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THE USE OF HIGH RESOLUTION MASS SPECTROMETRY TO STUDY PURINE METABOLISM IN PIGS

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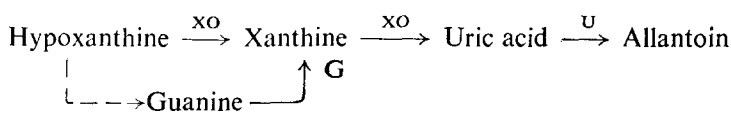
Abstract—Guanine and the xanthine oxidase inhibitor, allopurinol, were administered orally to pigs. The treatments included both separate and combined use of the drugs for varying periods. By means of high resolution mass spectrometry the concentrations of xanthine, hypoxanthine, uric acid, allopurinol, oxipurinol and guanine were measured in samples of kidney, synovial membrane, skeletal muscle and blood plasma from the slaughtered pigs. All tissues were essentially normal except the kidneys from pigs fed both drugs and no accumulation of guanine was observed in either skeletal muscle or synovial membrane tissue. These results are discussed in relation to leg weakness and "guanine gout" in pigs.

THE ENZYME guanase (EC 3.5.4.3) is normally responsible for the deamination of guanine to xanthine in most mammals.[1] However, some early work on purine metabolism established the absence of this enzyme in pigs and associated the deficiency with "guanine gout", i.e. the deposition of guanine crystals in skeletal muscle tissue.[2,3] Recent reports of leg weaknesses throughout the pig industry have renewed interest in the problem and the present investigation[4,5] was carried out to establish any correlation with the above reported enzyme deficiency; particular reference being paid to guanine deposition in joints.

The relevant purine metabolism involved in this study can be summarized as follows:



(XO—xanthine oxidase, U—uricase, G—guanase)

Allopurinol is an efficient xanthine oxidase inhibitor and blocks the reactions between hypoxanthine and xanthine and xanthine and uric acid. However, when administered to man the major excretory product is xanthine rather than hypoxanthine. This has been explained by postulating a resultant preferential operation at the alternative pathway in which hypoxanthine is converted, via a series of nucleotide intermediates, into guanine and then into xanthine by the enzyme guanase.[6] Assuming the conversion of hypoxanthine to guanine is also possible in pigs, the administration of allopurinol should raise the plasma and urinary levels of either guanine, if guanase is absent, or xanthine, if guanase is present. Similarly the administration of guanine

will only raise the plasma and urinary levels of guanine significantly if the enzyme guanase is absent. Since guanine is extremely insoluble in water[7] some deposition in various body-tissues is probable if guanase is absent or defective.

EXPERIMENTAL

As previously reported[4] litter mates were used of which four pigs were controls (group I), four were treated with guanine (group II) and four were treated with allopurinol (group III) over a 4 week period. In addition pigs pre-treated with either guanine or allopurinol were each given a supplement of the other drug for a period of up to 3 weeks (groups IVa and Va) following which, one animal of each group was returned to the initial drug for the remaining period of study (groups IVb and Vb). The treatments were as previously outlined[4] and animals were slaughtered at the end of each period of study to assess the effects of these treatments.

At slaughter X-rays were taken of the joints in the forelegs and samples of skeletal muscle and synovial membrane obtained from these joints. In addition, samples of blood plasma and cross sections of kidney were obtained for mass spectroscopic analysis.

All samples were freeze-dried and ground to a powder. Weighed portions (approx. 1 mg) were then admitted to the mass spectrometer (Varian M.A.T. SM1) by means of the direct insertion probe with no further processing. Low resolving power (approx. 1000) mass spectra of hypoxanthine, xanthine, uric acid, allopurinol, oxipurinal and guanine were obtained at a series of probe temperatures from 200° to 300° to establish the optimum evaporation temperature for each compound and the most intense characteristic ions for quantitative measurements. High resolving power (approx. 10,000) mass spectra of the various tissues were then examined to ensure that the selected peaks were free from interference from ions of the same atomic composition but arising from different precursors.[8] Quantitative measurements were made simultaneously on all six compounds by high resolution mass fragmentography[9–11] using the parent ions at m/e = 136,0385 (hypoxanthine and allopurinol), 151,0494 (guanine), 152,0334 (xanthine and oxipurinol) and 168,0283 (uric acid). The fragment ions at m/e = 52,0187 and 54,0218 were used to distinguish between the two isomeric pairs, hypoxanthine–allopurinol and xanthine–oxipurinol. Triplicate measurements were made where possible to reduce errors due to heterogeneous distribution of the compounds throughout the tissues. Since the results for some of the kidney tissues exhibited large biological fluctuations, a section through each kidney was re-examined to find the range of concentrations present. The mass spectra and evaporation characteristics of inosine (hypoxanthine riboside) and allopurinol riboside were also examined.

RESULTS

Mass spectra. The mass spectra of all six compounds were essentially similar to previously published spectra (hypoxanthine;[12,13] xanthine;[13,14] uric acid;[14] oxipurinol;[13] allopurinol;[13] guanine[12]) although the relative intensities of the fragment ions were, for some compounds, less in the present study. The optimum evaporation temperatures were 200–250° for hypoxanthine and allopurinol and 250–300° for the other four compounds. Spectral variations with temperature were insignificant within these ranges for all compounds.

