

Further studies on allopurinol-induced hyperuricaemia and visceral gout in red-tailed hawks (*Buteo jamaicensis*)

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To investigate the usefulness of allopurinol for the treatment of hyperuricemia in birds, experimental studies were performed using the physiologically occurring post-prandial hyperuricaemia in birds of prey as a model. Pre- and post-prandial plasma concentrations of allopurinol, oxypurinol, xanthine, hypoxanthine and uric acid were established by high performance liquid chromatography in red-tailed hawks (RTH, *Buteo jamaicensis*) at various time intervals after receiving allopurinol (50 mg/kg SID) or placebo. The dosage used caused slight, but significantly elevated plasma uric acid concentrations compared to controls, as well as vomiting in the majority of treated birds. Markedly elevated plasma concentrations of oxypurinol, xanthine and hypoxanthine were seen in experimental birds. Toxic signs were attributed to oxypurinol, the active (and toxic) metabolite of allopurinol. Xanthinuria was considered to be the cause of the observed renal function disorder. Extrapolation of data from studies in humans and combining these with those of the present study suggest that the maximum dose of allopurinol that can be safely administered to RTH is about half the dose given in the present study, but this needs verification.

Introduction

Uric acid is the main end product of protein metabolism in birds. Gout results when uric acid and urates fail to be excreted by the kidneys and are deposited within the body. The pathophysiology of avian gout is based on increased production of urates due to protein excess in the food or on reduced excretion due to renal failure. The drug which seems the most promising for the treatment of gout in birds with marginal renal function is allopurinol. Allopurinol is a competitive xanthine oxidase inhibitor which blocks the metabolic pathway from hypoxanthine via xanthine to uric acid. Although allopurinol is widely recommended for the treatment of gout, its use in birds is poorly documented (Lumeij, 1994). To investigate the effect of allopurinol on plasma uric acid concentrations in birds, experiments were performed using red-tailed hawks (RTH, *Buteo jamaicensis*) as a model (Lumeij & Redig, 1992). Birds of prey were considered a good model to study the effects of allopurinol since carnivorous birds show a marked

postprandial hyperuricemia (Lumeij & Remple, 1991). Surprisingly, three out of six birds developed hyperuricemia and visceral gout after allopurinol treatment, while in the three other birds no significant changes were seen in plasma uric acid concentrations. It was concluded that allopurinol should be used with care until further studies would prove the effectiveness of this drug in birds. It was suggested that the unexpected findings might be explained by the formation of oxypurinol, the relatively insoluble and nephrotoxic end product of allopurinol and/or that renal damage might have been caused by the deposition of the allopurinol precursor xanthine. To elucidate the aetiology of the aforementioned allopurinol induced hyperuricaemia, the present study was performed.

Materials and Methods

Birds and drugs

Ten adult red-tailed hawks were used for this experiment. The birds were from a group maintained for research purposes at the Raptor Center at the University of Minnesota. All these birds had been

Table 1. Mean \pm s.d. concentrations ($\mu\text{mol/l}$) of uric acid and its precursors xanthine and hypoxanthine, and the xanthineoxidase inhibitor allopurinol and its metabolite oxypurinol, before and after treatment with allopurinol (50 mg/kg SID orally, $n = 6$) or placebo ($n = 4$) for 4 days in red-tailed hawks (*Buteo jamaicensis*).

