Nucleotide metabolism and Urate excretion in the Dalmatian dog breed.

By

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Abstract

The purpose of this literature study is to give an overview of nitrogen and purine metabolism in general and in particular of the molecular mechanisms for the higher uric acid excretion in Dalmatian dogs. The character portrayed by the Dalmatian breed appears to be connected whit a malfunction within purine metabolism, some studies point at a kidney disease whereas other at a liver disease. Although there is a difference in renal handling of urate between purebred Dalmatians and other breeds, the transport mechanism of urate into hepatic cells is most likely a major contributing factor to the higher excretion of uric acid. Findings of different urine composition between the sexes may in the future give rise to new treatments. The differences in purine metabolism and the typical spots of the Dalmatian dogs appears to be affected by pleiotropy and are both inherited by means of a classic Medelian autosomal pattern. All purebred dogs of this breed are affected and portray a high uric acid excretion; the possible elimination of this trait is unlikely due to pleiotropic interactions. More studies on the subject are needed and are of importance as they provide, within comparative medicine, tools for understanding liver and kidney diseases connected to stone formation.

Sammanfattning

Denna litteratur studie har som syfte att ge en översiktlig inblick i nitrogen och purinmetabolismen samt att specifikt behandla de molekylära mekanismerna för den höga urinsyrautsöndringen hos Dalmatiner hundar. Denna egenskap verkar kopplad till en rubbning av purinmetabolismen, några studier pekar på en njursjukdom och andra på en leversjukdom. Även om man har uppvisat en skillnad i urathanteringen mellan Dalmatiner och andra hundraser verkar transportmekanismen av urat, in i lever celler, vara en viktig påverkande faktor för den höga uratutsöndringen. Skillnaderna i purinmetabolism och de rastypiska prickarna verkar kopplade genom pleiotropi och ärvs genom ett klassiskt Mendeliansk autosomalt mönster. Alla renrasiga Dalmatiner är påverkade och uppvisar den höga urinsyrautsöndringen; det verkar osannolikt att få bort denna egenskap genom avel på grund av de pleiotropiska interaktionerna. Ytterligare komparativa studier inom ämnet behövs och är av vikt eftersom de kan ge verktyg för en bättre förståelse av njur- och leversjukdomar kopplade till stenbildning.

Introduction

Urolithiasis, a process involving the formation of uroliths (stones) in kidney, bladder and/or urinary tract, has been observed in most mammals. Urolithiasis is not a single disease but rather a multifactorial disorder with the common denominator of an increased concentration of less soluble crystalloids in the urine. A multitude of different types of uroliths have been characterized and often recognised as organized crystal aggregates with intricate internal structures. It seems that the appearance of uroliths is primarily connected to their mineral composition. In dogs magnesium ammonium phosphate has been the mineral type most commonly encountered followed by the much less frequent recurrence of uroliths composed of ammonium urate, uric acid, calcium phosphate and calcium oxalate (Osborne & Clinton, 1986).

Between 1956 and 1982 it was estimated that 24 dogs per 10 000 in Sweden were surgically operated on for urolithiasis. During this period the recurrence of uroliths were confirmed in 95 different breeds (Wallerstrom & Wagberg, 1992). Although many dog breeds are affected by all types of uroliths it has been noted that the recurrence of urate uroliths is mostly seen within

the Dalmatian breed. All Dalmatian of purebred displays high quantities of uric acid in their urine, predisposing them to the formation of urate uroliths (Safra *et al.*, 2005). Even if all Dalmatian shows a high excretion of uric acid not all dogs of this breed develops uroliths (Osborne & Clinton, 1986).

The formation of urate uroliths in Dalmatians is connected with a dysfunctional purine metabolism. In Dogs of other breeds uric acid is metabolized to a more water soluble product, allantoin, which they excrete as the end product of purine metabolism (Sorenson & Ling, 1993). The first observation of the different metabolic handling of uric acid by the Dalmatian was recorded by Benedict (1916). Since then the causes of this phenomenon has been the subject of many studies.

Due to the predisposition of all purebred Dalmatians to develop urate urolithiasis it is of interest to investigate its mechanisms and it is needed for a better care of these dogs. The purpose of this literature review is to describe nitrogen and purine metabolism in general and to review the findings concerning the molecular mechanisms for the higher excretion of uric acid in the Dalmatian dog breed. A description of the possible reasons for the unexplainable occurrence of the disorder, in some Dalmatian dogs but not in others, is also presented as is the management of the disease. This review will also examine the possibility of confirming or rejecting the hypothesis postulating that the trait responsible for the higher excretion of uric acid in Dalmatian dogs could be eliminated.

