A pharmacokinetic and pharmacodynamic study with sodium salicylate in a model of acute non-immune inflammation in chickens

Eine pharmakokinetische und pharmakodynamische Studie mit Natrium-Salicylat am Beispiel einer akuten, nicht-immunologischen Entzündung beim Huhn

K. Baert, M. Schelkens and P. De Backer


Introduction

Classical non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed in different animal species and they often provide excellent symptomatic relief in many inflammatory disorders. The clinical signs associated with the process of inflammation have been recognised for thousands of years. Redness, swelling, heat, pain and loss of function can all be recognised to varying degrees in inflammatory foci in different species. The inflammatory response in tissues consists largely out of an active vasodilatation with increased permeability of white blood cells (WBC). The protein-rich fluid and the cells which accumulate in the injured tissues following this process make up the inflammatory exudate which is a major feature of acute inflammation (May et al., 1987). The inflammation process in birds and a comparison with mammal inflammation was described by Baert (2003). Practically no information about PG_E2 and bradykinin is available in literature about bird inflammation. The choice of these parameters was decided on the basis of comparative inflammatory processes, described in mammal inflammation.

The process of inflammation may sometimes be inappropriate to a healing process, as in hypersensitivity reactions or to severe as to damage tissue. In such circumstances a number of drugs are available to attenuate the symptoms and effects of inflammation. Sodium salicylate is one of the oldest NSAIDs and has been used for many years as an antipyretic, analgesic and anti-inflammatory agent in different species. NSAIDs inhibit symptoms associated with inflammation because they block the enzyme cyclo-oxygenase, which is necessary in the arachidonic acid cascade. This cascade forms an important group of inflammatory mediators, the eicosanoids such as prostaglandins and thromboxanes from arachidonic acid. However, in bird species scientific research on pharmacokinetics and pharmacodynamics of these drugs is limited (Baert, 2003). The plasma concentration of salicylic acid above which effective antipyretic, anti-inflammatory and analgesic activity can be achieved, has been reported to be 50 µg/ml for several species (Lees and Higgins, 1985; Lees et al., 1991). Based on the intravenous pharmacokinetics of sodium salicylate in broiler chickens (Baert and De Backer, 2002) and the earlier calculated good bioavailability, a dose of 50 mg/kg was chosen. Also, the methods to investigate inflammation processes in chickens are limited. In this study a model of non-immune inflammation, modified from Higgins et al. (1984) and Chansoriya et al. (1994) was used in chickens. Carrageenan, a mucopolysaccharide extract of Irish marine algae, was used to generate an inflammatory reaction. Since 1959, techniques using carrageenan were refined and these models became accepted as standard methods for screening and assessing the efficacy of anti-inflammatory drugs (Higgins et al., 1987). The objectives of this study were: the evaluation of the inflammatory effects of a mild subcutaneous inflammation model using sponges soaked in carrageenan in chickens, the time course of in vivo inhibition of synthesis of prostaglandin_E2 (PG_E2) in inflammatory exudate, and the pharmacokinetics of sodium salicylate in plasma and the penetration in the inflammatory exudate.

Materials and methods

Animals and experimental design

Twenty four clinically healthy broiler chickens (1.8 ± 0.22 kg BW, class Aves, order Galliformes, Gallus gallus, Ross breed) were used for the study. The animals were kept in groups of four chickens and fed a commercial chicken feed (Duvo, Wondelgem, Belgium) along with tap water ad libitum. A 12 h photoperiod was installed and ambient temperature was maintained at 22–24 °C. There was an acclimatisation period of 7 days before the start of the study. Twelve chickens received a placebo treatment and twelve chickens received an oral administration of the salicylate solution at 50 mg/kg BW and this was already done 0.5 hour before insertion of the sponges.