		Initial values	n h after 4th allopurinol treatment = (n - 1) h after feeding (see Fig. 1)					
			2 h after 3rd allopurinol treatment (fasting)	0 h after 4th = 24 h after 3rd allopurinol treatment	5 h	9 h	change in [uric acid] 24 h	Δ 9 h-24 h
Placebo								
uric acid	○	225 \pm 79	32 \pm 15	223 \pm 54	398 \pm 25	460 \pm 164	213 \pm 80	- 246 \pm 167
xanthine	□		46 \pm 8.5	5.5 \pm 0.6	40 \pm 15	37 \pm 17	12 \pm 9	
hypoxanthine	◇		59 \pm 21	48 \pm 14	141 \pm 49	70 \pm 8	24.5 \pm 16.5	
allopurinol			0	0	0	0	0	
oxypurinol			0	0	0	0	0	
Experiment								
uric acid	●	286 \pm 62	144 \pm 74	243 \pm 78	400 \pm 128	409 \pm 116	470 \pm 282	+ 120 \pm 180
xanthine	■		141 \pm 74	7.3 \pm 3.3	1184 \pm 280	1206 \pm 88	24 \pm 9	
hypoxanthine	◆		154 \pm 83	52 \pm 17	1449 \pm 447	821 \pm 543	70 \pm 32	
allopurinol	▼		37 \pm 33	0	48 \pm 34	1.2 \pm 2.9	0	
oxypurinol	▲		160 \pm 37	0	146 \pm 68	86 \pm 59	10.3 \pm 14.3	

^aThe birds were force fed half of a rat 1 h after the fourth allopurinol or placebo treatment. The symbols in the second column refer to those in Figure 1.

admitted to the centre with a unilateral wing fracture. None of the birds could fly well enough for release into the wild, but all were judged to be otherwise healthy. The following plasma chemical and haematological variables were determined to further document the absence of disease: haematocrit, total white blood cells and differentiation, total protein, plasma uric acid and urea concentration, aspartate aminotransferase and bile acids. One experimental group of six animals and one control group of four animals was formed. Birds from the experimental group were given allopurinol capsules in a dose of 50 mg/kg SID for 4 days at 09.00 a. m. Allopurinol capsules were made from commercially available tablets (Zyloric®, Glaxo Wellcome, Zeist, The Netherlands). Control birds were treated with a capsule containing a placebo. During the experiment, food and water was withheld from the birds except for one half-skinned rat which was force fed 1 h after the last medication. The fluid requirements of the birds were assumed to be covered by the fluid contents of the rats. Blood samples were collected just before any dosing, and 5, 9 and 24 h after the last allopurinol treatment (1 h before and 4, 8 and 23 h after feeding).

High performance liquid chromatography

Plasma concentrations of allopurinol, oxypurinol, xanthine, hypoxanthine and uric acid were established by high performance liquid chromatography (HPLC) according to Wung & Howell (1980) with some modifications.

A sample of 0.5 ml plasma was deproteinized with 0.05 ml 4.4 M perchloric acid (Baker Chemical Co., Phillipsburg, USA) centrifuged (10 min, 3000 g, 4°C), and 0.2 ml supernatant was neutralized with 0.015 ml 0.1 N sodium hydroxide (Merck, Darmstadt, Germany).

Stock standards of hypoxanthine, xanthine, uric acid, allopurinol and oxypurinol (Sigma Chemical Co, St Louis, USA) were prepared in a concentration of 1 M in distilled water. These were diluted with eluent (50 mM KH_2PO_4) to make calibration curves (1 to 100 μM). Concentrations were determined by HPLC (Beckmann Instruments, Inc., California, USA). HPLC separation was performed on a Lichrosper 100 RP-18, end capped 5 μm column (Merck Darmstadt, Germany) at a flow rate of 1.5 ml by isocratic elution with 50 mM potassium

dihydrogen phosphate pH 4.6 (eluent, Merck, Darmstadt, Germany). A standard volume of each standard or sample was injected into the column and the sensitivity was set at 0.01 \AA full scale. Absorption was monitored at 254 nm and the concentrations were calculated by evaluation of the peak area with an integrator (Hewlett Packard, Avondale, USA). Extraction efficiency was around 100%, for all compounds.

Statistics

Differences in concentrations of xanthine, hypoxanthine, and uric acid between birds of the experimental and placebo group at the various sampling times were tested for significance with Student's *t*-test for unpaired observations. The change in plasma concentration of uric acid between 8 and 23 h after feeding was compared between the experimental and control group and the difference tested for significance. In all calculations significance was assumed at $P < 0.05$.