Nitrogen metabolism

In order for more advanced organisms to function properly all metabolic processes need to be regulated. Nitrogen homeostasis is no exception and disorders as a consequence of its malfunction or unbalance are often serious.

Mammals are not able to fixate nitrogen by themselves and are depending on food as a source of nitrogen. With the diet there are a variety of nitrogenous compounds entering the body. The most important type of compound being the amino acids contained in dietary protein. Amino acids and nuclei acids are the compounds containing the highest amounts of nitrogen and are both essential for survival.

Amino acids unlike fat and carbohydrates are not stored for future utilisation. When the body's requirements for biosynthesis are fulfilled the amino acids in excess are rapidly degraded. There are processes involved in the disposal of nitrogen were it is metabolised and disposed as urea, ammonia and several other derivates of amino acid metabolism. Ammonia is a waste product formed in all body tissues and derives from sources as amino acid trans-deamination, bacterial action in the intestines, amines, purines and pyrimidines. Even if ammonia is produced all over the body, the production of urea by the urea cycle in the liver and the release of bound ammonia by many tissues, contributes in keeping the levels of blood ammonia very low. By this mode of action organisms can ensure a low concentration of ammonia, in cells and blood, which otherwise would quickly raise their pH to toxic levels. Urea is the perfect vehicle for nitrogen disposal as it is a neutral water soluble molecule composed of ammonia, carbon dioxide, water and aspartate. Transported by the blood urea reaches the kidneys were it is excreted with the urine, this being the most important route for disposal of nitrogen (Champe *et al.,* 2008).

Nucleic acids; macromolecules of monomeric nucleotide chains, are the molecules carrying the genetic information. The most common nucleic acids are ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), these being universal for all living things as they are found in all cells and viruses. Nucleotides are chemical compounds consisting of a nitrogenous base (purines: (A)denine and (G)uanine or pyrimidines: (T)hymine, (U)racil and (C)ystosine), a sugar (ribose or deoxyribose) and one or several phosphate groups, which combined in different ways get rise to chemical compounds involved in different metabolic processes. Nucleotides have a great variety of physiological functions. Their lack would make the production of DNA and RNA impossible leading not only to failure in cell proliferation but also in the production of proteins, carbohydrates, lipids and components of coenzymes. Nucleotides are involved in signal transduction pathways in which they function as secondary messengers (ex. cyclic Adenosine Monophosphate cAMP) and play an essential role as energy storage molecules (ex. Adenosine Monophosphate AMP, Adenosine triophosphate ATP). The pathways of intermediary metabolism are also depending on the presence of nucleotides as they have regulatory characteristics, activating and inhibiting key enzymes (Champe *et al.,* 2008).

Purine metabolism

The metabolism of purines, one of the main components of nucleotides, is comprehended within the nucleotide metabolism. The metabolic processes involving purines are well preserved amongst mammals. Although some differences as to synthesis and uricolysis, the degradation of uric acid, have been accentuated when comparing mammal whit amphibians, reptiles and birds (Young *et al.,* 1938).

The synthesis of purines and pyrimidines bases occurs by means of intracellular metabolism, either by salvage or de novo pathways. The salvage pathway is a way of economizing the expenditure of intracellular energy as the preformed free bases from nucleotide turnover and dietary substances are recuperated and reused. The de novo pathway mainly constructs purines and pyrimidines from non-purine/pyrimidine compounds foremost consisting of carbon and nitrogen. One of the synthesis pathways of purine anabolism is shown in Figure 1 were adenine and guanine are produced by metabolism of the non-purine compound D-ribose-5-phosphate. The formation of 5-phosphoribosyl-1-amine is the first committed step in purine synthesis and the primary point of negative feedback control. Adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are produced by metabolism of inosine monophosphate (IMP). The augmenting concentration of these produced molecules affects the strength exerted by the feedback control mechanisms (Berg *et al.,* 2001; Champe *et al.,* 2008).

