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The Effects of Cyclophosphamide on the Pharmacokinetics of Triiodothyronine in the Male Rat

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Abstract

In the present study, the possibility that cyclophosphamide or a cyclophosphamide metabolite may be accelerating the clearance of triiodothyronine has been examined.

Following administration of exogenous triiodothyronine to saline- and cyclophosphamide-treated rats, the area under the plasma–concentration time curve (AUC), apparent clearance (CL_{app}) and half-life of triiodothyronine were measured. AUC ($34{\cdot}43 \pm 12{\cdot}34$ compared with $33{\cdot}32 \pm 9{\cdot}92$ nmol h L^{-1}), CL_{app} ($36{\cdot}30 \pm 12{\cdot}89$ compared with $37{\cdot}51 \pm 11{\cdot}16$ mL h^{-1}) and half-life ($7{\cdot}50 \pm 1{\cdot}39$ compared with $6{\cdot}40 \pm 0{\cdot}96$ h) were not significantly different in the control rats compared with the cyclophosphamide-treated rats.

As cyclophosphamide does not appear to alter the elimination of triiodothyronine, it is likely that cyclophosphamide or a cyclophosphamide metabolite is acting at the hypothalamo-pituitary axis, reducing the synthesis or release of thyroid stimulating hormone and consequently decreasing the levels of triiodothyronine and thyroxine.

Cyclophosphamide is an oxazaphosphorine alkylating agent extensively used in cancer chemotherapy and as an immunosuppressant (Colvin & Hilton 1981; Newell & Gore 1991). It has a broad spectrum of antitumour activity and a comparatively high therapeutic index. Cyclophosphamide is itself devoid of alkylating activity and must undergo bioactivation by the cytochromes P450 in order to exert its therapeutic and toxic actions (Hohorst et al 1976; Marinello et al 1984). Phosphoramide mustard appears to be the therapeutically significant metabolite, while acrolein is the metabolite that has been associated with the toxicity of cyclophosphamide (Conners et al 1974; Colvin & Hilton 1981).

Over the past two decades, it has become evident that cytochrome P450 enzymes in the rat liver are subject to complex hormonal regulation via the gonadal steroids (Zaphiropoulos et al 1989). CYP2A2, CYP2C11 and CYP3A2 are enzymes expressed at a higher level in the male rat liver while CYP2A1, CYP2E1 and steroid 5α-reductase are expressed at a higher level in the female rat liver (Zaphiropoulos et al 1989). Further, rat hepatic CYP enzymes are also regulated by hormones such as growth hormone (Zaphiropoulos et al 1989), thyroid hormones (Waxman et al 1989; Waxman 1992; Ram & Waxman, 1991, 1992), insulin (Favreau et al 1987; Favreau & Schenkman 1988; Funae et al 1988) and the glucocorticoids (Schuetz et al 1984; Simmons et al 1987).

Le Blanc and Waxman (1990) examined the effect of cyclophosphamide (130 mg kg^{-1} i.p.) on individual cytochrome P450 enzymes in male rats in-vivo 3, 6 and 9 days after administration. They reported that the activities of the male enzymes CYP2A2, CYP2C11 and CYP3A2 were suppressed while the activities of the female enzymes CYP2A1 and steroid 5α-reductase were elevated over the 9-day period. They concluded that the suppression of CYP2A2, CYP3A2 and CYP2C11 and the elevation of CYP2A1 and steroid 5α-reductase by cyclophosphamide or a cyclophosphamide metabolite is via the hypothalamo-pituitary-gonadal axis, reducing testosterone levels and hence feminizing the profile of the various cytochrome P450 enzymes (Le Blanc & Waxman 1990). Their conclusion was made despite the fact that daily administration of human chorionic gonadotrophin after cyclophosphamide dosing did not prevent the cyclophosphamide-mediated changes (Le Blanc & Waxman 1990).