Results

No abnormalities in plasma chemical or haematological variables were seen before the experiment. Plasma chemical variables were not significantly different between the experimental and the control group. After 3 days withholding food and while treating with allopurinol, birds from the experimental group showed significantly higher plasma uric acid concentrations compared to controls (Table 1, $P = 0.01$). After force feeding, four out of six birds which had received allopurinol regurgitated the food partially (three birds within 5 h and one bird within 10 h). The control birds did not regurgitate the food.

Birds receiving allopurinol showed significant

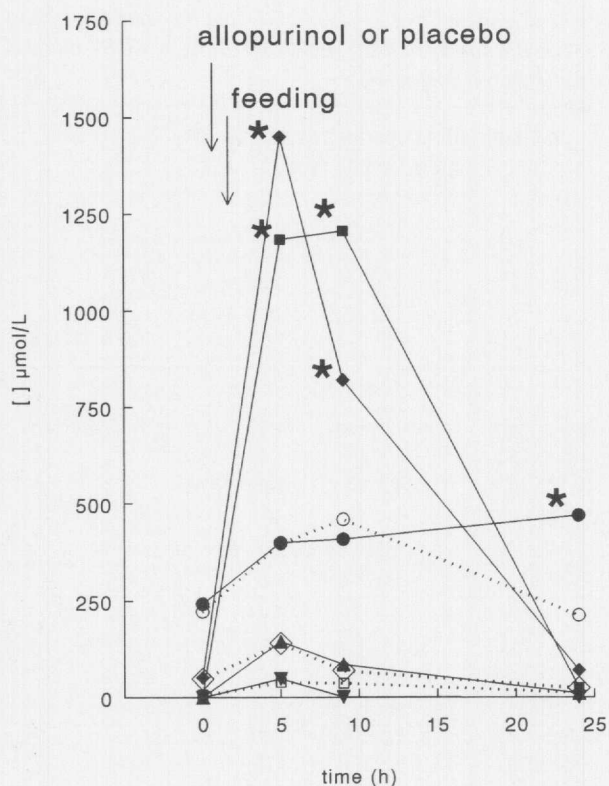


Figure 1. Mean concentrations of uric acid (●,○), and its precursors xanthine (■,□) and hypoxanthine (◆,◇), and the xanthineoxidase inhibitor allopurinol (▼) and its metabolite oxypurinol (▲), before and after treatment with allopurinol (50 mg/kg sid oral) or placebo for 4 days in red-tailed hawks (*Buteo jamaicensis*). Experimental group (n = 6) indicated with closed symbols. Placebo group (n = 4) indicated with open symbols and dotted lines. The birds were force fed 1/2 rat l h after fourth allopurinol (or placebo) treatment (arrows). For detailed information see Table 1. Asterisks indicate significant differences between experimental and control groups.

elevations of allopurinol, oxypurinol, xanthine and hypoxanthine at 5 h and 9 h after the last allopurinol treatment when compared to the placebo group ($P < 0.01$; Table 1 and Figure 1). Uric acid concentrations were increased in experimental animals (mean increase 120 mmol/l) and decreased in control animals (mean decreased 246 mmol/l) between 8 and 23 h after feeding. This difference was significant at $P = 0.012$ (Table 1 and Figure 1).

Discussion

The results from this study clearly show that allopurinol in a dose of 50 mg/kg SID leads to a marked rise in the plasma concentrations of the uric acid precursors xanthine and hypoxanthine. Treatment with allopurinol caused a slightly but significantly decreased renal function judged by differences in plasma uric acid concentrations after the third allopurinol treatment and different trends in the experimental and control groups between 8 and 23 h after feeding. Previous studies have shown

