Effect of amiodarone on non-deiodinative pathway of thyroid hormone metabolism

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Abstract Amiodarone, an iodine containing antiarrhythmic drug, causes a significant decrease in molar ratio of daily production rates of T\(_3\) and T\(_4\) from 0.75 in controls to 0.36 in amiodarone-treated rabbits. A model was constructed from the above data which showed that metabolism of T\(_4\) via non-deiodinative pathways (e.g. tetraiodothyroacetic acid and/or conjugates) increased from 29% in untreated controls to 66% in amiodarone-treated rabbits. In this study, we have examined the metabolic clearance rate of tetraiodothyroacetic acid in rabbits given amiodarone (20 mg·kg\(^{-1}\)·day\(^{-1}\) ip for 3 weeks) or saline (controls). Serum amiodarone and desethylamiodarone levels under the above experimental conditions were 0.20±0.067 and 0.17±0.058 mg/l, respectively, which were in the near-therapeutic range observed in humans. Control and amiodarone-treated rabbits were administered \(^{[\text{131}}\)I-tetraiodothyroacetic acid (10 μCi/rabbit) iv and blood was collected at 0.5, 1, 2, 4, 6, 10, 32 and 48 h. Serum tetraiodothyroacetic acid radioactivity was determined by trichloroacetic acid precipitation and ethanol extraction and metabolic clearance rates were calculated from the area under the curve of computer fits to tetraiodothyroacetic acid radioactivity data. Amiodarone treatment decreased metabolic clearance rates significantly from 0.107±0.008 in controls to 0.074±0.009 l/day in amiodarone-treated rabbits (p<0.05). However, when expressed per unit body weight (l·day\(^{-1}\)·kg\(^{-1}\)), the metabolic clearance rates were not significantly different between the controls and amiodarone-treated rabbits. The terminal serum elimination half-life in the two groups were similar (32.0±6.7 h in controls vs 49.2±12.4 h in amiodarone-treated). Our data suggest that a modest decrease in clearance rate and an increased production rate could result in an increase in serum tetraiodothyroacetic acid levels which may contribute to the reduction in 5'-monodeiodination of iodothyronines documented previously in amiodarone-treated animals. The marginal decrease in metabolic clearance rate of tetraiodothyroacetic acid found in this study suggests that the two-fold increase in the conversion of T\(_4\) metabolism to non-deiodinated metabolites/conjugates in amiodarone-treated rabbits results predominantly from an increase in production rate of tetraiodothyroacetic acid.

Amiodarone is an iodine-containing antiarrhythmic drug structurally similar to thyroxine. The myocardial electrophysiological actions of amiodarone resemble that of hypothyroidism (1-3). The clinical implications of amiodarone treatment on thyroid function have been reviewed recently (4). We have previously shown that chronic amiodarone treatment caused an inhibition in the peripheral conversion of T\(_4\) to T\(_3\) in rabbits in vivo (5). This inhibitory effect of amiodarone on the 5'-monodeiodinase has also been observed in other species (6-9) and in isolated cells (10). Our previous studies showed that the molar ratio of daily production rates of T\(_3\) and T\(_4\) was reduced significantly from 0.75 in controls to 0.36 in amiodarone-treated rabbits (5).

The present study was undertaken to gather fur-
ther insight into alterations in thyroid hormone physiology during treatment with amiodarone. On the basis of the experimental data on the production rates of \(T_4\), \(T_3\) and \(rT_3\), we hypothesized that \(T_4\) metabolism via the non-deiodinative pathway may be altered during amiodarone treatment. In the present study, we have determined the metabolic clearance rate of tetraiodothyroacetic acid (\(TA_4\)) in rabbits treated with amiodarone. Increased oxidative deamination of \(T_4\) (to \(TA_4\)) has been suggested to occur in situations associated with decreased conversion of \(T_4\) to \(T_3\) as in fasting and acutely ill patients with the low \(T_3\) syndrome (11). Increased serum \(TA_4\) concentrations and increased production of \(TA_4\) have been shown to occur under those conditions (11-14).