Induction of inflammatory reaction

A mild inflammatory response was induced subcutaneously under the wing above the pectoral muscle at both sides of each chicken. At this site, practically no feathers and featherfollicles are present. No feathers were pulled to minimise traumatic dermal inflammation. An incision of 1.5 cm was made under local anaesthesia (2% lidocaine hydrochloride, Astra Pharmaceuticals, Brussel, Belgium), care was taken not to cut visible blood vessels. Pressure
was exerted until bleeding subsided (1 min) and a sponge strip (35 × 20 × 5 mm), treated with sterile 1% carrageenan (Sigma Chemical, St Louis, MO, USA), was inserted in the subcutaneous pouch. The wound was not sutured for the duration of the study and was closed afterwards with 3 sutures. During the study asepsis was maintained by covering the wound with an adhesive non woven fabric (Fixomull® stretch, Beiersdorf AG, Hamburg, Germany). Only 2 subcutaneous pouches could be made on 1 chicken. To obtain a sufficient number of samples for each sampling point, we used 6 chickens per point. These sponges were removed at predetermined times up to 24 h.

Inhibition of bradykinin (BK)-induced swelling was evaluated as follows. Five µl of a 20 µg/ml solution of BK was injected intradermally in the sternal featherless region. After 30 minutes, the change in skin-fold thickness and 2 wheal diameters were measured, using spring gauge and vernier calipers, respectively. The volume of the wheal (as assumed to approximate to half an ellipse) was calculated by applying the following equation: \( V = \frac{2}{3} \pi r_1 r_2 r_3 \), where \( V \) is the volume of the wheal expressed in microlitres, \( \pi \) is the constant 3.1416, \( r_1 \) is the horizontal radius of the wheal, \( r_2 \) is the vertical radius of the wheal, and \( r_3 \) is the change in skin-fold thickness. Bradykinin was injected at 2 and 7 hours after administration of sodium salicylate.

Collection of blood and exudate samples

Blood samples for the collection of plasma were taken in the leg vein of six chickens at predetermined times up to 48 h into heparinized tubes. Plasma was collected by centrifugation (2500 × g, 7 °C, 10 min) and was stored at −20 °C until analysis for salicylate concentration. The sponges containing acute inflammatory exudate were removed at 4, 8, 12 and 24 h after insertion. Exudate volume was measured after removal. From each sponge, inflammatory exudate was collected into tubes containing 70 IU heparine and 10 µg BW540C (Glaxo Wellcome Research and Development, Stevenage, Hert., UK), a dual cyclooxygenase 5-lipoxygenase inhibitor, to prevent artefactual in vitro generation of eicosanoids (Higgins and Lees, 1984). Following removal of a 0.1 ml aliquot for measurement of leukocyte numbers, samples were centrifuged (2500 × g, 7 °C, 10 min) to separate cells. The supernatants were divided into aliquots prior to storage at −20 °C until analysed for PGE2 and salicylate concentration.

Analytical methods

Total leukocyte counts were done using a Coulter Counter (The Coulter Corporation, USA). PGE2 was analysed using a commercial ELISA method (Cayman Chemical, Ann Arbor, MI, USA). Salicylate and metabolites were quantitated using a HPLC method with a photodiode array detector (Baert et al., 2002). Briefly, plasma concentrations of salicylic acid and two possible metabolites gentisic acid and saliuric acid were analysed on a Thermo Separations Product (TSP, Fremont, CA, USA) HPLC-system using a P-4000 pump, Model AS 3000 autosampler and a Focus Forward scanning UV-detector set at 305 nm. A 250 × 4.6 mm internal diameter (ID) reversed-phase column (5 µm Spherisorb ODS-2, Chrompack, Antwerp, Belgium) attached to an appropriate guard column was used. The injection volume was 100 µl. The mobile phase comprised 85% water-acetic acid (99:1, v/v) and 15% acetonitrile. A gradient solvent programme was run: 0–4 min: 85/15; 4–20 min: 85/15–60/40; 20.1–25 min: 85/15. The flow rate was 1 ml/min. Samples were prepared by pipetting 0.5 ml of plasma or exudate into a 15 ml screw-capped tube, followed by the addition of 50 µl of I. S. (o-anisic acid in methanol, 100 µg/ml), 150 µl of 1 M HCl and 5 ml of diethylether. After centrifugation (2400 g, 5 min), the organic layer was transferred to a clean screwcapped tube and evaporated under nitrogen at 40 °C. The residue was redissolved in 250 µl of the mobile phase, briefly vortexed and 100 µl were injected.