The uricolysis of purines (Figure 1) occurs in the small intestine as well as within cells. In the small intestine dietary nucleic acids are hydrolysed to nucleotides, nucleosides and free bases by pancreatic enzymes. Within cells the degradation is carried out by other specific enzymes. Both of the free bases produced, adenine and guanine are catabolised to xanthine. Adenine is degraded by means of trans-amination to hypoxanthine and then by means of oxidation to xanthine. Guanine, however, is directly catabolised to xanthine. The important enzyme needed in this catabolic step and in the further transformation of xanthine into uric acid is xanthine oxidase, found in high amounts in blood, liver, lungs, intestines and also in other body tissues were it is present in smaller amounts. In the presence of the enzyme uricase, found in the peroxisomes of liver cells, uric acid is metabolized to allantoin, the end-product of purine metabolism excreted by mammals (Champe *et al.,* 2008).

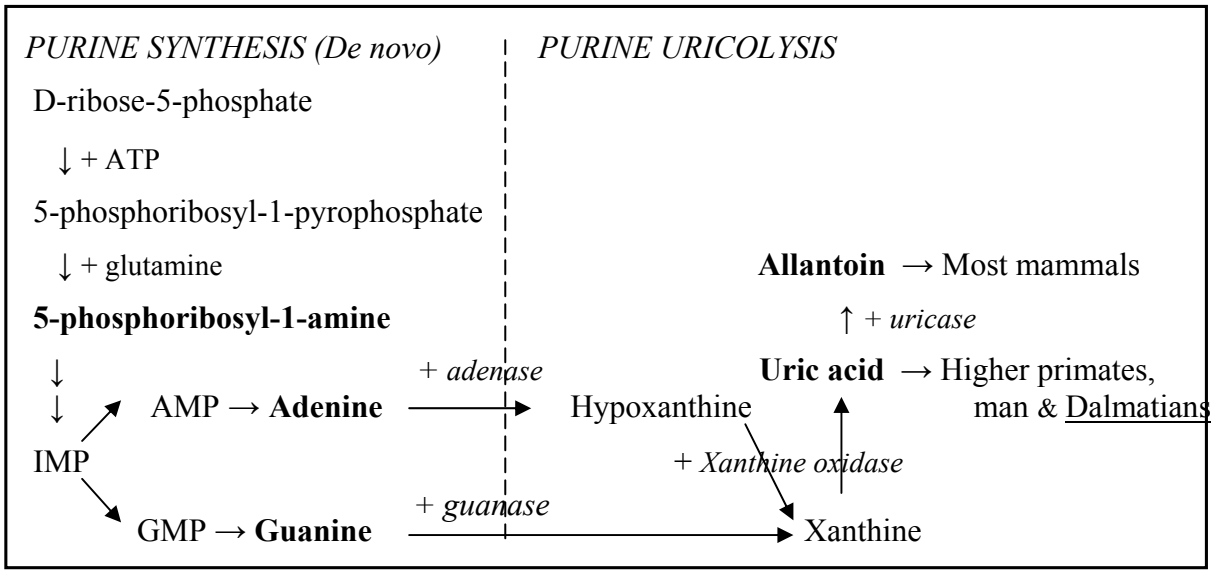

Figure 1. A Summarized overview of purine metabolism pathways, modified after Sorenson & Gerald (1993) and Champe *et al.* (2008).

A classification upon mammalian purine metabolism was made by Hunter & Givens (1914) were it appeared that some species degraded and then excreted purine metabolites as uric acid, such as man and chimpanzee. Others, such as carnivores and rodents, degraded and excreted purine metabolites as allantoin the more water-soluble end-product of purine metabolism (Figure 1).

Benedict (1916) reported the Dalmatian dog breed to have an unusual high excretion of uric acid, comparable to the excretion of the compound by adult men. It was clear that although other dog breeds did fit the classification made by Hunter & Givens (1914) the Dalmatians did not. Calculation of the uricolytic index, the percentage of uric acid metabolised to allantoin before secretion, revealed a variation within mammals, a ground upon which Hunter & Givens based their classification of different species. In higher apes and man the uricolytic index hade a range between 0-2 units, in most other mammals it ranged from 79-98 units. The uricolytic index of the Dalmatians was calculated, to be around 30 units, by Benedict (1916) when he firstly reported the unusual phenotype of this dog breed. This index deviated for that of other dog breeds as they were classified as carnivores with an index estimated to be around 90-98 units. The Dalmatians excretes most of their total nitrogen wastes as uric acid and very little as allantoin, as shown by their low uricolytic index (Wells, 1918; Friedman & Byers, 1948).

