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ORIGINAL ARTICLE

Metabolomic profiling of the effects of allopurinol on *Drosophila melanogaster*

Mohammed Al Bratty · Yahya Hobani · Julian A. T. Dow · David G. Watson

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Abstract Metabolomic profiling using hydrophilic interaction chromatography in combination with Fourier transform mass spectrometry was used to study the effects of the xanthine oxidase inhibitor allopurinol on wild type *Drosophila melanogaster*. Allopurinol treatment phenocopied the rosy mutation causing an elevation in the levels of xanthine and hypoxanthine and a fall in the levels of uric acid and allantoin. However, in addition there were some unexpected metabolic changes after treatment. Ascorbic acid levels were undetectable, glutathione levels fell and glutathione disulphide levels rose, methionine S-oxide levels rose and riboflavin levels fell. The origin of this oxidative stress was not immediately apparent; however, there was a strong suggestion that it might be related to a fall in NADPH levels linked to a reduction in glucose-6-phosphate dehydrogenase activity, resulting in reduced levels of some metabolites in the pentose phosphate pathway. In addition to producing oxidative stress there were marked effects on tryptophan metabolism with most of the metabolites in the kynurenine pathway being lowered by allopurinol treatment. The effects on the kynurenine pathway could be related to the established use of allopurinol in treating schizophrenia.

Keywords *Drosophila* · Metabolomics · Rosy · Fourier transform mass spectrometry · Allopurinol · Oxidative stress · Kynurenine pathway

1 Introduction

Metabolomics as a field of study is expanding rapidly and has the potential to impact in a number of areas including drug discovery, the management of the health of populations and crop improvement (Krastanov 2010; Wilcoxen et al. 2010; Spagou et al. 2010; Scalbert et al. 2009; Kamleh et al. 2009; Dunn 2008). In order to advance the field further simple model organisms are useful since genetic manipulation is more feasible than in more complex organisms such as human beings. *Drosophila melanogaster* offers a combination of genetic tractability, availability of well-characterised genetic mutant stocks, and organismal complexity (Dow 2007; Dow and Davies 2003) and is thus ideal for metabolomic studies. In an earlier paper we carried out a study of the metabolomics of the *rosy* (*ry*) *Drosophila* mutant (Kamleh et al. 2008). The expected changes in the pattern of metabolites directly related to the absence of the xanthine oxidase gene, such as the absence of uric acid and allantoin and the elevation of xanthine and hypoxanthine. In addition, perturbation both in the purine metabolic pathway at a distance from the lesion and in other metabolic pathways was observed.

Mohammed Al Bratty, Yahya Hobani were contributed equally to the work.

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M. A. Bratty · D. G. Watson (✉)
Strathclyde Institute for Pharmacy and Biomedical Sciences, 27, Taylor Street, Glasgow G4 0NR, UK
e-mail: d.g.watson@strath.ac.uk

Y. Hobani · J. A. T. Dow
Institute of Cell, Molecular and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

J. A. T. Dow
Department of Clinical Laboratory Science, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

The xanthine oxidase (XO) inhibitor allopurinol is used to treat gout via the inhibition of uric acid formation and is generally well tolerated (Pacher et al. 2006) although it can produce side-effects such as eosinophilia and vasculitis. In principle, administering allopurinol to *Drosophila* should phenocopy the *ry* mutant by inhibiting XO and causing xanthine and hypoxanthine to accumulate and uric acid to deplete. It could potentially also induce the more distant metabolic perturbations seen in the *ry* mutant. Allopurinol is a prodrug and is converted to oxopurinol by the action of XO. Oxopurinol is a suicide inhibitor of XO which replaces the hydroxyl ligand of the molybdenum ion at the active site of XO (Dietzel et al. 2009; Hille and Nishino 1995) and thus very effectively prevents conversion of hypoxanthine to xanthine and xanthine to uric acid. Inhibition of XO does not prevent xanthine being produced since it can be derived from guanine and the enzyme required for this conversions is not inhibited by allopurinol.