Analysis of data

Plasma and exudate concentration time relationships were evaluated using standard pharmacokinetic methods. Plasma pharmacokinetic parameters were estimated by fitting the concentration data to an appropriate model by means of a MW/PHARM computer program (version 3.15, Groningen, The Netherlands). All of the values reported are mean ± standard deviation (SD) and the significance of differences of means between drug and placebo treated animals was assessed by Student’s t-test for unpaired data. The level of significance was 0.05.

Results

Pharmacokinetics

The mean plasma and exudate concentrations of salicylic acid were plotted on a semi-logarithmic scale as a function of time and are shown in Figure 1. The plasma curve was best described as a one compartment open model. The pharmacokinetic parameters obtained for sodium salicylate are presented as the mean ± SD (n = 6) in Table 1. For the calculation of some of these parameters, a bioavailability of 80% was used, a value that was calculated in earlier experiments (Baert and De Backer, unpublished results).

The concentration of salicylate found in the exudate exceeds significantly the concentration found in plasma at the same time points and the half-life of salicylate in the
Table 1. Pharmacokinetic parameters of salicylic acid (SA) in plasma after a single oral administration of sodium salicylate at 50 mg/kg of SA (n = 6, mean ± SD). The pharmacokinetic parameters are: AUC – area under the curve from 0 to infinity; Cl – total clearance; Vd(area) – volume of distribution; t1/2el – half-life of elimination; kel – elimination constant; MRT – mean residence time; Cmax – maximum concentration after administration; Tmax – time to reach Cmax.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SA</th>
<th>MEAN</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>AUC (mg - h/l)</td>
<td></td>
<td>384.8</td>
<td>212.8</td>
</tr>
<tr>
<td>Cl (l/h - kg)</td>
<td></td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Vd(area) (l/kg)</td>
<td></td>
<td>1.03</td>
<td>0.34</td>
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<tr>
<td>t1/2el (h)</td>
<td></td>
<td>6.07</td>
<td>1.81</td>
</tr>
<tr>
<td>kel (h⁻¹)</td>
<td></td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>MRT (h)</td>
<td></td>
<td>8.83</td>
<td>2.58</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td></td>
<td>40.30</td>
<td>11.69</td>
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<tr>
<td>Tmax (h)</td>
<td></td>
<td>0.30</td>
<td>0.44</td>
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</tbody>
</table>

The volume of the exudate samples is presented in Figure 2. No differences could be found between the samples of the untreated birds and the birds who received an oral bolus of 50 mg/kg of sodium salicylate. The concentration of WBC in the exudate is presented in Figure 3. No differences in the concentration of WBC in the treated or the non-treated group were found. The mean PGE2 concentration (Figure 4) showed only at the 4 hour point a significant difference between the salicylate treated birds and the placebo treated birds. The injection of bradykinin intradermally gave macroscopically a very mild reaction in the skin. A slightly paler colour of the skin at the site of injection was apparent in most of the chickens, but no changes in skinfold thickness could be shown.

### Pharmacodynamics

The volume of the exudate samples is approximately 7.3 h, which is longer than in the plasma.

### Discussion

Ideally, a model of inflammation should provide the investigator with the ability to monitor quantitatively clinical, physiological, cellular and biochemical aspects of the inflammatory response in the species of interest. In practice, however, it is difficult and perhaps impossible to achieve all of these wide-ranging objectives in a single model. Additionally, particular problems are encountered when...
studying the chicken. The chicken is a relatively small animal, and the skin is covered with feathers. Also the skin is very thin and the subcutaneous connective tissue is very loose and easily disrupted. Each of the ‘cardinal signs’ of inflammation, redness, heat, swelling, pain and ‘loss of function’ can be used as an indicator of the progression of an inflammatory reaction. In chickens, few inflammation models have been used and information on efficacy of anti-inflammatory drugs is scarce. Studies on the increase of vascular permeability after chemically induced inflammation (turpentine, carrageenan) were done by Ito and Böhm (1986) and Jain et al. (1995). One research group recently developed an intra-articular inflammation model with sodium urate crystals in chickens. In this model, behavioural observations were done and were linked to the analgesic properties of the drug tested. (Gentle, 1997; Hocking et al., 1997). Others used naturally occurring musculo-skeletal disorders to evaluate anti-inflammatory drugs (Mc Geown et al., 1999). In this model, the classical signs of inflammation could not easily be assessed. Redness and heat could not be measured because of the anatomical features of the chicken skin: very thin and feathered, furthermore, the site of inflammation was situated under the wing. Swelling of the lesion could not be measured because the size of the inflammation site was largely dependent on the size of the foreign body. An attempt to measure the increased vascular permeability was done by assessing the volume of exudate and the number of leukocytes as a measure for diapedesis. Pain and loss of function are difficult to assess in birds. Current methods are based on behavioural studies. In this study, only detailed behavioural observations were done.