The Dalmatians "defect"

As it is shown in Figure 1, man and higher primates are categorized within mammals that excrete their nitrogen wastes as uric acid. The enzyme uricase, which plays the crucial part of further transforming uric acid to allantoin, has not been found in the livers of these species. In their genome, the gene urate oxidase (uox) coding for uricase, in the liver, has been subjected to mutations leading to its silencing and consequently to the excretion of urinary uric acid (Safra *et al.,* 2005). The molecular basis for the excretion of uric acid in humans and higher primates has not been applicable upon the Dalmatian dog breed and was until recently unknown. Klemperer *et al*. (1938) investigated further the findings made by Wells (1918) stating uricolytic activity in the Dalmatian liver.

By cloning and sequencing the canine uox gene, of a Dalmatian, and comparing it to the same cloned sequence in the DNA of a non-Dalmatian dog Safra *et al.* (2005) could exclude the canine uox gene as a cause of hyperuricosuria (excessive amounts of uric acid in urine). The sequences of the canine uox gene were the same in both dogs. The multipoint score analysis LOD, an analysis based on genotypes of microsatellites, within and flanking the uox gene sequence, gave negative results also indicating no differences.

With regard to the amounts and structure, the uricase in Dalmatian is the same as the one found in other non-Dalmatians dogs. Although this enzyme has not been distinguished between breeds, Klemperer *et al*. (1938) suggested that it wasn't the lack of uricase causing he high amounts of excreted uric acid in Dalmatians but rather a slower rate of transport of the compound into hepatic cells. By homogenising Dalmatians liver slices a higher uricolytic activity was reported in the mashed samples than in the intact liver slices. This indicated that the enzymatic activity was enhanced when liver cell structures were destroyed, thus an impaired transport of the compound into the hepatic cells could explain the lower amount of uric acid oxidized.

Further, in vivo experiments carried out by Friedman & Byers (1948) supported the theory of a defect hepatic uricolysis in Dalmatians. To eliminate renal excretion of uric acid, and consequently also of other substances, the ureters of two Dalmatians and two dogs of other breeds were ligated carefully so to not cause any injuries. The plasma concentration of uric acid increased rapidly in Dalmatians whereas in the dogs of other breeds it was minimally affected. The latter due to the ability to transform the excess uric acid to allantoin. The blood uric acid level was, in both dog strains, artificially raised. A higher plasma urate was in this case also reported in Dalmatians compared to dogs of other breeds, despite the intensified excretion of urate by the kidneys. Thus the livers of Dalmatians are not as efficient, in removing urate from the blood, as the livers of non-Dalmatians.

The kidneys handle the regulation of the water and ion content of the body fluids and have an important task in removing metabolic wastes (Sjaastad *et al.,* 2003). Uric acid can easily pass trough the glomerular filter of nephrons as it is an organic acid molecule rarely bound to plasma proteins. Due to its unbound state the glomerular filtration of urate is almost complete. The amount found in the primary urine depends on the glomerular rate of filtration and the plasma concentration of the compound (Lang *et al.,* 1979). In all mammals, with exception of the Dalmatian dog breed, the excretion of uric acid has been demonstrated to be carried out by a filtration-reabsorption mechanism (Friedman & Byers, 1948). In mongrel dogs (mixed breed dogs) renal clearance of urate, accounting for the speed with which the molecule passes trough the kidneys, has been found to be 20 % to 50 % of the glomerular filtration rate. That is, mongrel dogs have recurrence of reabsorption. To investigate the existing secretion of urate, the compound was given, to mongrel dogs, intravenously to observe variations in urate clearances. This technique conventionally used within clearance studies revealed, as hypothesised, the presence of a urate flux (Lathem *et al.,* 1960), approximately consisting of 2% of the filtrated fluid (Lang *et al.,* 1979). For Dalmatians, renal clearance has been found to exceed glomerular filtration leading to the conclusion of a urate tubular secretion rather than reabsorption (Roch-Ramel *et al.,* 1976). Using pharmacologic agents, inhibition of urate secretion in Dalmatians could be induced at such levels so to make the detection of urate reabsorption possible. These studies revealed that as, in mongrel dogs; also Dalmatians handled urate by means of secretion as well as reabsorption (Berger *et al.,* 1960; Weiner & Fanelli, 1975). A main difference in the handling of urate excretion between Dalmatians and dogs of other breeds is the site and amount of tubular secretion. For both strains of dogs,

reabsorption mechanisms have been located to the convoluted proximal tubules were a minor secretion of urate also is observed. By means of micropuncture studies, it was observed that, for Dalmatians only, further substantial secretion occurred in the early distal tubules. The Dalmatians appears to excrete more generous amounts of urate compared whit dogs of other breeds (Roch-Ramel *et al.,* 1976).