that raptors demonstrate a decline of plasma uric acid concentration between 8 and 23 h after feeding after first showing a postprandial rise in the first hours after ingestion of prey (Lumeij & Remple, 1991). This phenomenon was, indeed, seen in the control group. The experimental group, however, showed a significant increase during this time period, indicating reduced uric acid excretion, i.e. a renal function disorder. It would be expected that the rise of xanthine and hypoxanthine would be accompanied by a proportional fall in urate, but this was not seen in the experimental group. Since molecular weights of uric acid, xanthine and hypoxanthine are similar, concentrations of all three variables together are an indication of renal clearing capacity of nitrogenous waste products. The human kidney clears xanthine and hypoxanthine much more efficiently than uric acid. The rise of xanthine and hypoxanthine concentrations and the lack of a concurrent proportional decline of uric acid concentration are considered further evidence of reduced renal clearing capacity for nitrogenous waste, compared to controls. Although the initial dosage used in this experiment (50 mg/kg) was lower than in a previous experiment (100 mg/kg followed by 50 mg/kg) wherein visceral gout was induced by allopurinol in three out of six animals (Lumeij & Redig, 1992), the dosage used still induced signs of toxicity like vomiting and a renal function disorder. It seems that the drug-induced renal toxicity counteracts the uric acid decreasing effects of allopurinol, since plasma uric acid concentrations in the experimental group were significantly higher compared to controls.

In humans, the toxicity of allopurinol is determined by the concentration of the active metabolite oxypurinol. A guideline for maximum plasma concentrations in humans 6 to 9 h after ingestion is 30 to maximal 100 $\mu\text{mol/l}$ (Hande *et al.*, 1984). In the present study plasma oxypurinol concentrations between 127 and 222 $\mu\text{mol/l}$ were seen 2 h after the third administration of allopurinol, which is about twice the maximum recommended plasma concentration in humans. The high oxypurinol concentrations might have caused the toxicity signs (e.g. vomiting) in the experimental group. The relatively high oxypurinol concentrations in conjunction with toxicity signs (regurgitating, renal function disorder) indicate that the dosage of allopurinol used in the present study is too high. If these data from humans would be extrapolated to the present study, taking into consideration that the plasma drug concentration is directly proportional to the dose given, it seems that the maximum dose of allopurinol that should be given to RTH is about 50% of the dose given in the present study, i.e. 25 mg/kg. Further studies are needed to confirm the effects of this lower dose. The human kidney clears hypoxanthine and xanthine much more efficiently than uric acid, so the plasma concentrations of these precursors do not rise commensu-

rately with the fall in urate (Smith, 1979). Xanthine is as insoluble as uric acid, whereas hypoxanthine is highly soluble (Smith, 1979; Talbott & Yü, 1976). The pharmacological production of xanthinuria in humans is extremely rare. In Dalmatian dogs it has been recognized that excessive dosing of allopurinol may increase the concentrations of xanthine in the urine to values that may exceed the urinary solubility of xanthine, resulting in xanthine-containing urinary calculi (Ling *et al.*, 1991).

In the birds studied in this experiment it has been shown that in addition to the high plasma concentrations of oxypurinol, hyperxanthinemia occurs. Hyperxanthinuria can lead to xanthinuria. Both elevated plasma oxypurinol and xanthinuria are known in humans to cause reduced renal function. Further studies with a similar protocol as the present study might show that a reduced allopurinol dosage (25 mg/kg SID) can be safely administered in these birds.

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RÉSUMÉ

Etudes complémentaires sur l'hyperuricémie et la goutte viscérale induites par allopurinol chez des buses à queue rousse (*Buteo jamaicensis*)

Pour étudier l'utilité de l'allopurinol dans le traitement de l'hyperuricémie chez les oiseaux, des essais expérimentaux ont été entrepris, en prenant pour modèle l'hyperuricémie postprandiale survenant chez les oiseaux de proie. Des concentrations plasmatiques pré et postprandiales d'allopurinol, d'oxypurinol, de xanthine, d'hypoxanthine, et d'acide urique ont été établies en chromatographie liquide à haute performance chez des buses à queue rousse (RTH, *Buteo jamaicensis*) à intervalles réguliers après avoir reçu de l'allopurinol (50 mg/kg SID) ou un placebo. Le dosage utilisé a

entraîné des concentrations en acide urique faibles mais significativement élevées comparé aux témoins tout comme à la majorité des oiseaux traités qui présentaient des vomissements. Des concentrations plasmatiques très élevées d'oxypurinol, de xanthine et d'hypoxanthine ont été observées chez les oiseaux traités. La toxicité a été attribuée à l'oxypurinol, le métabolite actif et toxique de l'allopurinol. La présence de xanthine en excès dans l'urine a été considérée être la cause des désordres fonctionnels rénaux observés. L'extrapolation des données des études menées chez l'homme et de celles du présent essai, suggère que la dose maximale d'allopurinol qui peut être administrée sans risque au RTH correspond à environ la moitié de celle qui a été administrée sans le présent essai, mais ceci demande confirmation.