Material and Methods

Male New Zealand white rabbits with initial body weights of 2.40 ± 0.14 kg were used. Animals were divided into two groups of 7 rabbits each. One group was given amiodarone 20 mg·kg\(^{-1}\)·day\(^{-1}\) ip in a 5% aqueous solution for 3 weeks while the second (control) group received an equal volume of saline. The rabbits from both groups were allowed food and water ad libitum during the entire experimental period. At the end of three weeks, there was a significant difference (p<0.05) in the body weights of saline-treated rabbits (mean ± SEM, 2.9 ± 0.14 kg) and the amiodarone-treated rabbits (2.50 ± 0.13 kg). \(^{125}\text{I}]TA_4\) was prepared by radioiodination of 3,5,3'-triodothyroacetic acid as we have described previously (15): the specific activity of \(^{125}\text{I}]TA_4\) approximated 2000 mCi/mg. On the day of the experiment, rabbits from both groups were administered \(^{125}\text{I}]TA_4\) (10 μCi/rabbit) in 0.5 ml physiological saline as a single bolus iv into the ear vein. Blood (1-1.5 ml) was collected at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 32 and 48 h after isotope administration. The blood was centrifuged immediately upon collection and serum isolated for each time point. Serum \(TA_4\) radioactivity was determined by trichloroacetic acid precipitation, and ethanol extraction as described earlier (16). The recovery of \(^{125}\text{I}]TA_4\) added to control rabbit serum by the above procedure was 79.9 ± 3.2 (mean ± SD, N=5) and therefore a correction factor of 1.25 was applied in the calculation of total radioactivity in serum. Metabolic clearance rates (MCRs) were calculated from the area under the curve of \(TA_4\) radioactivity and expressed either as percent dose/l or as percent dose·l\(^{-1}\)·kg\(^{-1}\) (Fig. 1) as a function of time using the SAAM computer program (5).

Blood and myocardial tissues were obtained from control and amiodarone-treated rabbits at the termination of the kinetic study viz. 48 h after the administration of \(^{125}\text{I}]TA_4\). The rabbits did not receive any drug during the 48 h of the kinetic study. Serum and tissue amiodarone and desethylamiodarone concentrations were determined using a high-performance liquid chromatographic method standardized in our laboratory (17, 18).

The pharmacokinetic parameters of the control and amiodarone-treated rabbits were compared using Student’s t-test.

Results

Fig. 1 shows a semi-logarithmic plot of the elimination kinetics of \(^{125}\text{I}]TA_4\) in control and amiodarone-treated rabbits. Data are mean ± SEM from 5 to 6 rabbits from each group. The abscissa represents time after the administration of labeled \(TA_4\) while the ordinate represents the serum radioactivity as percent dose·l\(^{-1}\)·kg\(^{-1}\). The elimination curves for \(^{125}\text{I}]TA_4\) were similar for amiodarone-treated and control rabbits, with the data points for the former lying slightly above those of controls (Fig. 1). A similar pattern was also seen when the \(TA_4\) radioactivity was expressed as percent dose/l (not shown). The elimination kinetics followed a biexponential curve for both the control and experimental groups. The elimination half-life

Fig. 1
A semilogarithmic plot of the elimination kinetics of \(TA_4\) in control (●) and amiodarone-treated (▲) rabbits. The abscissa represents the time in hours after iv administration of radio-(\(^{125}\text{I}\)) iodine labeled \(TA_4\). The ordinate is the radioactivity in percent dose per liter per kilogram bodyweight. Values are mean ± SEM from 5-6 rabbits. The curves drawn are computer fits to the data.

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Table 1:
Kinetic parameters of TA₄ in controls and amiodarone-treated rabbits.

<table>
<thead>
<tr>
<th></th>
<th>MCR (l/day)</th>
<th>Vc (l/kg -1-day-1)</th>
<th>T½c(hours)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.123</td>
<td>0.098</td>
<td>0.107 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>0.106</td>
<td>0.088</td>
<td>0.087 ± 0.007</td>
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<tr>
<td></td>
<td>0.103</td>
<td>0.080</td>
<td>0.134 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>0.122</td>
<td>0.050</td>
<td>0.113 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>0.083</td>
<td>0.050</td>
<td>0.113 ± 0.007</td>
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<tr>
<td></td>
<td>0.079</td>
<td>0.028</td>
<td>0.110 ± 0.009</td>
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</tr>
<tr>
<td></td>
<td>0.008</td>
<td>0.009</td>
<td>0.007 ± 0.004</td>
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<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>NS</td>
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C: Controls; T: Amiodarone-treated

Table 2 gives the serum and myocardial concentrations of amiodarone and desethylamiodarone in amiodarone-treated rabbits. The serum concentrations of amiodarone averaged 0.20±0.067 mg/l while that of desethylamiodarone averaged 0.17±0.058 mg/l. The myocardial content of amiodarone and its metabolite, desethylamiodarone averaged 1.36±0.25 and 0.034±0.006 μg/g, respectively (Table 2). The data suggested a tissue to serum ratio of amiodarone to approximate 8.0. Although the serum and myocardial amiodarone values in the present study fell within the lower range of our previously reported values (19, 20) they are not different from the values obtained by us in a more recent study of 3 week amiodarone-treated rabbits (21).

Table 2.
Serum and myocardial amiodarone (AM) and desethylamiodarone (DEA) concentrations in AM-treated rabbits.

