PHARMACOKINETICS AND PHARMACODYNAMICS OF SALICYLATES IN BROILER CHICKENS

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Avian veterinarians always face problems when deciding the dosing regimen of drugs to be used for treatment of various conditions in birds because of the scarcity of information on the pharmacology of even the most commonly used drugs in birds. Class Aves contains more than 9500 species and such a large number makes it impossible to conduct pharmacokinetics in each and every wild avian species. Also, administration of drugs in endangered birds without any prior knowledge of their effects in birds can raise ethical problems. The extrapolation of pharmacokinetic parameters for birds from mammalian studies has been shown to generate a higher percent error compared to extrapolation between bird species (Hunter et al., 2008). There is no established model of drug research for avian species. This is also true for pharmacology of analgesic drugs in birds.

Aspirin (acetylsalicylate) and salicylic acid are popular and widely used traditional NSAIDs for treatment of pain in mammals. The pharmacokinetics of aspirin have been extensively studied in various mammals. There is only one study on the pharmacokinetics of salicylic acid in chickens (Baert and De Backer, 2002). Salicylic acid was injected intravenously at 50 mg/kg. The elimination half life was 4.04 hours, volume of distribution was 0.39 L/kg and clearance was 70 ml/h/kg. The pharmacokinetics were best described by a one compartment model and the therapeutic levels were maintained for five hours after the iv injection. They found that chickens cleared salicylic acid more slowly than ruminants and salicyluric acid was not found in chickens (one of major metabolites of salicylic acid in humans (Patel et al., 1990)).

There are few reports on analgesic efficacy of salicylic acid and other NSAIDs in chickens and domestic fowl. The walking ability of lame chickens was improved with the administration of the analgesic drug carprofen either orally (Danbury et al., 2000) or subcutaneously (McGeown et al., 1999). The effect of various NSAIDs was also evaluated by Hocking et al. (2005) in domestic fowl induced with acute pain by intra-articular injection of sodium urate crystals. Analgesic effects of carprofen, flunixin, ketoprofen and sodium salicylate were observed at dose rates much higher than mammals.

The objective of this study was to describe the pharmacokinetics and assess the pharmacodynamics in order to formulate the dose rate for either aspirin or salicylic acid to alleviate pain in lame broiler chickens. The pharmacokinetic parameters obtained from this experiment can be used to extrapolate dosing regimens for rare and endangered wild avian species found in New Zealand such as kiwis, kakapos and albatrosses, in which it will never be possible to study the pharmacokinetics of every analgesic drug.

Materials and methods

This study was approved by Massey University Animal Ethics Committee. Eighteen broiler chickens with an average weight of 2 kg were randomly assigned to each drug. These chickens were divided

into three groups of six chickens each. A medial metatarsal vein of each broiler chicken was catheterised under halothane anaesthesia. The birds were allowed to recover from the effect of anaesthesia before the injection of drugs. Aspirin injection was made by dissolving 100 mg of acetylsalicylic acid in 200µL of dimethyl sulphoxide (DMSO) and the resulting solution was diluted to 1 mL with 50% solution of polyethylene glycol (PEG)-300 in water. Salicylate (SA) injection was prepared by dissolving 100 mg of sodium salicylate in 1 mL of distilled water. The injection was filtered through 0.2µm syringe filters and immediately administered. Aspirin and salicylate were injected into the contralateral vein at 50 mg/kg. Four serial blood samples of 2 mL each were taken at 0, 5, 10, and 30 minutes from group 1; 1, 2, 4, 8 hours from group 2; and 12, 16, 24 and 32 hours from Group 3. This procedure was used to avoid taking excessive amounts of blood from any individual bird. The blood samples were collected in the heparinised vials which were chilled immediately after collection and centrifuged at 3000 rpm for 5 minutes. Plasma was pipetted out and flash frozen in dry ice. The plasma samples were stored at -70°C until the day of analysis. The plasma samples were prepared for analysis by a liquid-liquid extraction procedure. 300µL plasma was vortex mixed with 10% acetic acid in acetonitrile and centrifuged at 2400*g* for 15 minutes. The supernatant was separated and dried under gentle stream of compressed air at 20 psi . The dried samples were mixed thoroughly in 200µL of mobile phase on a vortex mixer and centrifuged at 4000 rpm for 10 minutes. 50µL of this supernatant was injected into the HPLC system. The plasma samples were analysed by High Performance Liquid Chromataography (HPLC) with Diode Array Detection (DAD). The HPLC system consisted of LC-20AD pumps, SIL-20AC HT auto-injector, diode array detector SPD-M20A, CTO-20A column oven, DGU-20A3 Degasser (Shimadzu, Japan). The chromatographs were analysed in LC solutions (Shimadzu, Japan). The separation of aspirin and Salicylic acid was achieved with a Phenomenex C18A (150 X 4.6 mm i.d, 5µm particle size) column at 32°C. The mobile phase consisted of 10mM phosphate buffer pH 5.8: acetonitrile: acetic acid (71:28:1) with flow rate of 1mL/min under isocratic conditions. The DAD detector was set at 254 nm wavelength (Broome et al., 2003; Pirola et al., 1998).

