

Kinetics of aldehyde oxidase in some tissues of the arabian one-humped camel, (*camelus dromedarius*)

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ABSTRACT

The specific activities of two molybdenum hydroxylases; aldehyde oxidase and xanthine oxidase from six tissues; (heart, jejunum, kidney, liver, lung and spleen), of the Arabian one-humped camel, (*Camelus dromedarius*) were measured. Three different substrates: 3-methylisoquinoline, phthalazine and phenanthridine were used for aldehyde oxidase activity. Only liver had aldehyde oxidase activity with phenanthridine as a substrate. Xanthine oxidase showed no activity in all the studied tissues, with xanthine as a substrate. The values of both V_{max} and K_m were determined, for hepatic aldehyde oxidase. The activity of aldehyde oxidase was completely inhibited using menadione and hydralazine.

INTRODUCTION

Two of the molybdenum hydroxylases namely aldehyde oxidase (EC 1.2.3.1) and xanthine oxidase (EC 1.2.3.2) are known to have vital roles in the hydrolysis of purines, pyrimidines, and aldehydes, (Clarke *et al.* 1997)⁽¹⁾. As drug metabolizing enzymes, aldehyde oxidase and xanthine oxidase are involved in some pathological conditions, and play an important task in the biotransformation of drugs and xenobiotics, (Pritsos, 2000 and Maia & Mira 2002)^(2,3). Aldehyde oxidase has been implicated in familial amyotrophic lateral sclerosis and hepatotoxicity of alcohol, (Berger *et al.*, 1995 and Mira *et al.*, 1995)^(4,5). Recently the involvement of aldehyde dehydrogenase in the oxidation of almotriptan, an antimigraine agent has been reported, (Salva *et al.*, 2003)⁽⁶⁾.

Aldehyde oxidase and xanthine oxidase are widely distributed throughout the animal kingdom from primitive species to the majority of mammals, (Beedham, 1985)⁷. Both enzymes from rat liver were purified by several investigators, (Moriwaki *et al.*, 1993 and McManaman *et al.*, 1996)^(8,9). Maia & Mira, 2002⁽³⁾, reported a method that allows simultaneous purification of these enzymes from the same batch of rat livers. The purified enzymes gave single bands of approximately 300 kDa on polyacrylamide gel gradient electrophoresis. In view of the limited information regarding the distribution of aldehyde oxidase and xanthine oxidase in the arabian camel tissues, this study was carried out to investigate the tissue distribution and the kinetics of these enzymes in the Arabian one-humped camel (*Camelus dromedaries*).

MATERIAL & METHODS

Chemicals

Phthalazine and phenanthridine were bought from Aldrich Chemical Company, (Gillingham, U.K.). 3-Methylisoquinoline was obtained from ICN Pharmaceuticals Inc. (Contra Costa, CA., USA). Xanthine, menadione, and hydralazine were purchased from Sigma Chemical Company, (St. Louis, MO, USA). All other chemicals were of analytical grade and supplied by BDH, (Poole, UK).

Tissues Samples

Fresh camel liver, heart, kidney, jejunum, spleen and lung samples were obtained from freshly slaughtered male camels, (aged 1-3 years) at Jeddah, Slaughter House, Saudi Arabia, within 20 min after slaughter. All tissue samples were kept in liquid nitrogen until arrival to the laboratory, after which they were stored for 2 days in a deep freeze at -80°C.

Enzyme Purification

Partially purified aldehyde oxidase and xanthine oxidase were prepared according to the method of Johnson *et al.* (1987)⁽¹⁰⁾ with some modifications. Frozen tissues were ground in liquid nitrogen and 50 ml of 1.15% ice-cold KCl solution containing 0.1mM EDTA were added to the resulting powder, (26.5g). The suspension was homogenized using a polytron homogenizer. The homogenate was placed in a flask and heated on a steam bath at 50-55°C for 15 min. The flask was then immediately cooled in an ice bath until the temperature dropped to 10°C or lower, and the suspension was

centrifuged at 20,000g for 30 min at 4°C. The resulting supernatant was then filtered through glass wool, and the volume of the clear solution was measured. Solid ammonium sulphate, (0.35 g per ml of extract), was slowly added with gentle stirring, so that 50% saturation was achieved at 0-4°C. The precipitate was collected by centrifugation at 6,000 g for 15 min at 4°C. The pellet from the previous step was dissolved in 5 ml of 0.1 mM EDTA solution, and stored in a deep freezer at -80°C.

