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SHORT COMMUNICATIONS

Allopurinol influence on iodine metabolism*

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THE EXTENSIVE use of allopurinol [4-hydroxypyrazolo-(3,4-*d*)pyrimidine] in the treatment of gout and hyperuricemia in man[1–3] has stimulated continued investigation of its biochemistry, pharmacology and toxicology.[4, 5] The drug is a potent inhibitor of xanthine oxidase *in vivo* and *in vitro* in several species.[6] Recent work has yielded new information as to its conversion to oxoallopurinol [4,6-dihydroxypyrazolo(3,4*d*)pyrimidine] and formation of ribonucleoside derivatives.[7]

The only other reports of the effect of allopurinol on other metabolic pathways have been concerned with iron metabolism.[8–10] The meager data on this aspect in man indicate the desirability of further study.[11]

In a review of histological data from thyroids from rats tested with allopurinol, the possibility of thyroid pathology was suggested. The present investigation explored this area by determining protein-bound iodine in dogs and monkeys before, during and after i.v. treatment with allopurinol.

Purebred young beagles of both sexes weighing between 7·1 and 10·1 kg and male and female rhesus monkeys weighing between 2·9 and 4·5 kg were used in the standard toxicology protocol.[12] Several clinical chemical parameters and protein-bound iodine (PBI) were evaluated weekly in order to select healthy animals and to obtain control values on each animal to be given allopurinol. Subsequently, total iodine was also determined and the study was extended to incluce 6-propylthiouracil-treated monkeys and thyroidectomized dogs. Thyroid suppression in 2 monkeys was accomplished by daily administration of a split dose of 6-propylthiouracil *per os*. Initial doses of 1·5 were increased to 4·5 and then 13·5 mg/kg in order to achieve a 50 per cent decrease in PBI. Two dogs were thyroidectomized by total surgical removal of the thyroid and parathyroid glands. Postoperatively they were maintained on thyroxin (25–100 μg daily) for 4–5 weeks, but no thyroxin was given 7–10 weeks prior to allopurinol treatment nor during treatment or recovery phases. Both dogs received daily calcium *per os* and one was supplemented with vitamin D throughout the study.

Allopurinol was administered i.v. daily for 14 days at dose levels between 6·25 and 100 mg/kg. The compound was formulated on a daily basis at 20 mg/ml in an alkaline saline, the final pH being 10·8.

PBI was determined by the Hycel dry ash method† which employs an oxalate–carbonate alkali solution for complete oxidation of the serum sample at 640° for 1 hr.[13] The catalytic effect of iodine on the reduction of ceric sulfate by arsenious acid provides the endpoint.[14] A commercial Iodo-trol‡ standard was run simultaneously. In a number of instances, butanol-extractable iodine (BEI)* was measured in parallel by extracting the serum sample twice with 1 ml of *n*-butyl alcohol, recovering the solvent phase by centrifugation and removing the organic solvent via a 180°C sand-bath with a stream of nitrogen. It was found necessary to employ 3–4 times the quantity of resin used for human and monkey serum for dog serum.[15] Estimates of total serum iodine were obtained by eliminating the treatment of serum with inorganic iodide-absorbing resin.

To confirm the results *in vivo*, some experiments were performed to establish whether additions of allopurinol or the alkaline saline diluent *in vitro* affected PBI values in monkey serum. The effect of allopurinol *in vitro* was evaluated by incubating 0·5 ml of serum for 30 min with varying quantities (125–1000 μg) of allopurinol. At the termination of the incubation period, a routine PBI was carried out.

At least two determinations of PBI and, at some of the doses of allopurinol, two measurements of BEI were obtained as pretreatment values permitting comparison of control and treatment values on

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† Hycel, Inc.; brochure on dry ash PBI determinations (1963).

‡ Dade Reagents, Inc.; control for serum protein-bound iodine.

TABLE 1. SERUM LEVELS OF PROTEIN-BOUND IODINE, BUTANOL-EXTRACTABLE IODINE AND TOTAL IODIDE BEFORE, DURING AND AFTER THE I.V. ADMINISTRATION OF ALLOPURINOL FOR 14 DAYS