There is an on-going interest in allopurinol as a cardioprotectant since the activity of XO is one of the major sources of reactive oxygen species (ROS) in the human body and it may play a major role in various forms of ischemic and vascular injury (Pacher et al. 2006). However, there is a balance between two effects, since XO also produces uric acid, which is an important antioxidant within the body; and it has been proposed that uric acid is responsible for about 25% the antioxidant capacity of human serum and that dietary antioxidants exert their effects indirectly via increasing plasma uric acid levels (Lotito and Frei 2004).

Metabolomics has the potential to provide a greater understanding of the action of drugs on wider metabolic networks than their intended targets. This might lead to appreciation of unexpected properties of the drugs and also give an indication of the physiological basis of side-effects. About 70% of human genes have *Drosophila* homologues, and so this fruit fly is potentially an experimental organism in which drug testing can be conducted with less expense and ethical considerations. The greatest challenge in metabolomics is to be able explain metabolic changes and the greatest confidence in explaining data obtained occurs when several closely linked metabolic changes are observed, e.g., in a single metabolic pathway.

In this paper we compare the effects of allopurinol on the *Drosophila* metabolome with those produced by the *ry* lesion which we reported previously (Kamleh et al. 2008). Allopurinol has previously been documented to mimic rosy mutations (Glassman 1965); but given the multiple uses of allopurinol, and unexpected metabolic sequelae of the rosy mutation, we sought both to confirm the classical results, and to look for novel, undocumented impacts of allopurinol on the metabolome.

2 Materials and methods

2.1 Chemicals

HPLC grade acetonitrile and water were obtained from VWR International Ltd (Lutterworth, UK). AnalaR grade formic acid (98%) was obtained from BDH-Merck (Dorset, UK).

2.2 *Drosophila* stocks

Drosophila melanogaster were kept in vials of standard medium at 25°C and 55% rh on a 12:12 photoperiod. Stocks used were the Oregon R (OR) wild type strain.

2.3 Treatment and extraction of flies

Allopurinol was dissolved in water at approximately 100 μg/ml. Following this the drug solution was placed on the nutrient medium for *Drosophila* and was kept for 1 day in order to allow the allopurinol to be completely absorbed by the agar. Then the 7 day old adult flies were transferred into drug vials and kept for 6, 24, 48 and 72 h before the extraction and homogenisation steps. Ice cold methanol/chloroform/water (3:1:1 v/v/v) was used in the sample quenching and extraction method. 10 adult flies from both gender were collected and anesthetized briefly under CO_2, they were then homogenised in the solvent mixture, followed by sonication for 15 s. The supernatant was then centrifuged for 5 min at 13000 *g* at 0°C, and the filtered supernatants were then stored in a freezer at −80°C until required. Prior to analysis, samples were brought to room temperature and were placed into chromatography vials for analysis by LC–MS.

2.4 LC–MS method

LC–MS data were acquired using a Finnigan LTQ Orbitrap instrument (Thermo Fisher Scientific, Hemel Hempstead, UK) set at 30,000 resolution. Sample analysis was carried out under positive ion mode. The mass scanning range was m/z 50-1200, while the capillary temperature was 200°C and the sheath and auxiliary gas flow rates were 30 and 10 arbitrary units, respectively. The LC–MS system (controlled by Xcalibur version 2.0, Thermo Fisher Corporation) was run in binary gradient mode. Solvent A was 0.1% formic acid in HPLC grade water and solvent B was 0.1% formic acid in acetonitrile. A flow rate of 300 μl/min was used and the injection volume was 10 μl, the gradient used was as follows: 90% B at (0 min)–50% B at (16 min)–20% B at (18 min)–20% B at (28 min)–90% B at (36 min). Samples were kept in a vial tray which was set at a constant

temperature of 3°C. The injection volume was 25 µl. Mass measurement was externally calibrated according to the manufacturer's instructions just before commencing the experiment, and was internally calibrated by lock masses (positive ion mode m/z 83.06037 and m/z 195.08625, due to acetonitrile dimer and caffeine, respectively, and negative ion mode 91.00368 due to formic acid dimer). Runs were carried out in negative ion mode using the conditions above after tuning in the negative ion mode and the assigning appropriate lock mass.