Previous studies conducted in this laboratory have concluded that 7 days following a single dose of cyclophosphamide (200 mg kg^{-1} i.p.) to male rats, there is a decrease in a range of cytochrome P450 enzymes and an accompanying decrease in the hormones testosterone, thyroid-stimulating hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) (McClure & Stupans 1992, 1995). Experiments were designed to examine whether perturbation of hormonal regulation was the mechanism by which cyclophosphamide alters cytochrome P450, NADPH P450 oxidoreductase and steroid 5α-reductase (McClure & Stupans 1995). Daily replacement doses of human chorionic gonadotropin, which stimulates testosterone release from the gonads, or T_3, were administered for 7 days after a single dose of cyclophosphamide. Conclusions made from this study were that cyclophosphamide alters the enzymes NADPH P450 oxidoreductase, steroid 5α-reductase and CYP3A2 via perturbation of the regulation of these enzymes by testosterone or thyroid hormones as hormone replacement following cyclophosphamide dosing prevented the cyclophosphamide-mediated changes in the expression and activity of these enzymes. However, while interference with regulation by testosterone or thyroid hormones may be part of the

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mechanism by which cyclophosphamide alters CYP2C11 and CYP2E1, hormone replacement did not prevent these cyclophosphamide-mediated changes to any significant degree.

Both sulphation and glucuronidation play an important role in the metabolism of T_3 and T_4. Sulphation of the phenolic group of T_4 or of the T_3's facilitates subsequent deiodination, leading to rapid and irreversible inactivation of thyroid hormones (Mulder & Jakoby 1990). At present, it is not clear which uridine diphosphoglucuronyl transferase (UDPGT) is responsible for the glucuronidation of T_4 and T_3. In one study, evidence was produced suggesting UDPGT type I catalyses the glucuronidation of T_3 and T_4 (Saito et al 1991). In another study, the type I and type II rat UDPGTs were shown to glucuronidate T_4 as well as reverse T_3 while T_3 itself was a poor substrate for these enzymes (Visser et al 1993). In the same study, type III UDPGT was shown to catalyse the glucuronidation of T_3 (Visser et al 1993). Both of these studies were conducted with microsomes from rats treated with known inducers of specific UDPGTs and then microsomal T_4 and T_3 glucuronidation examined in-vitro. The study by Visser et al (1993) was conducted in the presence of Brij 58. The significance of these in-vitro studies is not clear, particularly as native UDPGT activity appears to reflect in-vivo T_4 glucuronidation (Visser et al 1993). Studies from this laboratory have demonstrated that 200 mg kg^{-1} cyclophosphamide administered intraperitoneally to rats decreases the glucuronidation of morphine, a type II substrate, one day after dosing and increases the glucuronidation of morphine 4 and 7 days after dosing (Lear et al 1992). In contrast, the rate of glucuronidation of 1-naphthol, a type I substrate, was not altered (Lear et al 1992). This study assayed UDPGT activity in the native state. The effect of cyclophosphamide on sulphation has not yet been investigated.

As TSH was reduced in concert with T_4 and T_3, it is possible that cyclophosphamide or a cyclophosphamide metabolite was acting at the hypothalamo-pituitary-axis to decrease TSH and consequently T_4 and T_3. However, the possibility that cyclophosphamide may accelerate the clearance of T_3 and/or T_4 cannot be ignored. It was therefore hypothesized that cyclophosphamide may increase the clearance of T_3 via an increase in sulphation or glucuronidation. To examine this hypothesis, the pharmacokinetics of T_3 after exogenous administration to saline-treated controls was compared with the pharmacokinetics of T_3 after exogenous administration to rats pre-treated with cyclophosphamide 200 mg kg^{-1} 6 days earlier.

