SHORT COMMUNICATION

Effect of conjugated and unconjugated hyperbilirubinaemia on the plasma 25-hydroxy-vitamin D response to ultraviolet radiation in the rat

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Summary

1. Vitamin D deficiency was induced in anicteric Wistar rats, Wistar rats before common bile-duct ligation was performed and Gunn rats with unconjugated hyperbilirubinaemia.

2. The effect of ultraviolet radiation on plasma concentrations of 25-hydroxy-vitamin D was studied in a group of control anicteric Wistar rats \((n = 11)\), Wistar rats 8 days after common bile-duct ligation \((n = 7)\) with conjugated hyperbilirubinaemia and in Gunn rats \((n = 11)\).

3. The plasma 25-hydroxy-vitamin D response to ultraviolet radiation was similar in the three groups.

4. It is suggested that neither conjugated nor unconjugated hyperbilirubinaemia prevent cutaneous vitamin D\(_3\) synthesis or 25-hydroxylation in the rat.

Key words: 25-hydroxy-vitamin D, jaundice, ultraviolet radiation.

Abbreviation: 25-(OH)D, 25-hydroxy-vitamin D.

Introduction

Vitamins D\(_2\) and D\(_3\) are both ingested in the diet, but in man the most important source of vitamin D in England and the United States of America is the cutaneous synthesis of vitamin D\(_3\) from 7-dehydrocholesterol by the action of ultraviolet radiation of wavelength 280–310 nm (Haddad & Hahn, 1973; Preece, Tomlinson, Ribot, Pietrek, Korn, Davies, Ford, Dunnigan & O’Riordan, 1975). Bilirubin absorbs light at this wavelength and it has been suggested that cutaneous jaundice might be one of the factors in the pathogenesis of the low plasma concentrations of the hepatic metabolite 25-hydroxy-vitamin D [25-(OH)D] seen in jaundiced patients (Long & Sherlock, 1979). The purpose of this study was to test this hypothesis in an animal model by comparing the response of plasma 25-(OH)D to ultraviolet radiation in anicteric rats with that of rats with conjugated and unconjugated hyperbilirubinaemia.

Materials and methods

Male Wistar rats (340–410 g) were bred in the London Hospital colony. Male homozygous Gunn rats (370–480 g) with a deficiency of hepatic bilirubin-glucuronoside glucuronosyltransferase were obtained from the colony at the Royal Free Hospital, London. All rats were kept in surroundings devoid of ultraviolet radiation for 8 weeks and were on a low vitamin D diet (Guroff, De Luca & Steenbock, 1963) in order to reduce their plasma concentrations of 25-(OH)D from a basal value of 50–75 nmol/l.

Three groups of rats were studied. Group 1 consisted of anicteric control Wistar rats \((n = 18)\), 11 in the ultraviolet radiation subgroup and seven in the non-radiation subgroup. Group 2 consisted of Wistar rats 8 days after common bile-duct ligation with conjugated hyperbilirubinaemia \((n = 14)\), seven being in the radiation and seven in the non-radiation subgroups. Group 3 consisted...
of Gunn rats with unconjugated hyperbilirubinaemia \((n = 18)\), 11 being in the radiation and seven in the non-radiation subgroups. Groups 1 and 3 received the low vitamin D diet for 9.5 weeks before study whereas group 2 received it for 2 weeks pre-operatively and postoperatively because it was found that concentrations of 25-(OH)D fell very rapidly after common bile-duct ligation.

Common bile-duct ligation was performed on the group 2 rats by a standard technique (Lambert, 1965). The rats were anaesthetized with 310 \(\mu\)g of intramuscular fentanyl citrate/kg, 10 \(\mu\)g of fluanisone/kg (Hypnorm, Janssen Pharmaceutica, Belgium) and 5 \(\mu\)g of intraperitoneal diazepam/kg (Valium, Roche Products Ltd, England). The abdomen was opened through an upper midline incision. The common bile duct was identified and then ligated with silk just distal to the carina. A second silk ligature was tied around the duct approximately 3 mm distal to the first ligature. The segment of common bile duct between the two ligatures was excised and the abdomen was closed.