2.5 Data processing

Data files were processed using Sieve 1.3 (Thermo Fisher, Hemel Hempstead UK). The parameters used in Sieve were: time range 4–35 min, mass range 50–700 amu, frame width 0.02 amu and Rt width 2.5 min. The output from Sieve was transferred into Sieve Extractor, an Excel spreadsheet and an in-house macro written in Visual Basic, and used to search against a mass list of 65,000 compounds taken from the KEGG, Metlin, Human Metabolome and Lipid Maps databases.

3 Results and discussion

3.1 Allopurinol phenocopies rosy, but provides temporal resolution

All masses gave <2 ppm deviation (usually <1 ppm deviation) from the nominal mass of the metabolite assigned to them which meant generally that there was only one matching elemental composition in the data base of 65,000 compounds for compounds with a MW <300 Da, although isomers are always a possibility for many compounds. Table 1 shows groups of metabolites falling in specific pathways which were changed in OR flies as a result of treatment with allopurinol. 250 metabolites <700 Da, thus excluding most lipids, could be putatively identified in the extracts using the ZIC-HILIC column in positive and negative ion modes (Table 2 supplementary information). As expected there is a gradual build up of hypoxanthine and xanthine as a result of their metabolism to uric acid being blocked. Figure 1 shows extracted ion chromatograms showing the rise of hypoxanthine over 72 h and also a gradual increase in the peak for ingested allopurinol which is isomeric with hypoxanthine but elutes earlier from the ZIC-HILIC column. Surprisingly there was only a very slow change in uric acid levels during the course of the experiment indicating that the rate of elimination of uric acid from the flies is slow. This is consistent with the accumulation of uric acid crystals in tissues such as eye, fat body, or the lumen of the tubule of normal flies—termed "storage excretion" (Wigglesworth 1939). In *ry* mutants, urate is undetectable (Kamleh et al. 2008); this is because XO activity is never present throughout the life of the insect. Although the ultimate metabolic destination is similar, allopurinol application thus allows a *ry*-like phenotype to be imposed on an otherwise normal animal, revealing temporal information that is unavailable in the mutant. The levels of pterin also rose following allopurinol treatment; pterin is also a substrate for XO and is normally oxidised to produce isoxanthopterin, but this process is also blocked by allopurinol. It was found previously that isoxanthopterin was absent from *ry* mutants (Kamleh et al. 2008), however, in the current study isoxanthopterin levels were not affected by allopurinol over the time course of the experiment indicating that it is only metabolised slowly by the flies.

3.2 Allopurinol impacts on ascorbate metabolism

Strikingly, even after only 6 h of treatment with allopurinol, ascorbic acid levels in the flies fell to below the limit of detection and remained so up to 72 h; this can be seen in the extracted ion traces shown in Fig. 2. It can be observed that the levels of glutathione (GSH) fall rapidly after allopurinol treatment (Table 1) to around half the pre-treatment levels and correspondingly concentrations of GSSG rise. GSH is the co-factor for thioredoxin which recycles ascorbic acid and lowered levels of GSH may account for the loss of ascorbic acid. Methionine sulphoxide is another marker of oxidative stress and it was also found to increase following allopurinol treatment. What part of the metabolic network is being affected by allopurinol and causing the oxidative stress? GSH levels are maintained by glutathione reductase, an enzyme which uses NADPH as its co-factor. The main source of NADPH is from glucose-6-phosphate dehydrogenase (G6PDH) which oxidises glucose-6-phosphate to glucono 1,5 lactone 6-phosphate. This reaction is the rate limiting step in the pentose phosphate pathway. The levels of the pentose phosphate metabolites sedoheptulose phosphate, ribitol phosphate, deoxyribose phosphate and deoxyribose are lowered relative to the controls possibly indicating that allopurinol is somehow causing inhibition of G6PDH. The deficiency of NADPH is also reflected in the markedly lowered levels of glucitol which is derived from glucose by reduction with alditol reductase which requires NADPH as a co-factor. It is a matter of debate whether or not ascorbic acid can be biosynthesised by insects. Many mammals, apart from primates, biosynthesise ascorbic acid and thus do not require it as a vitamin. The sequence of metabolites in the biosynthesis of ascorbic acid is glucuronic acid-gulonic acid-gulonolactone-ascorbic acid. Gulonic acid and gulonolactone can be putatively identified in the extracts from *Drosophila* and are