Treatment of mg/kg/day for 14 days)	Time of determination of blood test	Protein-bound iodine			Butanol-extractable iodine			Total iodide		
		No. of animals	(μg/100 ml serum) (mean ± S.D.)	% of change	(μg/100 ml serum) (mean ± S.D.)	% of change	No. of animals	μg/100 ml serum) (mean ± S.D.)	% of change
Observations in monkeys									
100	Pretreatment	4	6·5 ± 0·6 (14)*		3·9 ± 0·3 (6)		4		
	Treatment	4	15·0 ± 2·5 (12)	+131	8·3 ± 1·2 (6)	+112	4	14·1† ± 1·8 (3)	+48
	Post-treatment	3	6·0 ± 0·6 (14)	−8	3·9 ± 0·3 (7)	+0	3	7·1 ± 0·3 (6)	−25
50	Pretreatment	4	6·2 ± 0·6 (22)		3·4 ± 0·5 (10)		2	9·4 ± 0·6 (12)	
	Treatment	4	11·8 ± 1·8 (11)	+92	4·3 ± 0·2 (2)	+27	2	11·9 ± 1·2 (4)	+15
	Post-treatment	3	6·7 ± 0·6 (10)	+ 8	3·0 ± 0·2 (5)	−12	2	9·4 ± 0·6 (4)	±0
25	Pretreatment	2	6·6 ± 0·9 (5)						
	Treatment	2	6·3 ± 0·9 (5)	−4					
	Post-treatment	1	8·8 ± 0·8 (5)	+33					
12·5	Pretreatment	2	8·1 ± 0·5 (9)						
	Treatment	2	11·8 ± 2·4 (5)	+46					
	Post-treatment	1	8·9 ± 0·9 (3)	+10					
6·25	Pretreatment	2	6·2 ± 0·2 (4)		4·8 ± 0·6 (4)				
	Treatment	2	5·7 ± 0·9 (6)	−8	4·2 ± 0·3 (6)	−12			
	Post-treatment	1	5·2 ± 0·3(3)	−16	3·8 ± 0·4 (3)	−21			
Observations in dogs									
100	Pretreatment	4	5·6 ± 0·4 (8)		3·9 ± 0·2 (2)		2		
	Treatment	4	13·9 ± 2·7 (19)	+149	9·9 ± 2·1 (19)	+154	2	39·2‡ ± 7·8 (3)	+63
	Post-treatment	2	10·1 ± 2·1 (22)	+80	8·4 ± 2·1 (22)	+115	2	29·9 ± 4·2 (20)	+24
50	Pretreatment	4	6·5 ± 1·2 (9)		5·4 ± 0·6 (4)		1	21·3 ± 1·0 (2)	
	Treatment	4	13·2 ± 1·5 (18)	+103	10·5 ± 1·5 (11)	+94	4	35·5 ± 3·6 (13)	+66
	Post-treatment	2	7·7 ± 1·8 (15)	+18	5·7 ± 1·2 (14)	+6	2	24·3 ± 2·5 (12)	+14

* Values in parentheses refer to number of determinations.

† Mean values from 11 untreated monkeys; total iodide = 9·5 ± 1·5.

‡ Mean values from 15 untreated dogs; total iodide = 24·0 ± 4·8.

each animal respectively. Although male and female animals were in each dose group, the values for the respective iodide fractions were averaged as shown in Table 1.

As the allopurinol dose was increased, there was a progressive elevation in PBI and BEI for both monkeys and dogs. At a dose of 12·5 mg/kg, only the male monkey showed an increment, while at 25 mg/kg the female animal responded in a delayed fashion. At 50–100 mg of allopurinol/kg all animals increased their PBI and BEI levels, but the response varied in intensity and duration. For example, PBI and BEI values remained above control values for part of the recovery period in dogs given 100 mg of allopurinol/kg.

A reduced number of animals in each group was studied during the revovery period because some animals were sacrificed at the termination of the treatment interval for histopathologic evaluation. Histopathology of the thyroids appeared to be within normal limits.

In some of the animals other iodide fractions were also estimated. Values for the total iodide are included in Table 1 and the mean obtained on 11 control monkeys, 9·5 ± 1·5 μg/100 ml (51 determinations)* was used for calculating the per cent change in total iodide. It can be seen that the corresponding mean for 4 monkeys treated with 100 mg of allopurinol/kg was 14·1 ± 1·8 μg/100 ml indicating a 48 per cent rise. A small elevation was seen at the 50 mg/kg dose.

In the dog, the control value for total iodide was 24·0 ± 4·8 (36 determinations on 15 animals) and at the 100 mg/kg dose of the drug 3 animals gave a mean value of 39·2 ± 7·8 μg/100 ml; this increase was also observed for dogs treated at the 50 mg/kg dose. Residual response was seen in the recovery period. Some determinations of thyroxin (T_4)† were performed on sera from dogs treated with 50 mg of allopurinol/kg. T_4 values of 1·0 ± 0·3 μg/ml (4)† during treatment did not significantly differ from those obtained on 15 control dogs, 0·8 ± 0·4 μg/100 ml (33).[16]