Enzyme assays

The activity of partially purified aldehyde oxidase under optimal conditions at pH 7.0 was measured as described by Johnson *et al.* (1984)⁽¹¹⁾. The oxidation rate of either phthalazine, (1mM) or 3-methylisoquinoline, (1mM) was monitored at 420 nm using 1mM potassium ferricyanide as an artificial electron acceptor, whereas that of phenanthridine, (0.05mM) was evaluated at 322 nm. The activity of xanthine oxidase was measured at 295 nm with xanthine (0.05mM) using molecular oxygen as an electron acceptor. All enzymatic determinations were performed using Aquarius visible/U.V. double beam spectrophotometer, (Cecil Instrument Limited), fitted to thermoelectric controller. K_m and V_{max} values were determined from Lineweaver-Burk double reciprocal plot. The enzyme activities were also measured after incubating the reaction mixture with either menadione or hydralazine at different concentrations.

Protein determination

Protein concentration of partially purified aldehyde oxidase and

xanthine oxidase fractions were determined by the method of Lowry *et al.* (1951)⁽¹²⁾ with bovine serum albumin as standard.

Statistical analysis

The data presented in this study are expressed as means \pm SD and comparisons were carried out using the student's t-test.

RESULTS

The specific activity of partially purified aldehyde oxidase from different tissues of the Arabian one-humped camel, (*Camelus dromedarius*), including liver, kidney, lung, jejunum, spleen and heart, was measured spectrophotometrically using three substrates; phthalazine, 3-methylisoquinoline and phenanthridine. Table (1), shows that on using phthalazine, camel liver exhibited the highest specific activity of aldehyde oxidase, (15.81 nmol/min/mg protein), compared with other tissues under the study. Furthermore it was noticed that the activities of kidney and lung aldehyde oxidase were 13%, ($p < 0.001$) and 32%, ($p < 0.001$) respectively as compared to liver. Alternatively with 3-methylisoquinoline lung and spleen

aldehyde oxidase had maximal specific activities, (17.14 and 14.72 nmol/min/mg protein) respectively. As compared with camel lung aldehyde oxidase, liver enzyme was 32%, ($p < 0.001$) whereas kidney enzyme was 20%, ($p < 0.001$). It could also be observed that only liver tissue showed aldehyde oxidase activity with phenanthridine, (4.02 nmol/min/mg protein). Using xanthine as a substrate for xanthine oxidase, no activity in any of the investigated camel tissues was detected. Table (2), shows the kinetics of partially purified aldehyde oxidase from camel liver. The K_m values were 0.15, 0.014, and 0.073 mM for phthalazine, 3-Methylisoquinoline and phenanthridine respectively. 3-Methylisoquinoline had the highest value of substrate efficiency (V_{max}/K_m). However, the lowest value was obtained by phenanthridine. It also shows that the camel hepatic aldehyde oxidase was completely inhibited by menadione when phenanthridine was used as a substrate at 2 μ M concentration of menadione. On the other hand, the enzyme was totally inhibited by only 1 μ M concentration of hydralazine when 3-methylisoquinoline was used.

Table1. Specific activities of aldehyde oxidase and xanthine oxidase in the arabian camel tissues using different substrates.

| Tissue | Specific activity (nmol/min/mg protein) of aldehyde oxidase and xanthine oxidase | | | |
|---------|--|----------------------|-----------------|----------|
| | Phthalazine | 3-Methylisoquinoline | Phenanthridine | Xanthine |
| Liver | 15.81 \pm 2.62 | 5.63 \pm 0.08* | 4.02 \pm 0.06 | NR |
| Kidney | 2.07 \pm 0.84* | 3.42 \pm 0.05* | NR | NR |
| Lung | 5.06 \pm 0.64* | 17.14 \pm 2.36* | NR | NR |
| Jejunum | 4.22 \pm 0.57* | 5.10 \pm 0.72* | NR | NR |
| Spleen | 3.55 \pm 0.62* | 14.72 \pm 1.61 | NR | NR |
| Heart | 2.52 \pm 0.07* | 5.08 \pm 0.84* | NR | NR |