Table 1 Metabolic pathways where several metabolites are significantly affected by allopurinol comparing each time point of treatment with untreated flies (batches of 10 male + 10 female flies, $n = 4$ for each time point)

Time of treatment	M_r	Δppm	Elution time	6 h		24 h		48 h		72 h	
				Fold change	*P* value	Fold change	*P* value	Fold change	*P* value	Fold change	*P* value
Purines/pterins											
Hypoxanthine	137.0460	0.82	11.5	2.3	0.0036	5.2	0.012	1.7	0.0035	6.0	0.0025
Xanthine	153.0407	−0.73	10.9	3.2	0.0077	7.8	0.0082	10.0	0.010	12.3	0.0001
Allantoin	159.0514	0.02	15.1	0.25	0.00073	0.52	0.007	0.10	0.0011	0.40	0.0028
Pterin	164.0568	0.81	12.7	1.3	0.035	1.7	0.000006	1.5	0.0024	1.4	0.000016
Urate	169.0357	0.26	14.04	1.1	0.38	1.1	0.085	0.85	0.14	0.72	0.002
Isoxanthopterin	180.0517	0.27	13.0	1.1	0.32	1.0	0.9	1.2	0.64	1.0	0.70
Inosine	269.0882	0.31	12.7	0.84	0.19	1.0	0.91	0.74	0.0012	0.67	0.00026
Oxidative stress											
Threonate	133.0300	−0.79	8.0	0.76	0.0035	0.88	0.063	0.51	0.000095	0.06	0.00027
Methionine S-oxide	166.0532	−0.3	19.1	0.99	0.98	2.77	0.00072	1.8	0.0019	1.4	0.83
Ascorbic acid	175.0248	0.11	13.7	ND	–	ND	–	ND	–	ND	–
GSH	308.0910	−0.37	17.0	0.51	0.0054	0.40	0.01	0.42	0.0024	0.45	0.0059
Riboflavin	377.1458	0.74	10.2	1.0	0.84	0.47	0.0067	0.30	0.00005	0.26	0.000048
GSSG	613.1592	−0.29	21.5	1.4	0.12	2.9	0.011	1.8	0.022	1.4	0.06
Pentose phosphate pathway											
Deoxyribose	133.0506	0.21	8.4	0.42	0.033	0.30	0.11	0.6	0.087	0.41	0.37
Deoxylulose/ribulose phosphate	213.0171	0.60	12.3	0.38	0.016	0.33	0.013	0.27	0.011	0.17	0.022
Deoxylulose/ribulose phosphate	213.0171	1.44	14.7	0.72	0.071	0.62	0.023	0.46	0.0076	0.19	0.037
Ribitol phosphate	231.0279	1.26	18.0	0.76	0.18	0.49	0.91	0.49	0.0046	0.23	0.018
Sedoheptulose 7-phosphate	289.0331	−0.26	20.3	0.73	0.022	1.0	0.33	0.75	0.0031	0.71	0.0075
Aldose reductase											
Gulonolactone	177.0405	0.11	12.2	0.30	0.029	0.32	0.034	0.26	0.026	0.52	0.090
Glucitol	181.0718	0.10	16.3	0.29	0.031	0.50	0.073	0.42	0.048	0.43	0.048
Gulonic acid/gluconic acid	195.0511	0.18	16.9	0.46	0.020	0.44	0.020	0.36	0.012	0.42	0.021
Kynurenine pathway											
Niacin/Nicotinate	124.0393	−0.12	8.2	0.72	0.029	0.79	0.034	0.44	0.0016	0.44	0.002
Oxoadipic acid	159.0300	0.65	12.2	0.28	0.003	0.31	0.029	0.25	0.0027	0.54	0.1
Dihydroxyquinoline	162.0548	0.03	9.1	1.4	0.039	1.7	0.00066	1.2	0.090	0.94	0.65
Xanthurenic acid	206.0448	−0.02	11.7	0.86	0.011	0.96	0.47	0.84	0.018	0.80	0.0011
L-Kynurenine	209.0922	0.68	13.4	0.71	0.00088	0.69	0.012	0.49	0.000093	0.41	0.000019
3-Hydroxy-L-kynurenine	225.0868	−0.86	15.1	0.90	0.15	0.83	0.027	0.66	0.00050	0.63	0.00069
N-Formylkynurenine	237.0871	0.24	13.9	1.1	0.58	0.45	0.30	0.60	0.06	0.53	0.0027
Miscellaneous											
L-Tyrosine	182.0812	0.77	15.7	0.73	0.024	0.87	0.043	0.66	0.001	0.75	0.0069
2-Amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine	196.0831	0.56	11.1	5.6	0.029	23.9	0.21	199	0.025	4.0	0.025
5-Hydroxy-L-tryptophan	221.092	0.10	13.6	0.41	0.0043	0.18	0.00021	0.26	0.00055	0.32	0.0023
Linolenyl carnitine	424.3421	0.13	10.1	0.66	0.003	0.95	0.5	0.63	0.0023	0.57	0.0013
Linolylcarnitine	426.3576	0.01	10.1	0.81	0.0088	1.1	0.21	0.75	0.0084	0.73	0.0020