Crosstransplantations experiments done between Dalmatians and dogs of other breeds point at a liver disease, rather than a kidney disease. Two Dalmatians and two mongrel dogs (mixed breed dogs) were each operated so to retain the kidneys of the other breed. The kidneys assumed the pattern of uric acid excretion of the recipient (Appelman *et al.,* 1966). Further transplantation of livers between Dalmatians and mongrel dogs revealed an opposite outcome. The recipients assumed the uric acid excretion pattern of the liver donor. Excretion of uric acid was monitored for 24-hours as well as urate serum level and clearance. In the same breed experiments all variables were statistically unchanged. For the reciprocally transplanted animals, all of the variables statistically deviated from the values of the liver donor registered prior to the transplantation. All of the three variables were however persistent with the values of the liver donor prior to the transplantation (Kuster *et al.,* 1972). These classic experiments were well performed giving a strong base to the statement that the high uric acid excretion in Dalmatians is an outcome of a metabolic liver disease.

Theories stating the liver to be the organ which determines the difference in uric acid handling between Dalmatians and mongrel dogs imply the production of hormones like factors promoting renal urate reabsorption and urate hepatic uptake. The lack of these factors in Dalmatian dogs would consequently cause a higher urate excretion. Experiments in which hepatocytes from mongrel dogs were transplanted in situ into Dalmatians showed, although for a limited time, a decrease in uric acid excretion and a raise in plasma uric acid concentration. Improvement of urate reabsorption and urate uricolysis was then reported (Kocken *et al.,* 1996). The theory of hormones like factors produced by the liver of mongrel dogs and not the liver of Dalmatians appears to be plausible. However these factors have yet to be discovered (Simkin, 2005).

Genetic aspects

The mode of inheritance of the high urate excretion trait is inherited by mean of an autosomal recessive Mendelian segregation pattern. The mode of inheritance was brought to the attention by Onslow (1923) and further investigated by Trimble & Keeler (1938). Dalmatians were crossed to other breeds and the first filial (F1) generations found to distinctly differ from their Dalmatian parent as regard to the high excretion of urate. The F1 generations had a low excretion of urate keener to that of their non Dalmatian parent. Further two sharply distinct classes, one whit high urate excretion and one with low urate excretion, were reported within the hybrid generations. No intermediate excretion of urate was found leading to the conclusion of a completely recessive trait. The number of genes involved in the process of hyperuricosuria (excessive excretion of uric acid) in Dalmatian dogs was studied by means of produced test crosses populations. The excretion of high uric acid is inherited as a simple recessive Mendelian unit-character. The trait was reported not to be sex-linked as mating of females excreting high amounts of urate with males excreting low amounts of urate produced some individual of the male sex which excreted low amounts of urate. If the trait would have been sex-linked, as it is a completely recessive character, all males produced by the mating should have portrayed a high urate excretion (Trimble & Keeler, 1938).

One character in particular is often associated with Dalmatians, their coat pattern. By breeding experiments the spotted pattern of the Dalmatians could be, with some differences, reproduced in hybrids with normal excretion of uric acid. These experiments lead to the conclusion that the genes coding for the spotted coat in Dalmatians could not be the same as those coding for the high urate excretion character. Spotted hybrids individuals portrayed larger and more uneven spots than the ones observed in Dalmatians which are small and very circular. Additionally the larger spots in the hybrid dogs hade white hairs interspersed within the spots which is not the case for the highly dark pigmented spots of the purebred Dalmatians. Linkage testing revealed the gene pairs not to be linked as they were located on different chromosomes. However a genetic linkage was found between the gene pairs coding for the high uric acid excretion and the genes responsible for the lack of white hairs within the spots of purebred Dalmatians (Trimble & Keeler, 1938; Keeler, 1940). As the breeders of the Dalmatians dog breed has preferred individuals with heavily pigmented small regular spots the most, an indirect selection for high uric acid excretion has probably been effectuated (Sorenson & Ling, 2005).