Materials and Methods

T_3 was obtained from Glaxo (Liverpool, UK); anaesthetic ether from Ajax Chemicals (Auburn, Australia); heparinized saline (10 int. units mL^{-1}) and NaCl (0·9%) from Delta West (Bentley, Australia); vinyl tubing, medical grade SV37, i.d. 0·58 mm from Paton Scientific (Victor Harbour, Australia). Sources of other materials were as previously described (McClure & Stupans 1992, 1995).

Assays

Plasma T_3 levels were analysed, using a rat hormone specific radioimmunoassay, by Dr J. Kennedy in the clinical chemistry department, The Queen Elizabeth Hospital, Adelaide, South Australia. The minimum quantifiable concentration was 0·21 nmol L^{-1}.

Animals

Adult male Hooded Wistar rats (180–220 g) were purchased from Gilles Plains Animal Resource Centre (Adelaide, Australia) and acclimatized under standard conditions. Animals were housed in metal cages and allowed free access to food and water.

Surgery

Catherization of the right external jugular vein was essentially by the method of Howe et al (1986) under ether anaesthesia. A vinyl tubing catheter was inserted into the vein and advanced approximately 2·2 cm so that the tip rested in the atrium of the heart.

Experimental

Rats were randomly assigned to two groups. A solution of 0·9% NaCl (saline) was administered to one group (controls) as a single intraperitoneal dose on day 0. The other group was treated with cyclophosphamide (200 mg kg^{-1}) freshly prepared in saline as a single intraperitoneal dose on day 0. On day 6, surgery was performed to catheterize the jugular vein. The rats were allowed to recover from the operation for 30 min before a blood sample (400 μL) was taken to determine basal T_3 levels. The recovery time was kept to a minimum as cyclophosphamide can cause leucopoenia (Fraiser et al 1991) and consequently the risk of infection following surgery is high. After collection of sample 0, T_3 (5 μg kg^{-1} 6·25 nmol kg^{-1}) was administered to both cyclophosphamide and saline-treated rats. Blood samples (400 μL) were taken after 0·5, 1, 2, 4, 8, and 24 h. Heparinized saline (400 μL) was used to restore the blood volume after each sample was taken. Blood samples were centrifuged and the resulting plasma stored at −70°C until used for analysis of T_3 levels.

Pharmacokinetic calculations

Plasma concentrations of T_3 for each rat were plotted on semilogarithmic graph paper as a function of time. The T_3 half-life was estimated by log-linear regression of the terminal slope of the concentration–time profile. The area under the curve (AUC) from time 0 to 24 h was determined by using the linear trapezoidal rule. Clearance was calculated using the formula:

$$CL = D.F/AUC \quad (1)$$

where D is the administered dose and F is the bioavailability factor. Therefore, apparent clearance (CL_{app}) can be calculated using the equation:

$$CL_{app} = CL/F = D/AUC \quad (2)$$

In the discussion it is assumed that the bioavailability (F) of T_3 is consistent between the control and cyclophosphamide-treated rats and therefore that CL_{app} represents CL.

Statistical analysis

Comparisons between the control and the cyclophosphamide-

treated groups were conducted using an unpaired Student's *t*-test.

Results

A representative concentration–time profile following exogenous T_3 administration is shown for a saline control rat (Fig. 1A) and a rat treated with cyclophosphamide six days previously (Fig. 1B). These profiles represent the sum of the exogenous T_3 and basal T_3. Six days after cyclophosphamide administration to male rats, basal plasma T_3 levels (i.e. at time 0 and immediately before exogenous T_3 administration) were significantly reduced relative to saline-treated controls (Table 1). Moreover, 7 days after cyclophosphamide administration and 24 h after administration of exogenous T_3 (5 $\mu g\,kg^{-1}$), T_3 levels were significantly reduced relative to controls as reported previously (McClure & Stupans 1995). Following exogenous T_3 administration the maximum plasma concentration (C_{max}) in the saline-treated rats was $3{\cdot}01 \pm 1{\cdot}06$ nmol L^{-1} compared with $3{\cdot}78 \pm 0{\cdot}96$ nmol L^{-1} in the cyclophosphamide-treated rats (Table 1). The AUC of T_3 following administration of T_3 5 $\mu g\,kg^{-1}$ was unchanged in the cyclophosphamide-treated rats compared with the saline controls (i.e. $34{\cdot}43 \pm 12{\cdot}34$ nmol h L^{-1} compared with $33{\cdot}32 \pm 9{\cdot}92$ nmol h L^{-1} (Table 1). In rats treated with cyclophosphamide 6 days before T_3 dosing, the mean estimated elimination half-life of $6{\cdot}40 \pm 0{\cdot}96$ h was not significantly different from the half-life value of $7{\cdot}5 \pm 1{\cdot}39$ h in the saline control group (Table 1).