There is also the question of the ethical applicability of any model developed for use in domestic animal species. Practically, all models that investigate inflammation cause pain and distress to the animals. A principal objective in designing new models, therefore, was to minimise any distress and discomfort to the chickens being used. In this study, chickens seemed to tolerate the procedure well. No stress reactions were noted during the incision and insertion of the foreign body and during the study, chickens showed a normal feeding and drinking behaviour. After the study, wounds were closed and healing was rapid and complete.

Pharmacokinetic parameters after administration of sodium salicylate are already described for several animal species, including dogs (Waters et al., 1993), cattle and goats (Short et al., 1990a) and rabbits (Short et al., 1990b). In chickens, very few reports are available about plasma concentrations after administration of sodium salicylate (Nouws et al., 1994; Baert and De Backer, 2002). The findings in this study show a half-life of 6.07 h, which is slightly longer than the half-life found after intravenous administration (4.04 h) by the same authors. This could be due to delayed absorption processes. However, a generally fast absorption was found with a Tmax after 0.30 h and a Cmax of about 40 mg/l. The exudate concentrations exceed significantly the concentrations in the plasma at the same time points. Also a longer half-life was seen in the exudate (Figure 1). The present study confirms that salicylate penetrates into and was slowly cleared from inflammatory exudate in the chicken. This finding is in accordance with published results in other species and other NSAIDs (Landoni and Lees, 1995; Scherkl et al., 1996; Lees et al., 1999).

The exudate samples were practically uncontaminated with blood. This is in contrast with the data obtained in horses by Higgins and Lees (1984). The volume of the exudate samples are shown in figure 2. Volumes rose from 3 ml at the 4 h point to more than 10 ml at 24 h. However, no difference could be shown between the sodium salicylate treated birds and the placebo treated birds. As indicated by figure 3, leukocyte infiltration into exudate occurred following the insertion of the carrageenan treated sponges. Cell numbers rose at 4, 8, 12 and 24 h. Cells were relatively slow to mobilise but by 12 hours numbers were still rising and at 24 hours the mean count had almost reached $10^6$ cells/litre. Higgins et al. (1987) found a value of $100 \times 10^9$ cells/litre in the equine sponge model. The somewhat lower number of cells could be due to the less vascularised subcutaneous tissue and the fewer number of macrophages present in the chicken. Again, no difference could be shown between the sodium salicylate treated birds and the placebo treated birds. Thus, a single oral administration of sodium salicylate previous to the inflammatory stimulus seems to have no influence on chemotaxis into the inflammatory exudate. This was also the case for meloxicam in an identical study design in chickens (Baert and De Backer, unpublished results). In other animal species, e.g. in rat and equine carrageenan induced inflammation models, phenylbutazone or flunixin could not inhibit leukocyte accumulation (Higgs et al., 1980; Lees and Higgins, 1984). Earlier experiments in rats, however, showed a depression of leukocyte migration into plastic sponge exudate in rats after administration of 150 mg/kg aspirin (Di Rosa, 1979). This could mean that a concentration of more than 50 $\mu$g/ml in inflammatory exudate or an oral dose of 50 mg/kg might be insufficient to obtain an effect on the migration of leukocytes.

