quercetin (Sigma-Aldrich; Poland) in phosphate buffered saline – PBS (Biomed-Lublin, Poland) was prepared including Sonic-2 ultrasound bath (POLSONIC Pałczyński, Poland) and shaken for 30 min in 37 °C. Final doses in the study were infused using solution based on 30 mg of quercetin in 5 mL of PBS. All doses in the study were given immediately after morning milking at 9.00 am. Immunomodulating impact after intramammary administration is difficult to limit to the effect located only in the inflammatory quarter. It was decided to administer the drug to all quarters among all tested animals. Administration of quercetin to all quarters was supposed to minimize the fluctuation of SCC. Initially, a high dose (HD) 30 mg/quarter/day was proposed. Study cows were treated for all quarters so total dose per udder was 120 mg of quercetin/udder/daily. Such a dose was continued between the first and third day dose (D1–D3). After baseline monitoring (B1–B3) and three days of HD treatment (D1–D3), daily dose was corrected into a lower one (LD) – 10 mg/quarter/day (D4–D8) and consequently last phase of the study was performed with a total dose 40 mg of quercetin/udder/daily. In final clinical investigation, immune parameters were analyzed within three days after the last dose (D8) in recovery period (R1–R3). The sequence of work was B1–B3 → D1–D3 → D4–D8 → R1–R3 (Fig. 1).

2.2. Animals

The animal studies were conducted with the consent of the Ethics Committee (Resolution No. (33/2017), Local Ethical Committee for Animal Experiments in Lublin). Based on SCC and clinical investigation, 9 dairy cows with clinical mastitis of one quarter were selected for the pilot study. SCC > 300,000 cells/mL in one from 4 quarters was taken as inclusion criterion. Only cows with only one inflamed quarter were included in the study. The study was conducted on cows weighing ≈ 700 kg each, of black and white race, in the age between 4 and 12 years. The animals were fed with farming feed concentrates and fodder (oats, barley) grain alternated with raw corn, pasture grazing grass silage, green forage, straw, and meadow hay with water access *ad libitum*. The analyses were carried out in one selected farm (Agromarina Sp. z o. o., Poland). Prior to the drug administration and milk delivery, each udder was disinfected. Milk samples for analyses (0.5 L) were collected once per day from inflamed quarter of each cow, immediately before daily morning milking. Milk samples (15 per each cow) were collected at baseline (B1–B3), HD (D1–D3) LD (D3–D8) and after the last dose (R1–R3). Blood sampling for immune response analysis (6 samples per each cow) was done once daily before morning milking at baseline (B1–B3) and after the last dose (R1–R3).

Baseline analysis			Single dose per quarter every day								Recovery phase		
			30 mg/quarter/day			10 mg/quarter/day							
B1	B2	B3	D1	D2	D3	D4	D5	D6	D7	D8	R1	R2	R3

Fig. 1. Scheme illustrating study design. B1–B3 – baseline analyzed within 3 subsequent days before first dose; D1–D3 – high dose treatment (120 mg/udder); D4–D8 – low dose treatment (40 mg/udder).

2.3. Adverse drug reactions monitoring

The side effects which could be related to the intramammary administered quercetin, were monitored during the whole study. No medicines were given to the animals during the study. Every day the veterinarian examined the condition of the udder and the general condition of the animals. Milk was also monitored daily.

2.4. Data analysis & statistical methods

Baseline analysis (hematology, TNFα, SCC) was performed every 24 h among all cows three days before the first dose (B1–B3). The blood samples were analyzed for selected blood parameters like: WBC – white blood cells volume; RBC – red blood cells volume; Hb – hemoglobin; Hct – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDW – red cell distribution width; Plt – platelets; Fib – fibrinogen. All parameters were analyzed utilizing previously described methods and equipment (Burmanczuk et al., 2016). SCC analysis was performed using the Bentley BactoCount IBCm analyser (Bentley Instruments Inc.) immediately after milk sampling. Immune response was measured with the use of TNFα levels in blood serum utilizing ELISA method. The concentration of TNFα in peripheral blood serum was measured in duplicate, using a commercial bovine TNFα specific kit (LifeSpan Biosciences, Inc., Seattle, USA, cat. number LS-F5014), according to the manufacturer's instructions. The assay was based on the sandwich ELISA principle. The manufacturer claims that no significant cross-reactivity or interference between TNFα and analogs could be observed. The intra-assay of the method and inter-assay coefficients of variation were 10 and 12%, respectively and the detection range was 7.81–500 pg/mL. The sensitivity of the method was < 7.81 pg/mL. Color developed in the sample was read as an absorbance at 450 nm wavelength using Spectramax M2 Microplate/Cuvette Reader (Molecular Devices, Pennsylvania, USA). Half-life effect – time to reach 50% reduction of SCC ($t_{1/2SCC}$) based on slope of SCC median was calculated based on the first order process equation $t_{1/2SCC}$ = Ln(2)/slope. The slope value based on linear fit was calculated by GraphPad Prism® v. 6.01 (GraphPad Software Inc.). An effective dose (ED_{50}) based on inhibitory dose for SCC (ID_{50}) from sigmoidal 4PL interpolation was calculated using GraphPad Prism® v. 6.01.

The analysis of raw data was conducted with the use of GraphPad Prism® v. 6.01 (GraphPad Software Inc.). Unpaired two tailed *t*-test was used for data comparison in subsequent groups and chi^2 test was used for median SCC trend analysis. The first group was the SCC mean for baseline (B1–B3), and the second one was the SCC mean value on a particular day of the study. The differences with $p < 0.05$ were considered as statistically significant.

3. Results

3.1. SCC levels in milk samples

Fig. 2 shows the SCC changes in milk in baseline and during quercetin administration in two different doses. SSC Arithmetical mean in baseline amounted to 2,632,741 ± 1,718,167 cells/mL. Starting from D1 and continuing to D8, a decrease of SCC in relation to baseline was characterized by declining trend ($p < 0.0001$). After 3 days of quercetin administration in a 120 mg/udder (D1–D3) dose, SCC decreased by 14.12% with reference to arithmetic mean of baseline. However, this decrease was not statistically significant as $p > 0.05$. The slope of median for SCC in D1-D3 phase pointed out that $t_{1/2SCC}$ with reference to baseline while maintaining used dose would amount to ≈ 85.45 h. The same prediction assumed on the bases of arithmetical mean indicated that $t_{1/2SCC,}$ with reference to baseline while maintaining the used dose, could reach ≈ 333.07 h (≈ 2 week's treatment). After the next 5 days of quercetin administration in a lower dose of 40 mg/udder