ND metabolite not detected in sample group

significantly lower in the allopurinol treated samples; again, this could be a consequence of a lack of NADPH which is required as a co-factor for the reduction of glucuronic acid to gulonic acid. It has recently been observed that *Drosophila* over-expressing G6PDH have an increased lifespan, which gives an indication of the

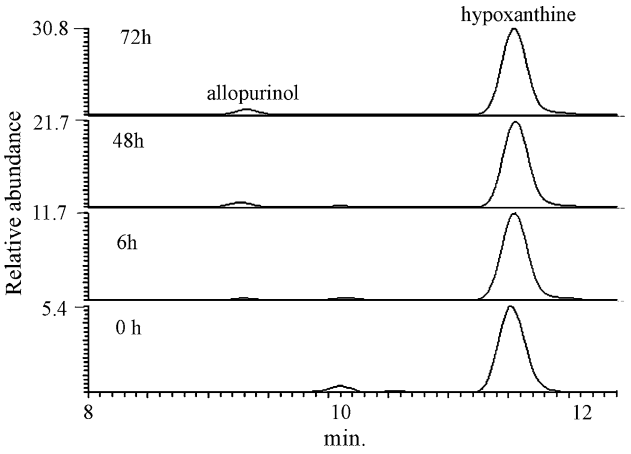

Fig. 1 Extracted ion traces in positive ion mode (m/z 137.04–137.05) showing an increase of hypoxanthine and allopurinol levels with time in *Drosophila* treated with allopurinol. The intensity of the hypoxanthine peak gradually increases so that at 72 h it is 5.7 times greater than the time 0 concentration. Absorption of allopurinol by the flies can be observed through an increase in the peak corresponding to allopurinol up to 72 h. (Analysis conditions as described in 2.4)

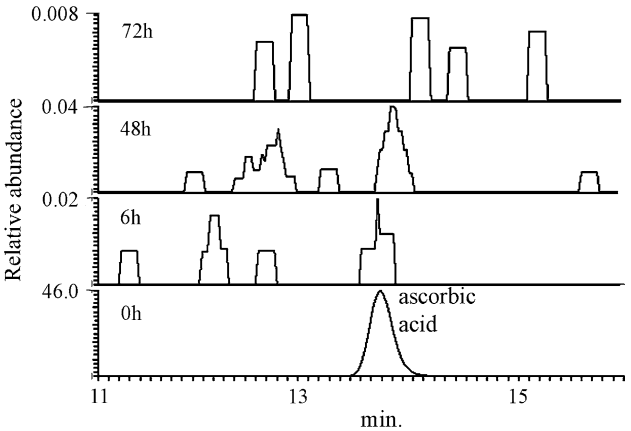

Fig. 2 Extracted ion traces in negative ion mode (m/z 175.02–175.03) showing Loss of ascorbic acid in *Drosophila* following treatment with allopurinol. The ascorbic acid peak can be observed at time 0 but by the 6 h time point it is no longer detectable (Analysis conditions as described in 2.4)

importance of G6PDH as a source of NADPH and thus in protection against oxidative stress (Legan et al. 2008).

