Study of the effect of an orally administered oligopeptide complex of embryonic origin (Humanofort®) in the medicinal management of inflammatory musculoskeletal disorders accompanied by chronic pain in dogs

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Magyar Állatorvosok Lapja **136,** 235–240 (2014/4)
Received for publication: 26 November 2013

Summary

The efficacy of a standardised oligopeptide complex containing growth factors (IGF-1, IGF-2, FGF, NGF, EGF and CTGF), prepared from chicken embryo extract (Humanofort®, a veterinary product having curative effect) was studied in the treatment of inflammatory musculoskeletal disorders accompanied by chronic pain in dogs, after oral administration for 42 days. A multicentric, randomised, placebo-controlled experiment was carried out in accordance with the relevant EU directive. The clinical examinations and the treatments took place according to the rules of Good Clinical Practice. The trial proved that Humanofort® treatment significantly improved the willingness to move of dogs suffering from chronic musculoskeletal disorders as compared to the placebo group (n = 49; p = 0.0006).

Introduction

In the everyday veterinary and human clinical practice, pain is the commonest complaint indicative of a possible musculoskeletal disease, and the commonest reason for taking affected animals to a veterinarian. According to its course and duration, pain may be acute or chronic, persisting or intermittent. A chronic pain is defined as pain that has persisted for more than three months. While acute pain often ceases spontaneously, without any residual signs, chronic pain may induce hypersensitivity of the central nervous system and often lead to the development of depression (2, 4).

Recently, degenerative joint diseases accompanied by chronic pain have increasingly become a focus of attention. This large group of diseases is associated with severe deterioration of the quality of life and prolonged pain, and the developing deformities often lead to limited mobility resulting in reduction or complete loss of the animal's usual everyday activities (13, 16, 20, 21).

Joint diseases accompanied by chronic pain result in severe deterioration of the quality of life

Dogs suffering from chronic musculoskeletal diseases are frequently presented to veterinary clinics for examination. Depending on the type and severity of the lesions, these animals receive conservative or surgical treatment, and medicinal treatment may last for a very long time. For this purpose, primarily steroids or nonsteroidal anti-inflammatory drugs (NSAIDs) having analgesic and anti-inflammatory effects are used (24). As a basic requirement, such medicines should be devoid of major side effects, simple to administer and have a predictable duration of effect. Several publications have reported that anti-inflammatory drugs, including even the most advanced active substances, may have substantial side effects, especially when given in a relative or absolute overdose or when administered over a long period of time (3, 9, 33, 38, 39). It has also been reported that oxidative stress plays an important role in the aetiology of inflammation (10, 11, 23, 40).

These diseases are treated with steroids or NSAIDs which may have substantial side effects

Reducing the excessively high dose of anti-inflammatory drugs or shortening their time of administration may not only result in the animal's improved quality of life but may also reduce the risk of development of life-threatening adverse events. However, pain relief still remains a primary objective (5, 12).

Numerous growth factors have an outstanding role in the regulation of inflammatory processes. Insulin-like growth factors 1 and 2 (IGF-1, IGF-2) are biologically active proteins having a structure similar to that of insulin. In young animals, they are responsible for the growth and maturation processes, while in sexually mature animals they exert an anabolic effect. They are the most important natural activators of the Akt signalling pathway, and thus they participate in the processes of cell growth and cell proliferation and in the inhibition of programmed cell death. IGF-1 has been found to exert a favourable, neuroprotective effect on the brain, and the inhibition of the IGF-1 signalling pathway results in a longer lifespan. Regular movement has an impact on the production of free radicals considered to be among the decisive factors of ageing as well as on ageing-related factors such as sirtuins or IGF-1. In case of skeletal muscles, it exerts its anti-ageing effect by normalising the redox status and improving the reduced mitochondrial functions, while in the brain it can bring about improvement through the formation of new nerve cells (26, 27, 28). Fibroblast growth factor (FGF) plays a role in vasculogenesis and wound healing, while the lack of nerve growth factor (NGF) results in the death of sympathetic and sensory neurons. Additional growth factors include epidermal growth factor (EGF), which facilitates the growth, propagation and maturation of cells, and the matricellular protein connective tissue growth factor (CTGF) plays an important role in the attachment, migration and propagation of cells and in the formation of blood vessels (8, 15, 25, 34, 39). A joint characteristic of these growth factors is that they play a role in the growth, propagation, maturation and especially in the anabolic metabolism of cells (13, 14, 28, 29, 30, 31, 35, 37, 40).