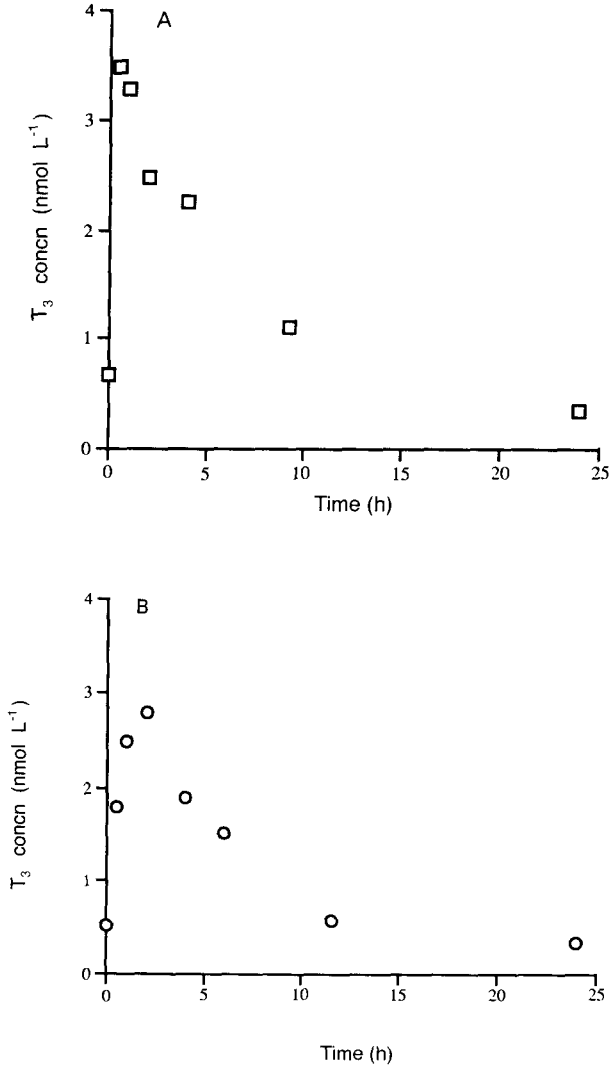

FIG. 1A. Representative graph of a concentration–time profile in an individual saline control rat following exogenous T_3 administration. B. Representative graph of a concentration–time profile in an individual cyclophosphamide-treated rat following exogenous T_3 administration.

Table 1. Plasma T_3 concentrations and pharmacokinetic parameters in control versus cyclophosphamide-treated rats after a dose of T_3 (5 $\mu g\,kg^{-1}$).