The concentration of the eicosanoid PGE2 in the exudate was relatively low in comparison with findings by Higgins et al. (1987). They found values ranging from 5–60 ng/ml. This could be due to the nature of the tissue that was stimulated. In the chicken, the subcutaneous tissue is much less vascularised and fewer macrophages are present. A major difference between the chicken and the horse date is seen in the shape of the curve. In chickens, only in the first time point a significant difference could be found between the sodium salicylate treated birds and the placebo treated birds. In horses the maximal concentration of PGE2 is found at 12 hours. This could mean that in chickens, the role of the eicosanoids in this chicken inflammation model is restricted to the first hours after the inflammatory stimulus. This is in accordance with the findings of Ito and Böhm (1989). They concluded that prostaglandin mediation of inflammatory oedema after carrageenan induced foot pad oedema occurred to some degree in the later stages of the inflammation (2.5–5 hours after the inflammatory stimulus). Since maximal numbers of leukocytes and maximal concentration of eicosanoids not coincide, these data suggest that other cells or tissues may be involved to a greater extent in the synthesis of these compounds.

Bradykinin injected intradermally induced oedematous lesions, with peak volume at 30 minutes, in calves and horses. The mechanism of action is unknown, but it may be cyclo-oxygenase dependent, because BK is known to release inflammatory eicosanoids through activation of phospholipase A2 (Landoni et al., 1995; Auer et al., 1991; Dray and Perkins, 1993). Thus, treatment with an NSAID can attenuate the swelling caused by BK, as has been shown by Landoni et al. (1995) for flunixin in calves. In this experiment in chickens, practically no swelling was seen, only a paler colour of the skin at the site of injection of most of the chickens. Bradykinin induced changes in vascular permeability in the chicken skin were studied by
Awadhiya et al. (1980). These researchers found that the venular response to BK was slightly delayed up to two hours after injection and not as strong as histamine.

This inflammation model in chickens has advantages and disadvantages. Only a few characteristics of inflammation can be assessed and none of the cardinal signs of inflammation can easily be measured in this model. Based on the present findings sodium salicylate may be used to suppress the inflammatory cascade in chickens, but further research is needed to characterise the inflammatory response in this chemical inflammation model and the actions of the non-steroidal anti-inflammatory drug in chickens.

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Summary

The current knowledge about inflammation in birds and the influence of non-steroidal anti-inflammatory drugs on inflammation is very limited. Sodium salicylate is an NSAID that possesses favourable pharmacokinetic properties in chickens and can be used to treat inflammations in birds. In this experiment, an acute inflammatory reaction was generated by implanting a carrageenan impregnated sponge strip in the subcutaneous tissue of broiler chickens. Half of the chickens received 50 mg/kg BW sodium salicylate orally and the other half received a placebo treatment. Blood and exudate samples were taken from these chickens at predetermined times. The pharmacokinetics of sodium salicylate were investigated in blood and exudate. Other parameters that were investigated were the volume of the exudate, the number of leukocytes in the exudate and the prostaglandin E2 concentration in the exudate. Also bradykinin was injected intradermally and the effects of salicylate on bradykinin induced oedema were studied. Maximum salicylic acid plasma concentrations occurred within the hour after administration and maximum exudate levels were seen at the first exudate sampling point (4 h). Salicylic acid exudate concentrations exceeded plasma concentrations at the same time points and the exudate half-life of elimination was longer than the plasma half-life of elimination. No differences were found in the volume of the exudate and the leukocyte numbers between the treated and the untreated group. Sodium salicylate reduced the PGE2 concentration in the inflammatory exudate at the 4 hour time point, but at the later time points, no significant differences were found. The intradermally injected bradykinin did not produce significant effects in the chicken skin. Based on these findings, sodium salicylate may be used to suppress the inflammatory cascade in chickens, but further research is needed to characterise the inflammatory response in this chemical inflammation model and the actions of this non-steroidal anti-inflammatory drug in chickens.

Keywords

Chickens, pharmacokinetics, sodium salicylate, inflammation model, carrageenan

Zusammenfassung

Eine pharmakokinetische und pharmakodynamische Studie mit Natrium-Salicylat am Beispiel einer akuten, nicht-immunologischen Entzündung beim Huhn


Stichworte

Huhn, Pharmakokinetic, Natrium-Salicylat, Entzündungsmodell, islandisches Moos

References


Correspondence: Kris Baert, Department of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium; e-mail: kris.baert@rug.ac.be