Being an oligopeptide complex, the purified and standardised chicken embryo extract (Humanofort®) used in the study contains numerous growth factors (FGF, NGF, EGF, CTGF, IGF-1, IGF-2) that have an effect on the course of inflammation. The degree of inflammation (and its decrease or increase) may be monitored on the basis of changes in the experimental animals' pain and, indirectly, by studying their willingness to move (11, 40).

The growth factors contained by the product exert an effect on
– inflammation,
– anabolic processes,
– vasculogenesis,
–wound healing
– nerve cells, and
– cell metabolism

Materials and methods

For the double-blind study we used the veterinary product of curative effect, called Biogenic Pet Small currently having a marketing authorisation in Hungary (marketing authorisation number: 475/1-2/2012 NÉBIH ÁTI – Directorate of Medicinal Products of the National Food Chain Safety Office; batch number: 25060510) containing Humanofort® 25 mg (manufacturer: S. C. Hipocrate 2002 SERV S. R. L., Bucharest, Romania, batch number: 0702092009) as active substance, or tablets of identical weight (1,000 mg), size and colour as the active product, but not containing active substance. The active substance was a chicken embryo extract (SECO) purified, standardised and produced by biotechnological means.

The investigational product contains a purified, standardized chicken embryo extract

Into the investigation we included dogs that were not pregnant and lactating and were not planned to be bred within the subsequent 6 weeks. Another criterion was the presence of a musculoskeletal disorder accompanied by chronic pain that required conservative therapy. The owners' consent was obtained and documented in all cases. Exclusion criteria included a severe pain requiring medicinal treatment as well as the use of any joint-protecting or regenerating product for a period of at least 14 days within the last 28 days preceding the treatment.

In the experiment, half of the dogs received the product containing the active substance, and the other half were given a placebo. Animals receiving the active substance and those treated with placebo were selected randomly.

The investigations were carried out in four independent veterinary clinics operating in four different regions of Hungary.

The experiment was performed in a multicentric, randomised, double-blind and placebo-controlled design, in conformity with the relevant EU directive (18). The clinical examinations and the treatments were carried out according to the rules of Good Clinical Practice (17).

The double-blind, placebo-controlled experiment was performed on dogs suffering from chronic musculoskeletal disorders

A total of 49 dogs were included in the experiment. According to the randomisation performed before the study (MICROSOFT® EXCEL 2010), 24 dogs received the placebo and 25 dogs were administered the active substance. To meet the requirements of a double-blind study, the primary packaging was marked as SO (original) and SF (placebo) . During the experiment, two dogs of the group receiving the active substance were excluded (one because of side effect and the other because it was not brought back for the control examination), while all dogs of the placebo group completed the study. The sex distribution was 26 males (53%) and 23 females (47%). By binomial test, the sex ratio did not differ markedly from 1:1 ($p = 0.7754$).

The dogs included in the study were subjected to general veterinary examination on day 0 and day 42 (on the last day of treatment) of the study, and to physical examination on days 14 and 28. During the time of the study, the dogs were staying in their usual environment, their diet was not changed, and they did not receive any other veterinary medicinal product or product of curative effect.

During the study, changes in the dogs' behaviour, motility and musculoskeletal signs were recorded with the help of a scoring sheet, by processing the partial data or the WOMAC osteoarthritis index (Western Ontario and Macmaster Osteoarthritis Index) as modified by Lascelles (26) and the Glasgow Pain Scale (GPS), as well as by the use of other technical publications (32, 36).