TABLE 1. AVERAGE CONCENTRATIONS OF HYPOXANTHINE (HX), XANTHINE (X), GUANINE (G), ALLOPURINOL (AL) AND OXIPURINOL (OX) IN THE MEDULLA AND CORTEX REGIONS OF KIDNEY TISSUE FROM CONTROL PIGS (GROUP I) AND PIGS FED GUANINE (GROUP II), ALLOPURINOL (GROUP III), ALLOPURINOL AND GUANINE UP TO SLAUGHTER (GROUPS IVa AND Va) AND ALLOPURINOL OR GUANINE SUBSEQUENT TO THE COMBINED DRUGS (GROUPS IVb AND Vb)

	Concentrations (ng/mg of dry tissue)									
	Medulla					Cortex				
Group	HX	X	G	AL	OX	HX	X	G	AL	OX
I	43	10	17			36	5	20		
II	39	35	55			35	16	50		
III	47	35	16	17	3	47	23	15	22	5
IVa + Va	121	1620 to 42	32	19	910 to 16	88	530 to 20	41	24	141 to 37
IVb + Vb	51	94	34	19	25	50	100	14	19	14

The molecular ions of the six compounds were the most suitable for quantitative measurements and no significant interference was detected at any of the peaks of interest below 280°, for any of the tissues examined. However, above this temperature a large peak appeared close to the molecule ions of hypoxanthine and allopurinol. It was tentatively identified as $C_4H_8O_5$ on the basis of its accurate mass (m/e = 136,037). This ion was not observed in human tissue and its origin remains obscure. Since the resolving power of the mass spectrometer was insufficient to resolve completely the two ions, the temperature was not raised above 280° until the profiles of hypoxanthine and allopurinol were completed.

Tissue analyses. The concentrations of xanthine, allopurinol, oxipurinol and guanine in muscle tissue from all pigs, except those in groups IV and V killed when on the mixture were very low and near the detection limit of the mass spectrometer (approx. 1–5 ng/mg of dry tissue). The concentration of hypoxanthine in these animals was 28 ± 10 ng/mg of dry tissue. All of these tissues were essentially normal and there was no indication of crystal deposition in any of the specimens analyzed.[5] Similar results were obtained for the blood plasma from all groups except the pigs in groups IVa and Va namely, hypoxanthine 26 ± 6 ng/mg dry tissue and little evidence of the other compounds. The groups IV and V animals on the mixture showed slightly elevated concentrations in muscle tissue, but these were not indicative of crystal deposition.[5] The blood plasma levels were also elevated but varied markedly between animals. No uric acid was found in any of the samples and no significant profiles were obtained for any of the compounds in the samples of synovial membrane. The results for the kidney tissues are given in Table 1.

The mass spectra of allopurinol and hypoxanthine could not be distinguished from the mass spectra of their ribosides,[15] nor could the compounds be separated by differential evaporation in the ion source. Hence the concentrations measured for hypoxanthine and allopurinol probably arose partly from their ribosides since these were identified in the excretion studies.[4] Some protein bound purines may also have contributed.

DISCUSSION

Of all the tissues studied by mass spectrometry only the kidneys of the pigs killed when on the mixture were grossly abnormal. Large localized deposits were formed in both the cortex and medulla regions of these kidneys. These deposits were identified on the basis of their mass spectra and evaporation characteristics as a mixture of xanthine and oxipurinol in the ratio of approx. 2:1. The histology studies also showed severe kidney damage and the presence of very large numbers of crystals in all animals which had received the combined drugs up to the time of slaughter.[5] No comparable accumulations of xanthine or oxipurinol were found by mass spectrometry in the kidneys of the pigs which had been subsequently taken off the combined drugs for several weeks prior to slaughter. However an increase in the concentration of xanthine was measured These kidneys were also found to be histologically abnormal but the damaged tissue was regenerating and although there were clear indications that crystals had been deposited only a few small ones remained.[5] The kidney results suggest that the elevated levels found in the blood plasma and skeletal

muscle tissue from group IV pigs were due to the acute renal failure caused by the combined drug treatment.

All the other animals demonstrated a remarkedly good tolerance for the massive doses of drugs administered with no visible or measurable consequences. The mass spectroscopic results clearly demonstrate that no accumulation occurred in the skeletal muscle or synovial membrane as a result of either guanine, allopurinol or the combined drugs. The X-ray of the joints from which the synovial membranes were removed also showed no abnormalities. Considering the very large quantities of these compounds which were administered, these experiments suggest that guanine is readily converted to xanthine in pigs. This was substantiated by the excretion studies which showed a marked increase in xanthine only during allopurinol treatment. It was concluded that the enzyme guanase is present in pigs and that the leg weaknesses reported were not related to "guanine gout".

As expected from the metabolic studies the levels of uric acid found in pigs were much lower than in humans since these animals utilize the enzyme uricase to excrete allantoin, rather than uric acid, as the end product of purine metabolism. However, the concentrations of hypoxanthine and xanthine found in normal pig blood plasma and skeletal muscle tissue were very close to the previous measurements on normal humans.[9] Since the pig demonstrates many similarities to humans regarding purine metabolism it is of interest, in view of the high dosage regime, that no evidence of crystal deposition or even raised tissue levels was found, of either purines or drug metabolites during allopurinol therapy in pigs with normal renal function.

Although the high allopurinol dosage used had produced maximal xanthine oxidase inhibition in these animals, the results are in contrast to those obtained in xanthinurics, with hereditary xanthine oxidase deficiency, where xanthine levels 10 times normal human levels were found in muscle.[9] However, the increased levels of these compounds (without crystallization) in both plasma and muscle in the pigs with acute renal failure would indicate that the long term effect of a combined purine/pyrazolopyrimimidine load on the kidney in chronic renal failure should be evaluated. Where crystals have been reported in human muscle following long term allopurinol therapy the highest levels have been recorded in the patient with the most extensive renal damage.[11,16]

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