Sex differences

Utilising the Woolf method comprehending statistical analyses, calculation of crude odds ratios and 95% intervals, it was assessed whether sex was a risk factor in the formation of uroliths in dogs of the Dalmatian dog breed. The material upon which the study was performed was retrieved from records of all Dalmatians compiled by the veterinary medical Database of North America from 1981 to 2002. Results showed that the odds of uroliths formation were higher in males compared to females Dalmatian. Further investigation revealed the proportions of the recurrence of different kinds of uroliths within the sexes. Both for uroliths composed of urate and non-urate compounds their recurrence was higher for the males. Of the uroliths found in females the majority was composed of urate compounds. Although within the portion of non-urate uroliths the recurrence of struvite and mixed compound uroliths was observed to be higher than for the males which instead had a higher number of different types of non-urate uroliths (Hasan *et al.,* 2005).

There are several plausible reasons for the predominant incidence of the disorder in males. One of the reasons, most mentioned, is the anatomic difference between the sexes lower part of the urinary tract. In males, uroliths, if of substantial size tend to be caught up in the narrow groove of the os penis, a “bone” which makes the urethra of the male dog a lot less stretchable than the urethra of the female that lacks such “bone”. The urethra of females is also shorter and wider meaning that small uroliths can be voided before any clinical signs can be detected. In males as the uroliths obstruct the urinary flow, clinical sign are detected much earlier. An obstructed urethra has to be taken seriously if not treated in a reasonable time frame. Further it has been observed that the urine composition of females Dalmatians differs from that of males Dalmatians (Hasan *et al.,* 2005). In vitro studies of human urine revealed the presence of urate uroliths formation, growth and aggregation inhibitors. It seems as urate crystals are kept in solution by these metabolites (Grases *et al.,* 1999). Studies performed on Dalmatians to enlighten the workings of these inhibitory metabolites showed that urate uroliths forming Dalmatians excretes less amounts of the inhibitory substances mentioned than do Dalmatian of comparable age which are not urate uroliths formers (Carvalho *et al.,*2003). The different composition of urine between sexes may imply that the dietary and drug protocols designed to minimise urate uroliths formation in Dalmatian males may not have the same effect in female Dalmatians (Hasan *et al.,* 2005).

Diagnosis and management

The major predisposing factors to urate urolithias beside high urate excretion are high renal excretion of ammonium ion, production of microbial urease, low urine pH and presence or absence of inhibitor metabolites for urate formation (Sorenson & Ling, 1993). The onset of the disorder is highest between the ages of 4-9, if an individual is not affected within that period it will probably not be affected at all (Hasan *et al.,* 2005). The diagnosis of urate urolithiasis can be difficult as uroliths of this type are strongly radiopaque, meaning that they are not well detected by means of plain radiographies. Signs of irritation in the dog, coupled to the lower urinary tract, should be enough to start looking for uroliths. Clinical signs could be bloody urine, difficulty to urinate, small amount of urine frequently urinated and the recurrence of gritty material in the dogs urine (www.marvistavet.com). When suspecting the presence of uroliths analyses to determine their composition are very important as there is a variation in their appearance and composition (Sorenson & Ling, 1993).

Uroliths can be removed by mean of dissolution or surgery. The dissolution protocol involves bringing the uric acid, of uroliths, back to solution. Protocols for dissolution of uric acid uroliths have been designed to minimize uric acid supersaturation in humans. Lack of comparable models has lead to the design of the canine protocols based on the human's ones and canine uncontrolled clinical studies (Osborne *et al.,* 1986). Canine dissolution of urate uroliths requires a combination of diuresis, low protein diet, urine alkalinization and administration of allopurinol, a xanthine oxidase inhibitor, to reduce uric acid formation. Surgery is a faster although more invasive approach, involving physical removal of uroliths. A combination of surgery and dissolution may be the case for animals were all uroliths can not be removed. Surgery is more often required by individuals suffering from serious obstruction of renal pelvis, ureters or/and urethra (Osborne *et al.,* 1986; Sorenson & Ling, 1993; www.marvistavet.com).