ZUSAMMENFASSUNG

Weitere Untersuchungen über die durch Allopurinol induzierte Hyperurikämie und Eingeweidegicht bei Rotschwanzbussarden (*Buteo jamaicensis*)

Zur Untersuchung der Brauchbarkeit von Allopurinol für die Behandlung der Hyperurikämie bei Vögeln wurden experimentelle Studien durchgeführt, für welche die physiologisch auftretende postprandiale Hyperurikämie bei Greifvögeln als Modell benutzt wurde. Die prä- und postprandialen Plasmakonzentrationen von Allopurinol, Oxypurinol, Xanthin, Hypoxanthin und Harnsäure wurden bei Rotschwanzbussarden (*Buteo jamaicensis*) in verschiedenen Zeitabständen nach der Verabreichung von Allopurinol (50 mg/kg SID) oder einem Placebo mittels Hochdruckflüssigkeitschromatographie festgestellt. Die verwendete Dosis bewirkte bei der Mehrzahl der behandelten Tiere im Vergleich zu den Kontrollen leicht, aber signifikant erhöhte Plasma-Harnsäurekonzentrationen sowie Erbrechen. Bei den Versuchstieren wurden deutlich erhöhte Plasmakonzentrationen von Oxypurinol, Xanthin und Hypoxanthin festgestellt. Die toxischen Symptome wurden Oxypurinol, dem aktiven (und toxischen) Metaboliten von Allopurinol, zugeschrieben. Die Xanthinurie wurde als Ursache der beobachteten Nierenfunktionsstörung betrachtet. Die Extrapolation von Daten aus Untersuchungen bei Menschen und deren Kombination mit denen der vorliegenden Studie deuten darauf hin, daß die maximale Allopurinol-Dosis, die gefahrlos an Rotschwanzbussarden verabreicht werden kann, ungefähr halb so groß ist wie die in der vorliegenden Studie gegebene Dosis, aber das bedarf einer Nachprüfung.

RESUMEN

Nuevos estudios sobre la inducción de Hiperuricemia y gota visceral del Alopurinol en ratoneros de cola roja (*Buteo jamaicensis*)

Para determinar la utilidad del alopurinol en el tratamiento de la hiperuricemia en aves, se realizaron estudios experimentales evaluando la hiperuricemia postprandial en rapaces como modelo. Se determinaron, mediante cromatografía líquida de alta precisión, las concentraciones pre- y postprandiales en plasma de alopurinol, oxipurinol, xantina, hipoxantina y ácido úrico en ratoneros de cola roja (RTH, *Buteo jamaicensis*) a diferentes intervalos de tiempo, después de recibir alopurinol (50 mg/kg/día) o placebo. Las dosis utilizadas daban lugar a una ligera, pero significativa elevación de la concentración de ácido úrico en plasma con respecto a los controles, así como vómitos en la mayoría de los animales tratados. Se observaron concentraciones plasmáticas muy elevadas de oxipurinol, xantina e hipoxantina en los animales tratados. Los síntomas de intoxicación se atribuyeron al oxipurinol, el metabolito activo (y tóxico) del alopurinol. La xantiniuria se consideró la causa de la disfunción renal observada. Mediante la extrapolación de datos procedentes de estudios en seres humanos y combinándolos con los nuestros, se sugiere que la máxima dosis de alopurinol que puede ser administrada, en condiciones de seguridad, a RTH, es aproximadamente la mitad que la utilizada en este estudio, aunque esto requiere posteriores verificaciones.