The induction of oxidative stress caused by allopurinol is unexpected since there is an extensive literature indicating that it functions indirectly as an anti-oxidant by decreasing production of superoxide as a by product of XO activity (Pacher et al. 2006), much of the superoxide produced in the human body results from XO activity. However, it is also possible that there is an impact on anti-oxidant levels due to a reduction in uric acid levels, the major concentrations of uric acid are in the Malpighian (renal) tubule and the slow changes uric acid in the whole fly may not reflect the changes in the specific regions of the body. The *ry* mutant, which lacks uric acid, has been shown to be hypersensitive to oxidative stress (Hilliker et al. 1992). Uric acid is believed to be an important anti-oxidant in human serum where it protects ascorbic acid against oxidation possibly via acting as an iron chelating agent (Glantzounis et al. 2005). Depletion of uric acid in serum results in rapid oxidation of ascorbic acid (Sevanian et al. 1991). It was found that mutants of *Drosophila* with impaired XO activity produced higher levels of ROS particularly within their guts (Massie et al. 1991) which might account in the current results for the depleted levels of ascorbic acid in the whole fly. It has been shown that the highest levels of XO in mice occur in the proximal small intestine (Mohamedali et al. 1993). It may be that the allopurinol can affect oxidative stress in two ways; when allopurinol was administered to mice it was found to cause a fall in the levels of both uric acid and ascorbic acid in brain dialysate, but ascorbate levels in brain tissue itself were not reduced (Enrico et al. 1997). It was suggested that the presence of uric acid in extracellular fluids might be more important for stabilising ascorbic acid in extracellular fluid than within tissues.

3.3 Allopurinol and the kynurenine pathway

Lowered levels of antioxidants may have an effect on the kynurenine pathway. The rate determining step in the pathway is the conversion of tryptophan into formylkynurenine. The enzyme carrying out the conversion is indolamine dioxygenase (IDO) which is a haem based enzyme requiring the iron to be in the iron (II) form for the conversion to occur (Botting 1995; Batabyal and Yeh 2007). In vitro ascorbic acid is used as a co-factor for the enzyme since it restores it to its iron (II) form by reducing iron (III), however, in vivo the co-factor is not known. In the allopurinol treated flies, levels of formylkynurinine and the downstream metabolites kynurenine, hydroxykynurenine, xanthurenic acid, nicotinic acid and oxoadipic acid are lower than those in the untreated flies. Dihydroxyquinoline, which is also in the kyurenine pathway, goes against the trend and is significantly elevated in allopurinol treated flies. It is possible that the depletion of ascorbic acid impacts on the kynurenine pathway either directly or through failure to maintain a reducing atmosphere and as a consequence failure to maintain iron in its iron (II) state. However, alternatively the reduction in levels of metabolites in the kynurenine pathway may due to a reduction in the availability of the superoxide anion which is a co-factor for IDO (King and Thomas 2007). The effects on allopurinol on tryptophan metabolism are of interest since allopurinol is well established as a drug in the management of schizophrenia (Buie et al. 2006; Dickerson et al. 2009). It

has been proposed that its effectiveness in treating schizophrenia is through inhibiting the conversion of adenosine into guanosine since lowered adenosine levels may have a role in the condition. However, it is also well established that schizophrenia and other types of brain disorder can be correlated to increased degradation of tryptophan via the kynurenine pathway (Barry et al. 2009). In addition, in recent years allopurinol has been used as an experimental drug in the treatment of patients who have suffered a stroke (Muir et al. 2008). The severity of a stroke has been correlated with increased degradation of tryptophan via the kynurenine pathway (Darlington et al. 2007). IDO also has a key role in modulating the immune response (King and Thomas 2007). The picture here is not entirely clear with IDO being induced by interferon-γ in response to infection resulting in increased tryptophan degradation which boosts immune response but at the same time IDO induction has a role in the adaptive immune response and causes immune-suppression which has led to an interest in it as a target for anticancer drugs (Macchiarulo et al. 2009). Some of the difficulty in interpreting such contradictory roles for IDO might be attributed to specific co-regulation of enzyme activity and also to localisation of its activity. It has been proposed that IDO and inducible nitric oxide synthase (NOS2) are regulated in a reciprocal fashion (King and Thomas 2007) and it was found that in the *ry* mutant, both innately and following infection, higher levels of NO were observed (Kim et al. 2001). Thus, an increased production of NO can be linked to the removal of XO activity and hence possibly to the reciprocal regulation of NOS2 and IDO with XO also playing a role. In our earlier work we observed down regulation of kynurenine and hydroxykynurenine in male *ry* flies but not female (Kamleh et al. 2008). Such arguments become increasingly tenuous but highlight the benefits that would be obtained from bringing in transcriptomics data to support such observations of the metabolome.