Diets

The main goal of specific diets given to animals with high urate excretion is to promote undersaturation of urine with respect to urate. A desired low urine gravity of < 1.020 can be kept by generous water consumption generated by encouragement, *ad libitum* access or by mixing water with the fodder. Diets containing low amounts of purines could further promote the undersaturation of urate in the urine, however the effectiveness of giving such low purine diets has been and still is debated (Sorenson & Ling, 1993). A study by Young *et al.* (1938), investigating the material for synthesis of purines, feeding Dalmatians with different protein sources, lead to the conclusion of an endogenous production of uric acid, not affected by the source of protein. Although under normal condition purines are synthesised by dietary proteins. Trough the years several low purine diets have been designed for prevention and dissolution of urate uroliths (Sorenson & Ling, 1993). Only two commercial diets, Canine s/d and Canine u/d (http://www.petrx.com), which are nutritionally complete, have been recommended for these purposes (Sorenson & Ling, 1993). The first product is combined with a supplementation of sodium bicarbonate to ensure a balanced urine pH as it is an acidifying diet. However this diet should not be given to dogs requiring sodium restriction. The second product is an alkalizing diet and sodium bicarbonate is administrated only when the urine pH needs to be stabilized. The urine pH desired is 7.0 and shouldn't be higher than 7.5 to minimize the risk of precipitation of other substances which could form uroliths (Sorenson & Ling, 1993).

Allopurinol

Lowering the concentration of uric acid in the urine is achieved most effectivly by administration of allopurinol. This agent is an inhibitor of the enzyme, xanthine oxidase, responsible for the catalytic transformation of hypoxanthine and xanthine to uric acid (Figure 2). Dalmatians remove allopurinol from their system trough catabolism of the agent to oxypurinol as well as by urinary and faecal excretion. Oxypurinol, also an inhibitor of xanthine oxidase, is less potent than allopurinol however having a longer plasma half-life it lowers plasma levels under a longer period of time. The reduction of urate excretion is related to the dosage of allopurinol and the administration of this drug should in the ideal case be adjusted for each individual based upon the amount of excreted uric acid. Consequently to the administration of such agent, plasma levels of xannthine and hypoxanthine will increase. These substrate as well as allopurinol, oxypurinol and urate will be excreted trough the faeces and urine. If allopurinol is wrongly administrated the risk for formation of xanthine uroliths will increase as it is the substrate with the lowest urine solubility (Massey *et al.,* 1970; Ling & Sorenson, 1993). Reformation of urate uroliths is likely to occur; up to 50% of the affected dogs will have a new episode (Kruger & Osborne, 1986). The administration of allopurinol is often practiced as prevention for reformation, after the first treatment for presence of uroliths (www.marvistavet.com).

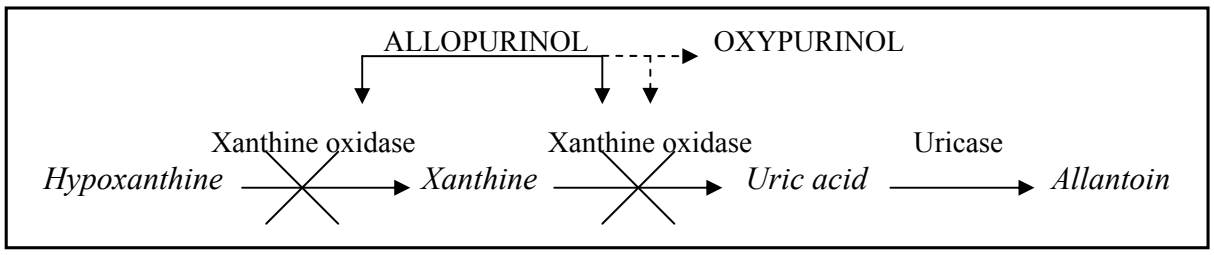

Figure 2. An overview of the inhibitory action upon the action of xanthine oxidase, exerted by allopurinol and its metabolite oxypurinol. Modified after Modabberzadeh, (1995).