Eight days later (day 1) under similar anaesthesia, the dorsal region from the base of the neck to the base of the tail of all the rats was shaved and 3 ml of blood was withdrawn from the internal jugular vein for estimation of plasma 25-(OH)D and bilirubin. The animals were randomly divided into ultraviolet radiation and non-radiation subgroups. The radiation subgroups were immediately irradiated for 1 h with their dorsal region exposed 15 cm from a Westinghouse FS20 sunlamp fluorescent tube with a wavelength spectrum of 270–360 nm with a maximum of 310 nm (Corless, Gupta, Switala, Barragry, Boucher, Cohen & Diffey, 1978). The radiation subgroups were again anaesthetized and irradiated for 1 h with the sunlamp on the next day (day 2). The irradiation did not cause cutaneous burns. Further blood collections were taken from all the animals on the following 2 days (days 3 and 4).

After venesection the blood was heparinized, centrifuged and the plasma was placed in the dark, within 1 h, at \(-20^\circ\)C for storage until assay. Plasma concentrations of total and conjugated bilirubin were measured by a standard autoanalyser technique. Plasma concentration of 25-(OH)D was measured by the competitive protein-binding method of Edelstein, Charman, Lawson & Kodicek (1974) with the rat-kidney binding protein of Haddad & Chyu (1971). All plasma samples from the rats with common bile-duct obstruction were subjected to Sephadeox LH20 chromatography to remove material producing non-specific quenching before assay of 25-(OH)D (Skinner & Wills, 1977). No falls in assayable concentrations of 25-(OH)D occurred when such chromatography was performed on plasma from normal or Gunn rats, but this procedure was only routinely performed on the plasma samples from bile-duct ligated rats.

Results are reported as means \(\pm\) SEM. The response to irradiation was assessed by the paired Student's \(t\)-test and the difference between groups by the unpaired Student's \(t\)-test, and when there was gross non-normality, as with the response to ultraviolet radiation in group 2, by the Mann–Whitney \(U\)-test.

### Results

The mean \((\pm\) SEM) plasma concentration of total bilirubin on day 1 in the group 1 Wistar rats was less than 8 \(\mu\)mol/l, in the group 2 common bile-duct ligated rats it was 160 + 16 \(\mu\)mol/l and in the group 3 Gunn rats it was 124 \(\pm\) 11 \(\mu\)mol/l. The conjugated fraction of bilirubin was more than 90% in the common bile-duct ligated rats and the unconjugated fraction more than 90% in the Gunn rats. Ultraviolet radiation was not associated with significant changes in plasma concentrations of bilirubin in any of the three groups.

#### Plasma 25-(OH)D response after ultraviolet radiation (Table 1)

The plasma concentrations of 25-(OH)D were similar \((P > 0.05)\) in the three groups of rats before irradiation. After ultraviolet irradiation on days 1 and 2, the plasma concentrations of 25-(OH)D on days 3 and 4 were significantly higher \((P < 0.001)\) in all three groups. The mean rise was highest in the common bile-duct ligated rats and lowest in the Gunn rats but in the numbers studied here there was no significant difference in the response between the groups.