4 Concluding remarks

The current paper has demonstrated the potential value of *Drosophila* as a model organism for uncovering the wider effects of drug molecules. The goal of the paper was to phenocopy the effects of the *ry* mutation and observe the metabolic effects. While the expected effects on uric acid metabolism were observed there a number of unexpected metabolic changes. Most marked was the disappearance of ascorbic acid in the flies. Of most interest in terms of the therapeutic indications for allopurinol was its effect in reducing trypophan metabolism which tie into the established use of allopurinol in treating schizophrenia and its experimental use in the treatment of patients who have suffered a stroke. It would also be possible to hypothesise on the basis of the effects of allopurinol of tryptophan metabolism that allopurinol might have some value as an immune-modulator. These insights could be accomplished using a simple organism which costs little to procure and maintain and also is very amenable to the production of knock out lines. The introduction of transcriptomic methods to support metabolomic experiments with *Drosophila* will provide a very powerful technique for understanding the ramifications of both genetic mutations and drug action.

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References

Barry, S., Clarke, G., Scully, P., & Dinan, T. G. (2009). Kynurenine pathway in psychosis: Evidence of increased tryptophan degradation. *Journal of Psychopharmacology, 23*, 287–294.

Batabyal, D., & Yeh, S. R. (2007). Human tryptophan dioxygenase: A comparison to indoleamine 2, 3-dioxygenase. *Journal of the American Chemical Society, 129*, 15690–15701.

Botting, N. P. (1995). Chemistry and neurochemistry of the kynurenine pathway of tryptophan metabolism. *Chemical Society Reviews, 24*, 401–412.

Buie, L. W., Oertel, M. D., & Cala, S. O. (2006). Allopurinol as adjuvant therapy in poorly responsive or treatment refractory schizophrenia. *Annals of Pharmacotherapy, 40*, 2200–2204.

Darlington, L. G., Mackay, G. M., Forrest, C. M., Stoy, N., George, C., & Stone, T. W. (2007). Altered kynurenine metabolism correlates with infarct volume in stroke. *European Journal of Neuroscience, 26*, 2211–2221.

Dickerson, F. B., Stallings, C. R., Origoni, A. E., Sullens, A., Khushalani, S., Sandson, N., et al. (2009). A double-blind trial of adjunctive allopurinol for schizophrenia. *Schizophrenia Research, 109*, 66–69.

Dietzel, U., Kuper, J., Doebbler, J. A., Schulte, A., Truglio, J. J., Leimkuhler, S., et al. (2009). Mechanism of substrate and inhibitor binding of rhodobacter capsulatus xanthine dehydrogenase. *Journal of Biological Chemistry, 284*, 8759–8767.

Dow, J. A. T. (2007). Model organisms and molecular genetics for endocrinology. *General and Comparative Endocrinology, 153*, 3–12.

Dow, J. A. T., & Davies, S. A. (2003). Integrative physiology and functional genomics of epithelial function in a genetic model organism. *Physiological Reviews, 83*, 687–729.

Dunn, W. B. (2008). Current trends and future requirements for the mass spectrometric investigation of microbial, mammalian and plant metabolomes. *Physical Biology. 5*, 011001.

Enrico, P., Esposito, G., Mura, M. A., Migheli, R., Serra, P. A., Desole, M. S., et al. (1997). Effects of allopurinol on striatal dopamine, ascorbate and uric acid during an acute morphine challenge: Ex vivo and in vivo studies. *Pharmacological Research, 35*, 577–585.

Glantzounis, G. K., Tsimoyiannis, E. C., Kappas, A. M., & Galaris, D. A. (2005). Uric acid and oxidative stress. *Current Pharmaceutical Design, 11*, 4145–4151.

Glassman, E. (1965). Genetic regulation of xanthine dehydrogenase in *Drosophila melanogaster*. Federation Proceedings 24, 1243–1251

Hilliker, A. J., Duyf, B., Evans, D., & Phillips, J. P. (1992). Urate-null rosy mutants of *Drosophila melanogaster* are hypersensitive to oxygen stress. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 4343–4347.