Discussion

It has been hard to get an accurate estimate of the situation regarding the prevalence of urate urolithiasis in Sweden. Inger Hagbohm, the chairwoman for the Swedish Dalmatians Society, (Svenska Dalmatiner Sällskapet) stated in a personal communication (2008), that no records over the occurrence of urate urolithiasis in Dalmatians are kept in Sweden. The problem is better recorded in the US were there is, for this disorder, a good register. The prevalence of the disease in Swedish Dalmatian dogs could be estimated from the health records of the major Swedish insurance companies. Animals treated for uroliths are given allopurinol as a prevention measurement for the rest of their life and this medication, in addition to other medical procedures, is most likely paid for by the animal's insurance.

The transporter protein of uric acid, named URAT1, in human has been found by Enomoto *et al.* (2002). This transmembrane protein located in the luminal cells lining the proximal tubule of the kidney is suggested to be the carrier responsible for the reabsorption of urate from the glomerulus's filtrate. A homologous gene coding for a similar transporter protein has been found in dogs by searching the published genome of a Boxer dog (Simkin, 2005). For the theory postulated, regarding the livers regulation of renal urate reabsorption, the liver would produce a smaller factor secreted through the blood to interact whit renal cells and regulate the transporter protein just mentioned. In humans such a hepatic factor has not yet been found. Further more it was also hypothesised that the liver may regulate the hepatic uptake of uric

acid, however URAT1 has not been found in the human liver. It would be of interest to further analyze the genome of the dog, in search of additional sequences analogous to the sequences found for URAT1. Perhaps there is an analogous anion transporter protein in the liver, which could be called URAT2, responsible for the hepatic uptake of uric acid. If a comparison could be made between the URAT2 found in dogs of other breeds and the URAT2 found in Dalmatians, a difference in urate transport rate could be explained at a molecular level. If there were an inefficient URAT2 transporter in the liver of Dalmatians, the capacity of the system in removing uric acid from the blood would be greatly reduced.

As the genes responsible for high urate levels in Dalmatians seem to be connected to some extent to genes responsible for the breed's coat pattern, it appears improbable that the trait will be eliminated by breeding efforts. Dog owners will most likely still want the Dalmatians to have their typical coat pattern. Thus the trait of high uric acid is not likely to be affected by means of purebred breeding. Another factor supporting this is the onset of the disease late in the animal's life. The disorder usually is detected at 4-9 years of age when individuals have already been mated, long before a possible detection of the disease. An effort in minimizing the occurrence of urolithiasis within the Dalmatian breed could be made by waiting with breeding until the males have reached the critical age for the onset of the disorder. This proceeding could make a difference since it is not enough to chemically analyse the excreted levels of uric acid. All Dalmatians excrete high amounts of urate.

As a result of these conclusions it is important to develop a way to manage this dog breed that will provide them with a good life. Males for many reasons urinate more often than females leading to an enhanced passage and clearance of ions in their urine. Males because of their urinary pattern may not naturally need in their urine as many inhibitors for stone formation compared to females. Studies on the origin of the differences in urine composition between the sexes could provide insight for the development of sex specific treatments. A question for future research is if the composition of the urine is connected or not to the different urination behaviour of the sexes.

The studies suggested in this discussion could be of economical interest for the Swedish insurance companies. Although if the number of affected Swedish Dalmatians turns out to be very low the investigation wouldn't be cost efficient. Epidemiological research is however important in order to acquire information that could clarify the molecular mechanisms of multifactorial disorders. This information often leads to management improvements regarding prevention, diagnosis and treatment. Improvements that could be valuable in veterinary medicine as well as in human medicine.

Conclusions

The deviating handling of urate by the Dalmatian dog breed has been known for almost a hundred years. At first it was suggested that the defect was caused by a different handling of urate by the kidneys. However further studies pointed at the liver as the organ most likely to be responsible for the differences in urate transport across liver cells in Dalmatian dogs. Many theories have been postulated and will be investigated further in the future. Today as the canine genome has been published, new possibilities arises. The genes responsible for the Dalmatian dog's higher urate excretion will most likely be identified giving new insight to the molecular mechanisms involved in the handling of urate by the liver and kidneys. These processes are not yet well understood. Although further studies are needed it can be concluded that the trait responsible for the high excretion of urate in Dalmatians will probably not be

eliminated by genetic efforts due to its connection to the breeds coat pattern. Improvements in the quality of life of these dogs can and should be made by further developing the ways of management of the disease as these dogs will have to live with their unusual purine metabolism. By studying the multifactorial disorder of urolithiasis, using a dog model, valuable knowledge for comparative medicine and animal science is acquired.

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