TABLE 2. SERUM LEVELS OF PROTEIN-BOUND IODINE, BUTANOL-EXTRACTABLE IODINE AND TOTAL IODIDE BEFORE, DURING AND AFTER THE I.V. ADMINISTRATION OF 50 mg OF ALLOPURINOL/kg FOR 14 DAYS TO MONKEYS WITH PROPYLTHIOURACIL-SUPPRESSED THYROIDS AND TO THYROIDECTOMIZED DOGS

Species	Time of determinations of blood tests	Protein-bound iodine (μg/100 ml serum) (mean ± S.D.)	Butanol-extractable iodine (μg/100 ml serum) (mean ± S.D.)	Total iodide (μg/100 ml serum) (mean ± S.D.)
Monkey (+ 6 PT)* ♂	Pretreatment	5·0 ± 0·1(2)	2·5 ± 0·2(2)	8·6 ± 0·5(2)
	Treatment	4·8 ± 0·1(3)	3·6 ± 0·3(3)	12·2 (1)
	Post-treatment	6·5 ± 0·7(4)	4·6 ± 0·4(4)	11·4 ± 1·2(4)
Monkey (+ 6 PT) ♀	Pretreatment	3·3 ± 0·2(3)	2·5 ± 0·2(3)	6·7 ± 0·1(3)
	Treatment	4·9 ± 0·5(3)	3·4 ± 0·4(3)	8·6 ± (1)
	Post-treatment	6·3 ± 0·5(6)	3·6 ± 0·2(6)	9·0 ± 0·7(6)
Dog† thyroidectomized ♂	Pretreatment	3·7 ± 0·3(6)	3·5 ± 0·5(6)	19·8 ± 3·3(5)
	Treatment	3·4 ± 0·3(2)	2·5 ± 0·3(2)	13·5 (2)
	Post-treatment	3·0 ± 0·3(4)	2·0 ± 0·1(4)	31·9 ± 2·4(4)
Dog† thyroidectomized ♂	Pretreatment	3·3 ± 0·3(8)	2·6 ± 0·3(8)	27·1 ± 3·6(8)
	Treatment	1·9 ± 0·3(3)	1·2 ± 0·3(3)	12·8 ± 4·4(3)
	Post-treatment	1·8 ± 0·2(3)	1·4 ± 0·2(3)	16·1 ± 0·6(3)

* 6 PT = 6-propulthiouracil.

† Mongrel dog.

are outlined in Table 2. It required approximately 17 weeks and increasing doses of 6-PT to suppress PBI and BEI levels approximately 50 per cent. At this time, 50 mg of allopurinol/kg was administered

In an attempt to localize the action to the thyroid gland, allopurinol was tested in 2 monkeys maintained on a dose of 6-propylthiouracil (6-PT) sufficient to suppress thyroid function. These results

* Values in parentheses indicate the number of determinations performed.

† Bio-Science Laboratories, Van Nuys, Calif.

while the animals were maintained on 6-PT. There was a definite but delayed increase in both parameters. In addition, total iodide was elevated approximately 32 per cent.

Similar measurements on 2 thyroidectomized dogs are also presented in Table 2. Neither dog received supportive hormone therapy for several weeks before the allopurinol trial, nor during allopurinol treatment or the recovery phase. The data in Table 2 reveal that allopurinol did not influence the iodine parameters determined. Several estimations of T_3 (sponge kit)* failed to exhibit any significant changes.[17] T_3 values obtained on 4 control dogs gave a mean of 54 $\pm$ 2 per cent (10), while the mean obtained on the 2 thyroidectomized dogs treated with allopurinol was 51 $\pm$ 2 per cent (4).

Sera from 4 untreated monkeys and a commercial preparation of human sera (Iodo-trol) were used for incubations *in vitro* with allopurinol. Only the alkaline saline diluent was added to corresponding aliquots of serum. No increase in PBI was detected in serum exposed to allopurinol for 30 min. This finding also eliminates the possibility of contamination of allopurinol with iodine.

In the absence of marked thyroid histopathology over a 14-day treatment period with allopurinol, it would seem reasonable to interpret the elevation in protein-bound iodide as a release of nonfuctional iodoamino compounds. The limited measurements of thyroxin suggest no marked quantitative change in this iodoamino acid and do not favor increased thyroxin binding.

The maximum tolerated nontoxic dose (MTD) for repeated daily i.v. injection for 14 days was 12·5 mg/kg/day for monkeys and less than 50 mg/kg/day for dogs. Injection of larger doses led to temporary dose-related reversible nephrotoxicity, slight hepatotoxicity and slight protracted anemia. Although elevation of serum BUN and PBI were generally dose related, the two underlying mechanisms were clearly independent. Diminished renal clearance did not account for the elevated iodine parameters.

The relatively high doses of allopurinol as well as the i.v. route used do not permit the prediction of possible iodine derangement in man receiving this drug.

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