Ille, R., & Nishino, T. (1995). Flavoprotein structure and mechanism 4. Xanthine oxidase and xanthine dehydrogenase. *Faseb Journal, 9*, 995–1003.

Kamleh, M. A., Dow, J. A. T., & Watson, D. G. (2009). Applications of mass spectrometry in metabolomic studies of animal model and invertebrate systems. *Briefings in Functional Genomics & Proteomics, 8*, 28–48.

Kamleh, M. A., Hobani, Y., Dow, J. A. T., & Watson, D. G. (2008). Metabolomic profiling of *Drosophila* using liquid chromatography Fourier transform mass spectrometry. *Febs Letters, 582*, 2916–2922.

Kim, Y. S., Nam, H. J., Chung, H. Y., Kim, N. D., Ryu, J. H., Lee, W. J., et al. (2001). Role of xanthine dehydrogenase and aging on the innate immune response of *Drosophila*. *Journal of the American Aging Association, 24*, 187–193.

King, N. J. C., & Thomas, S. R. (2007). Molecules in focus: Indoleamine 2, 3-dioxygenase. *International Journal of Biochemistry and Cell Biology, 39*, 2167–2172.

Krastanov, A. (2010). Metabolomics-the state of the art. *Biotechnology & Biotechnological Equipment, 24*, 1537–1543.

Legan, S. K., Rebrin, I., Mockett, R. J., Radyuk, S. N., Klichko, V. I., Sohal, R. S., et al. (2008). Overexpression of glucose-6-phosphate dehydrogenase extends the life span of *Drosophila* melanogaster. *Journal of Biological Chemistry, 283*, 32492–32499.

Lotito, S. B., & Frei, B. (2004). The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. *Free Radical Biology and Medicine, 37*, 251–258.

Macchiarulo, A., Camaioni, E., Nuti, R., & Pellicciari, R. (2009). Highlights at the gate of tryptophan catabolism: a review on the mechanisms of activation and regulation of indoleamine 2, 3-dioxygenase (IDO), a novel target in cancer disease. *Amino Acids, 37*, 219–229.

Massie, H. R., Shumway, M. E., & Whitney, S. J. P. (1991). Uric-acid content of *Drosophila* decreases with aging. *Experimental Gerontology, 26*, 609–614.

Mohamedali, K. A., Guicherit, O. M., Kellems, R. E., & Rudolph, F. B. (1993). The highest levels of purine catabolic enzymes in mice are present in the proximal small intestine. *Journal of Biological Chemistry, 268*, 23728–23733.

Muir, S. W., Harrow, C., Dawson, J., Lees, K. R., Weir, C. J., Sattar, N., et al. (2008). Allopurinol use yields potentially beneficial effects on inflammatory indices in those with recent ischemic stroke a randomized, double-blind, placebo-controlled trial. *Stroke, 39*, 3303–3307.

Pacher, P., Nivorozhkin, A., & Szabo, C. (2006). Therapeutic effects of xanthine oxidase inhibitors: Renaissance half a century after the discovery of allopurinol. *Pharmacological Reviews, 58*, 87–114.

Scalbert, A., Brennan, L., Fiehn, O., Hankemeir, T., Kristal, B. S., et al. (2009). Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. *Metabolomics, 5*, 435–458.

Sevanian, A., Davies, K. J. A., & Hochstein, P. (1991). Serum urate as an antioxidant for ascorbic-acid. *American Journal of Clinical Nutrition, 54*, S1129–S1134.

Spagou, K., Tsoukali, H., Raikos, N., Gika, H., Wilson, I. D., & Theodoridis, G. (2010). Hydrophilic interaction chromatography coupled to MS for metabonomic/metabolomic studies. *Journal of Separation Science, 33*, 716–727.

Wigglesworth, V. B. (1939). *The principles of insect physiology*. London: Methuen.

Wilcoxen, K. M., Uehara, T., Myint, K. T., Sato, Y., & Oda, Y. (2010). Practical metabolomics in drug discovery. *Expert Opinion on Drug Discovery, 5*, 249–263.