The behaviour and motility of the dogs and the changes in the musculoskeletal signs were evaluated by scoring methods

Results

By randomisation, 24 dogs were classified into the SF (placebo) and 25 dogs into the SO (original) treatment group. During the experiment, 2 dogs had to be removed from the SO group, and thus the number of dogs suitable for evaluation was 23 in that group. Overall, 47 dogs completed the study.

No adverse effects attributable to the treatment were observed during the study.

The treatment was considered effective if the score characterising the severity of the disease had decreased by at least 1 by the end of the treatment (day 342) as compared to the score determined at the start of treatment **(Table 1)**.

Comparing the values of the two treatment groups by two-tailed Fisher's exact test, it can be stated that SO treatment was significantly more effective ($p = 0.0006$). The 95% lower confidence limit for the odds ratio of SO versus SF efficacy was 3.9, i.e. it can be stated with 95% confidence that the chance of efficacy of SO treatment of the study dogs is at least 3.9 times higher than that of the SF treatment. The severity scores obtained during the study are presented in **Table 2.**

Humanofort treatment was significantly more effective than the placebo

Table 3 shows how the clinical signs of the treated dogs changed as compared to the scores measured on the day of inclusion.

Table 1. *Effectiveness of the treatment*

Test groups	**Not successful**	**Successful**
Treated (SO)	0	23
Placebo (SF)	10	14

Table 2. *Change of the mean score and its standard deviation in the treated group over time*

Scores, mean (SD)			
Day 0	**Day 14**	**Day 28**	**Day 42**
8.52 (5.20)	5.91 (4.06)	3.43 (2.84)	2.43 (2.74)

Table 3. *Change of the mean score and its standard deviation compared to Day 0 by treatment group*

Test groups	**Reduction of scores, mean (SD)**		
	Day 14	**Day 28**	**Day 42**
Treated	2.61 (2.29)	5.08 (3.76)	6.09 (4.12)
Placebo	0.75 (1.65)	1.58 (2.72)	2.00 (2.75)

Based upon the results obtained in the groups treated with the product containing the active substance and the placebo, it can be established that the active substance was significantly more successful in terms of both pain reduction and mitigation of the severity of motility disturbance. In the group treated with the product containing the substance, the score indicating the severity of the locomotor disease signs decreased by 6.09 points on the average, while in the placebo-treated group a decrease of 2.00 points could be observed.

The deviations arising from the different study sites and from the subjectivity of the investigators were not studied, although there may have been substantial differences between the practical experiences and the judgement of data of the evaluation table.

The 'unique and unrepeatable' (*'unicum neque iterabile'*) signs can be evaluated objectively only through a consensus of several experts reached after the evaluation of video recordings made at each examination (1, 6, 7, 18, 19, 22, 23).

By the use of Humanofort®
chronic joint pain can be
diminished without side effects

Discussion

The results of our experiments indicate that the motility (willingness to move) of dogs suffering from chronic joint pain can be improved by the use of Humanofort®. This improvement is attributable to the anti-inflammatory effect of the active substance, and could be measured through the reduction of pain, which is one of the main signs of inflammation. The advantage of Humanofort® over steroid and nonsteroidal products is that, according to the present state of knowledge, it does not have adverse effects.

It was beyond the scope of the present experiment to determine exactly which growth factor(s) contained by Humanofort® were responsible for the anti-inflammatory effect.

Numerous data are available in the literature about the mechanism of action of natural growth factors. However, in the present experiment we did not aim to determine the effects bound to specific molecules. The objective of our future research will be to elucidate whether the natural growth factors present in the Humanofort® product in specific quantities and ratios influence the effects of one another and, if yes, in what way and to what extent.

Acknowledgements

The authors thank the veterinarians who participated in the clinical trial. Particular thanks are due to Dr. Kinga Marton for designing and organising the clinical trials. The research was co-financed by grant GOP 1.1.1-08/1-2008-0032.

References