Pharmacokinetic parameters were calculated by a non compartmental approach using standard equations in a spreadsheet. These included half life of the terminal phase ($T_{1/2\gamma z}$), area under the curve extrapolated from time zero to infinity ($AUC_{0-\infty}$), area under the moment curve extrapolated from time zero to infinity ($AUMC_{0-\infty}$), volume of distribution (V_d ml/kg), clearance (Cl ml/min/kg) and mean residence time (MRT min). The non-compartmental pharmacokinetics were calculated from the mean of pooled data. The samples from each of the three groups at various time points were pooled to give one mean concentration time curve (Gagnon and Peterson, 1998; Cheung et al., 2005).

A blinded randomised experiment was conducted to evaluate the analgesic efficacy of salicylic acid in lame broiler chickens. 12 broiler chickens were randomly assigned to each group (using a Javascript randomisation procedure); lame salicylic acid, lame placebo, lame control, sound salicylic acid, sound placebo and sound control. The birds in the salicylic acid treatment groups were injected iv with salicylic acid at 50 mg/kg. The placebo birds were injected with a similar volume of normal saline. The birds in the control were not injected with anything, but were handled in the same way as the other groups. The analgesic efficacy test were based on walking ability and standing time on lame broiler chickens. Naturally occurring lameness in broiler chickens is painful. Lame chickens are unable to stand for as long and also walk less and slower as compared to their healthy compatriots. Our hypothesis for this experiment was that an intravenous injection of salicylic acid at 50 mg/kg would increase the walking ability and standing time in lame broiler chickens without any effect on healthy broiler chickens.

Results and discussion

After intravenous administration, aspirin rapidly hydrolysed to salicylic acid, which is its major metabolite in mammals (Hutt et al., 1986). Aspirin has short half life in all species excluding cats. Chickens have a higher volume of distribution and faster clearance as compared to other species. This trend was also seen in a pharmacokinetic study of morphine in chickens (Singh et al., 2010). Aspirin had a very short half life in chickens; only 12.6 minutes (table 1). The maximum plasma concentration achieved in chickens was 46.72µg/mL. The peak plasma concentration for salicylate achieved 1 hour after aspirin injection was 93.75 µg/mL and at this time point, plasma concentration of aspirin was lowest. The volume of distribution was 0.25 L/kg, terminal half life 2.9 hours and clearance 60.6 mL/hr/kg. Salicylic acid was detectable in plasma until 16 hours after iv injection at 50 mg/kg. Pharmacokinetic parameters obtained after intravenous injection of salicylic acid in chickens were similar to the previous study conducted by Baert and Backer (2002). Broiler chickens had lower volume of distribution for salicylic acid as compared to morphine and butorphanol (Singh et al., 2011), which is typical for all the NSAIDS (Davis 1980). NSAIDS have lower tissue distribution as compared to opioids (Lin et al., 1987).

Salicylic acid provided good analgesia in lame broiler chickens. There was a significant increase in walking pace (Fig 1) and standing time (Fig. 2) in lame broiler chickens injected with salicylic acid. This effect did not last longer than 2 hours after administration. There was no effect of salicylic acid injection in sound birds in the analgesic efficacy tests. All the results from the placebo group in both the studies were non-significant, but the trend suggested that the lame birds progressively became worse with time. The results of the control group were similar to the placebo. This shows that there was no stress or pain caused by injection but cannot rule out stress induced analgesia caused by handling. When the lame birds were not given any treatment as in the control or placebo groups, the severity of lameness increased with time.

It is believed that major side effects in mammals such as gastric irritation and ulcers associated with use of NSAIDs are due to inhibition of COX-1 enzyme (Vane et al., 1998). In our study no bird showed any clinical signs attributable to ulcers, possibly because the birds only got a single dose.

The results from the pharmacodynamic study suggest that salicylic acid provides good analgesia at 50 mg/kg intravenous bolus dose rate but the effects lasted only 1 hour. The effective plasma concentration for salicylic acid in broiler chickens ranged from 110 to 150 ng/mL.

References

Baert K, De Backer P (2002) Disposition of sodium salicylate, flunixin and meloxicam after intravenous administration in broiler chickens. Journal of Veterinary Pharmacology and Therapeutics 25, 449-453

Cheung BWY, Cartier LL, Russlie HQ, Sawchuk RJ (2005) The application of sample pooling methods for determining auc, aumc and mean residence times in pharmacokinetic studies. Fundamental and Clinical Pharmacology 19, 347

Danbury TC, Weeks CA, Chambers JP, Waterman-Pearson AE, Kestin SC (2000) Self-selection of the analgesic drug carprofen by lame broiler chickens. The Veterinary Record 146, 307-311

Davis LE (1980) Clinical pharmacology of salicylates. Journal of American Veterinary and Medical Association 176, 65-66

Gagnon RC, Peterson JJ (1998) Estimation of confidence intervals for area under the curve from destructively obtained pharmacokinetic data. Journal of Pharmacokinetics and Pharmacodynamics 26, 87-102

Hocking PM, Robertson GW, Gentle MJ (2005) Effects of non-steriodal anti-inflammatory drugs on pain related behaviour in a model of articular pain in the domestic fowl. Research in Veterinary Science 73, 1785-1794

Hutt AJ, Caldwell J, Smith RL (1986) The metabolism of aspirin in man: A population study. Xenobiotica 16, 239-249

Hunter RP, Mahmood I, Martinez MN (2008) Prediction of xenobiotic clearance in avian species using mammalian or avian data: How accurate is the prediction? Journal of Veterinary Pharmacology and Therapeutics 31, 281-284.