<table>
<thead>
<tr>
<th></th>
<th>AM</th>
<th>DEA</th>
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<tbody>
<tr>
<td>Serum (mg/l)</td>
<td>0.20±0.067</td>
<td>0.170±0.058</td>
</tr>
<tr>
<td>Myocardium (μg/g)</td>
<td>1.36±0.25</td>
<td>0.034±0.006</td>
</tr>
</tbody>
</table>

Mean±SEM (N=5-6).
Discussion

Although conversion of T₄ to T₃ and to rT₃ accounts for over 70% of T₄ metabolism in normal man, this pattern is altered under pathological conditions (11). It has been suggested that diversion of T₄ metabolism away from deiodination is associated with increased deamination of hormone to TA₄. Thus, in acutely ill patients with low serum T₃ concentrations, serum TA₄ levels were increased (12). Similarly, Pittman et al. (14) reported that the mean serum TA₄ concentrations increased two-fold and the fraction of T₄ metabolism by deamination doubled in fasting patients. These changes were associated with a decrease in T₃ disposal rate as has been reported to be the case in patients on chronic amiodarone therapy (8).

We have noted in our previous publications that the rabbit is a clinically relevant model for studying both acute and chronic hemodynamic and electrophysiological effects of amiodarone therapy (reviewed in 2,22) although some species differences exist between the rabbit and man with respect to the metabolism of the drug. The serum amiodarone and desethylamiodarone levels after 3 week amiodarone treatment in the present study were 0.20 ± 0.067 and 0.170 ± 0.058 mg/l, respectively, which are in the sub-therapeutic range observed during chronic therapy in man.

Using the data from our previous findings in the chronic rabbit model (5), we developed a scheme to estimate the route of metabolism of T₄ in control and amiodarone-treated rabbits Fig. 2. This scheme predicted that the diminution in the conversion of T₄ to T₃ in amiodarone-treated rabbits is associated with a significant increase in T₄ conversion to TA₄ and/or conjugates (from 1190 ng/day in controls to 4170 ng/day in amiodarone-treated rabbits). In the present study, we have found that amiodarone treatment of rabbits for 3 weeks caused a decrease in MCR in amiodarone-treated rabbits (about 18%) which did not reach statistical significance when calculated on a per unit body weight basis. We have not calculated the production rate of TA₄ in the present study because methods for measurement of serum TA₄ concentration were not available. However, the model depicted in Fig. 2 clearly shows that the inhibition of the conversion of T₄ to T₃ from 63% to 29% in amiodarone-treated rabbits can be accounted for almost fully by the increased diversion of T₄ to non-deiodinative routes of metabolism (29% in controls vs 66% in drug-treated rabbits). Thus, keeping in view the model in Fig. 2, it is evident that marginal decrease in TA₄-MCR in amiodarone-treated rabbits cannot account for the more than two-fold increase in their T₄ conversion to non-deiodinative metabolites. The data actually suggest that there must be an increase in the production rate of TA₄ in amiodarone-treated rabbits. The increased production of TA₄ and associated serum level may play a role, through inhibition of T₄-5’-monodeiodination (23), in the cardiac hypothyroidism-like effects during chronic amiodarone therapy. Clearly, further work is needed to substantiate this hypothesis.

Whether or not diversion of T₄ metabolism from T₃ to rT₃ and to deamination pathways by protracted amiodarone administration makes any direct contribution to the observed antiarrhythmic

Fig. 2.
Chart showing estimates of routes of metabolism of T₄ in controls (top) and amiodarone-treated rabbits (bottom). The values for the production rates were obtained from our earlier studies (5). The bottom most box on the right hand side in control and amiodarone-treated groups represents conversion of T₄ to T₃ conjugates, T₃ excreted intact in urine and tetraiodothyroacetic acid (Tetrac).
and electrophysiological properties of the drug is not clear at the present time. Patterson et al. (3)
have suggested that action of amiodarone is medi-
ated by changes in thyroid hormone levels. Their
suggestion was based on their observations of de-
crease in T₃ levels, similarity of cardiac effects of
hypothyroidism and chronic amiodarone treat-
ment, and reversal of the electropharmacological
effects of the drug by T₃. On the other hand, io-
panoic acid, a potent inhibitor of intracellular con-
version of T₄ to T₃, did not produce sinus brady-
cardia in rats (6) as seen following amiodarone
administration, suggesting that amiodarone may act by
mechanisms other than and/or in addition to its
effect on thyroid hormones (22). Increase in serum
rT₃ probably does not contribute importantly to
cardiac effects of amiodarone. We have recently
shown that repeated administration of rT₃ at a dose
one hundred times the production rate of rT₃ did
not cause any appreciable hemodynamic and elec-
physiological changes in the rabbit myocardium
(24). Because of its low biological potency as well as
low serum levels, it is unlikely that changes in TA₄
levels directly mediate the cardiac effects of amio-
darone; however, the possibility that changes in serum TA₄ and/or production may contribute by
inhibiting 5'-monodeiodination of T₄ to T₃ cannot
be excluded at this time.

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