- Results are presented as mean \pm SD, n=6
- * = $p < 0.001$
- NR = no Reaction

Table 2. Kinetics of partially purified aldehyde oxidase of the arabian camel liver.

| Substrate | V_{max} nmol/min/mg protein | K_m (nM) | V_{max}/K_m nmol/min/mg protein | Inhibitor concentration required for total inhibition | |
|-----------------------|-------------------------------------|---------------|---|--|---------------------|
| | | | | Menadione (mM) | Hydralazine (mM) |
| Phthalazine | 12.39 | 0.15 | 0.083 | 0.10 | 5.0 |
| 3- Methylisoquinoline | 7.14 | 0.014 | 0.510 | 2.0 | 1×10^{-3} |
| Phenanthridine | 3.79 | 0.073 | 0.052 | 2×10^{-3} | 2.0 |

DISCUSSION

The present study shows that the investigated tissues of the Arabian one-humped camel, (*Camelus dromedarius*), don't display any xanthine oxidase activity. These results are consistent with the findings of Mura *et al.*, (1986)¹³ that in camel liver, hypoxanthine is converted into inosine monophosphate rather than being oxidized to uric acid. This investigation demonstrates that camel aldehyde oxidase is present predominantly in liver, lung and surprisingly spleen, (Table 1). These findings coincide with those of Li Calzi *et al.*, (1995)¹⁴ who reported a high level of expression of the bovine enzyme in liver lung, and spleen. However, the incidence of aldehyde oxidase at a relatively high level in the spleen can be accounted for the unique nature of the spleen as one of the specialized lymphoid tissues where much of the immune response occurs. As compared with hepatic aldehyde oxidase activities in other species, (human, guinea pig, rabbit and rat), reported by Rashidi *et al.*, (1997)¹⁵ using phthalazine at a concentration of 1mM as a substrate, camel hepatic enzyme showed the lowest enzyme activity. From this view, the findings of our study are in

full agreement with other studies carried out on other drug-metabolizing enzymes other than molybdenum hydroxylases, which revealed that camel seemed to have the lowest enzyme activity when compared to the other species, (el Sheikh *et al.*, 1988, Damanhour & Tayeb 1993 and Damanhour 2002)^(16,17,18).

The complete inhibition of camel aldehyde oxidase by low concentration (1×10^{-3} mM) of hydralazine when 3-methylisoquinoline was used as a substrate, (Table 2), coincides with an earlier report on different species, (Johnson *et al.*, 1985)¹⁹. On the other hand, menadione, at a relatively high concentration, (2mM), was needed to cause complete inhibition of the enzyme with the same substrate, (Table 2). Although these results demonstrate that camel hepatic aldehyde oxidase activity seemed to be the lowest among the other mammals, however, its role could be the protection of the animal against plant toxins. From this view, our data is in agreement with the findings of Moriwaki *et al.*, (1997)⁽²⁰⁾. It was shown by Raza *et al.*, (1998)⁽²¹⁾ that the maximum expression of P450 protein was seen in the camel liver, and kidney, therefore P450 enzymes are highly involved in the metabolism

of a wide array of xenobiotics and pollutants. It could be concluded that the involvement of camel hepatic aldehyde oxidase in the metabolism of xenobiotics is still controversial.

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الملخص العربي

تم قياس النشاطية النوعية لاثنتين من إنزيمات الموليبيدينوم هيدروكسيلاز (ألدهايد أوكسيداز وزانثين أوكسيداز) من ستة أنسجة (القلب والصائم والكلية والكبد والرئة والطحال) في الجمل العربي. استخدمت ثلاثة مواد تفاعل مختلفة (3- ميثايل أيزوكينولين وثالازين وفينانثريدن لقياس نشاطية ألدهايد أوكسيداز. فقط الكبد أظهر نشاطية لألدهايد أوكسيداز مع فينانثريدن كمادة تفاعل. لم يبد زانثين أوكسيداز أي نشاطية في جميع الأنسجة المدروسة مع زانثين كمادة تفاعل. تم تعيين قيم كلاً من V_{max} و K_m للألدهايد أوكسيداز الكبدية. باستخدام مينادايون وهيدرالازين كانت نشاطية ألدهايد أوكسيداز مثبته بالكامل.