Lin JH, Cocchetto DM, Duggan DE (1987) Protein binding as a primary determinant of the clinical pharmacokinetic properties of non-steroidal anti-inflammatory drugs. Clin Pharmacokinet 12, 402-432.

McGeown D, Danbury TC, Waterman-Pearson AE, Kestin SC (1999) Effect of carprofen on lameness in broiler chickens. The Veterinary Record 144, 668-671.

Patel DK, Notarianni LJ, Bennett PN (1990) Comparative metabolism of high doses of aspirin in man and rat. Xenobiotica 20, 847-854.

Pirola R, Bareggi SR, De Benedittis G (1998) Determination of acetylsalicylic acid and salicylic acid in skin and plasma by high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications 705, 309-315

Singh P, Johnson C, Gartrell B, Mitchinson S, Chambers P (2010) Pharmacokinetics of morphine after intravenous administration in broiler chickens. Journal of Veterinary Pharmacology and Therapeutics 33, 515-518.

Singh PM. Johnson C. Gartrell B. Mitchinson S. Chambers P. 2011. Pharmacokinetics of butorphanol in broiler chickens. Veterinary Record 168, 588

Vane JR, Bakhle YS, Botting RM (1998) Cyclooxygenases 1 and 2. Annual Review of Pharmacology and Toxicology 38, 97-120.

Pharmacokinetic parameter	Aspirin	Salicylic acid after aspirin injection	Salicylic acid
$AUC_{(0-\infty)}$ (µg.hr/mL)	19.5	728.1	823.9
$AUC_{(0-t)}$ (µg.hr/mL)	20.4	689.6	810.4
$AUMC_{(0-\infty)}$ (µg.hr2/mL)	5.5	4097.6	3802.0
$Vd_{(area)}$ (mL/Kg)	754.5	399.70	251.7
Cl (mL/hr/Kg)	2456.9	68.67	60.68
$T_{1/2\,z}$ (hours)	0.2	4	2.9
MRT (hours)	0.3	5.8	4.6

Table 1: Pharmacokinetics parameters calculated from the mean plasma concentration time curves for Aspirin and Salicylic acid after administration at 50 mg/kg, intravenously in broiler chickens calculated from a composite mean concentration time curve.

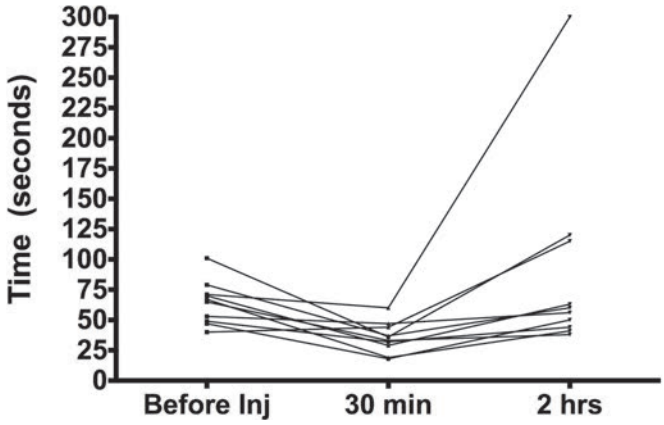

Figure 1: Salicylic acid was able to increase the walking pace after an iv injection at 50 mg/kg. The effect did not last more than 2 hours after the injection. There was significant decrease in time to finish a course 30 minutes after an injection of salicylic acid. P=0.0013. Lines represent individual birds.

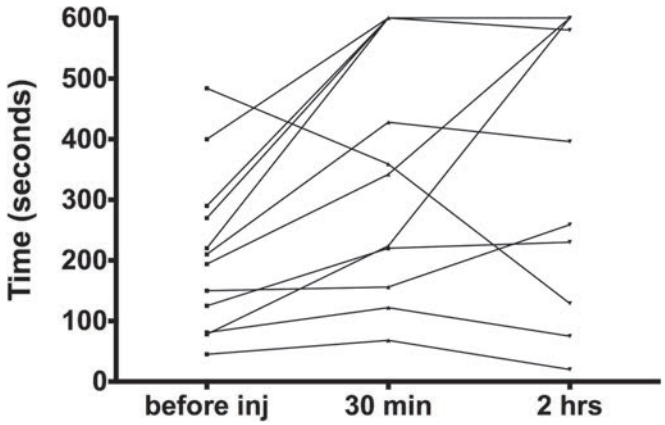

Figure 2: Lame broiler chickens were able to stand for longer duration after an iv injection at 50 mg/kg. There was significant increase in standing time 30 minutes after an injection of salicylic acid. =0.0115. Lines represent individual birds.