#### Plasma 25-(OH)D response in non-irradiated controls (Table 1)

Basal mean plasma concentration of 25-(OH)D in the common bile-duct ligated rats were higher on day 1 than in the other two groups and this difference was significant compared with the group 3 Gunn rats. The difference between the plasma concentrations of 25-(OH)D of group 2 irradiated and non-irradiated rats was not significant. Mean plasma concentrations of 25-(OH)D of the non-irradiated rats were below day 1 concentrations when measured on days 3 and 4.
Table 1. Plasma concentrations of 25-(OH)D in ultraviolet-irradiated and non-irradiated rats

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>Ultraviolet-irradiated</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Before irradiation (day 1)</td>
<td>5.5 ± 2.5</td>
<td>4.3 ± 3.3</td>
<td>6.8 ± 1.8</td>
</tr>
<tr>
<td>1 day after irradiation (day 3)</td>
<td>26.5 ± 3.3</td>
<td>29.3 ± 12.0</td>
<td>21.0 ± 2.5</td>
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<tr>
<td>2 days after irradiation (day 4)</td>
<td>27.8 ± 3.8</td>
<td>28.5 ± 7.8</td>
<td>18.5 ± 2.3</td>
</tr>
<tr>
<td>Non-irradiated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before irradiation (day 1)</td>
<td>5.5 ± 4.3</td>
<td>12.0 ± 1.5</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td>2 days later (day 3)</td>
<td>1.8 ± 0.8</td>
<td>11.5 ± 2.3</td>
<td>1.3 ± 1.3</td>
</tr>
<tr>
<td>3 days later (day 4)</td>
<td>3.5 ± 2.5</td>
<td>7.5 ± 2.8</td>
<td>0.8 ± 0.5</td>
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Discussion

In this animal study exposure to ultraviolet radiation on two consecutive days was associated with marked rises in plasma concentrations of 25-OH(D) in all three groups of vitamin D-deficient rats tested. In contrast, in the animals not irradiated with ultraviolet radiation, the plasma concentrations of 25-(OH)D fell rapidly, presumably as a result of removal of circulating plasma 25-(OH)D and the continuing low vitamin D diet. The difference between the plasma concentrations of 25-(OH)D of group 2 and group 3 non-irradiated and non-irradiated rats is explained by the animals being from two separate groups studied at different times. It is concluded that the rise in plasma 25-(OH)D in the jaundiced subgroup resulted solely from the cutaneous synthesis of vitamin D3 and subsequent hepatic 25-hydroxylation.

The main effective wavelength for phototherapy of hyperbilirubinaemia is 400–500 nm (Gläuser, Lombard, Gläuser & Sisson, 1971; Ostrow, 1972) and consequently no significant change was seen in the plasma concentrations of bilirubin of the irradiated rats. The rise in plasma 25-(OH)D in the jaundiced animals cannot therefore be explained by a fall in plasma concentrations of bilirubin. The mean response in the irradiated common bile-duct ligated rats was slightly greater than in the Wistar rats but was a little reduced in the Gunn rats. A possible reason for the lesser (but not significantly less) response in the Gunn rats is the large black and brown pigmented areas on their skin being associated with reduced cutaneous vitamin D3 synthesis.

In studies in man, reduced plasma concentrations of 25-(OH)D can usually be corrected to normal by vitamin D supplements in patients with alcoholic cirrhosis (Posner, Russell, Absood, Connor, Davis, Martin, Williams, Norris & Merchant, 1978) and primary biliary cirrhosis (Skinner, Long, Sherlock & Wills, 1977). The subsequent renal synthesis of dihydroxy-vitamin D metabolites 1,25-, 24,25- and 25,26-dihydroxy-vitamin D) has also been shown to be normal in patients with cirrhosis (Long, Skinner, Wills & Sherlock, 1978). In the foregoing studies patients were not divided into icteric and anicteric groups but ultraviolet radiation therapy has been used in two jaundiced patients and in both there was a rise in plasma 25-(OH)D (Jung, Davie, Siklos, Chalmers, Hunter & Lawson, 1979). Ultraviolet radiation of appropriate wavelength and of a suitably controlled dosage is an inexpensive and physiological method of treating vitamin D deficiency and should be further assessed in the prophylaxis and treatment of vitamin D deficiency and osteomalacia in patients with chronic liver disease, whether icteric or anicteric.

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References