	Saline control	Cyclophosphamide
T_3 (nmol L^{-1}) Day 6 (0 h)	$0{\cdot}67 \pm 0{\cdot}13$	$0{\cdot}52 \pm 0{\cdot}07$*
T_3 (nmol L^{-1}) Day 7 (24 h)	$0{\cdot}61 \pm 0{\cdot}34$	$0{\cdot}27 \pm 0{\cdot}08$*
C_{max} (nmol L^{-1})	$3{\cdot}01 \pm 1{\cdot}06$	$3{\cdot}78 \pm 0{\cdot}96$
AUC (nmol h L^{-1})	$34{\cdot}43 \pm 12{\cdot}34$	$33{\cdot}32 \pm 9{\cdot}92$
CL_{app} (mL h^{-1})	$36{\cdot}30 \pm 12{\cdot}89$	$37{\cdot}51 \pm 11{\cdot}16$
Half-life (h)	$7{\cdot}50 \pm 1{\cdot}39$	$6{\cdot}40 \pm 0{\cdot}96$

Values represent the mean ± s.d. for n = 5–7 rats.
* Significantly different relative to saline controls $P < 0{\cdot}05$.

Discussion

Cyclophosphamide is an alkylating agent that has been reported to produce decreased plasma thyroid hormone levels (i.e. TSH, T_3, and T_4) in rats over a 14-day period following its administration (McClure & Stupans 1995). The observed decrease in TSH was accompanied by a corresponding decrease in T_3 and T_4. The effect of cyclophosphamide was temporal and the nadir of the plasma thyroid hormone levels was reached 7 days after the administration with some recovery after 10 and 14 days (McClure & Stupans 1995). This is suggestive that cyclophosphamide or a cyclophosphamide metabolite mediates a decrease in the synthesis or release of thyrotrophin releasing hormone (TRH) from the hypothalamus, or TSH from the anterior pituitary and consequently decreases T_3 and T_4 as a secondary effect. The decreases in thyroid hormones were accompanied by a decrease in the activity and expression of a number of enzymes that are partly or completely regulated by thyroid hormones, i.e., CYP3A2, NADPH P450 oxidoreductase and steroid 5α-reductase (McClure & Stupans 1995). Although cyclophosphamide is likely to be altering plasma T_3 concentrations indirectly via its action at the hypothalamo-pituitary-axis, in the present study we have explored the possibility that in concert, cyclophosphamide may also be increasing the clearance of T_3.

Previous reports from this laboratory have shown that cyclophosphamide increased the activity of morphine-UDPGT (Lear et al 1992) and it was hypothesized that cyclophosphamide may be accelerating the clearance of T_3 via induction of the UDPGTs or sulphotransferases responsible for the clearance of T_3. However, in the present study,

cyclophosphamide treatment decreased T_3 plasma concentrations and altered CYP enzyme activities via a mechanism independent of a change in T_3 half-life, AUC or CL_{app} (and hence CL) (Table 1). Despite the differences in the basal T_3 levels between the saline and cyclophosphamide-treated rats on days 6 and 7, the C_{max} values for both groups of rats were similar and 6–10 fold the T_3 basal levels (Table 1). Therefore, the potential discrepancy in AUC values between the two groups because of differences in the basal T_3 levels on days 6 and 7 was minimized. The reported half-life for T_3 in the rat varies from 6 to 12 h (Higueret & Garcin 1982; Garcin et al 1984; Golstein et al 1988) which is consistent with our mean estimated elimination half-life of T_3 of about 7 h.

In conclusion, this study has demonstrated that cyclophosphamide or a cyclophosphamide metabolite does not appear to decrease plasma T_3 concentrations via an alteration in the pharmacokinetics of T_3. In particular, cyclophosphamide does not mediate an increase in the CL of T_3. Likely mechanisms for the reduction in thyroid hormones following cyclophosphamide administration include: cyclophosphamide or a cyclophosphamide metabolite acting at the hypothalamus to interfere with TRH synthesis and/or release and hence indirectly decreased TSH and subsequently T_3 and T_4; cyclophosphamide or a cyclophosphamide metabolite acting at the pituitary to interfere with TSH synthesis and/or release and hence indirectly decreased T_3 and T_4; and cyclophosphamide or a cyclophosphamide metabolite impairing the synthesis and/or release of TSH from the anterior pituitary and simultaneously impairing the synthesis or release of T_3 and T_4 at the thyroid gland.

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