

Dexamethasone and Some Commonly Used Drugs Inhibit MCT8-mediated T_3 Transport *in Vitro*

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Abstract

Monocarboxylate transporter 8 (MCT8) is the first thyroid hormone transporter that has been linked to a human disease. Besides genetic alterations other factors might impair MCT8 activity. To investigate whether some common drugs can inhibit MCT8-mediated T_3 transport, [^{125}I] T_3 uptake and efflux were measured in COS-7 cells transiently transfected with hMCT8 before and after exposure to increasing concentrations of drugs. The mode of inhibition was also determined. Dexamethasone inhibited T_3 uptake at 10 μ M; hydrocortisone reduced T_3 uptake at 500 and 1000 μ M; prednisone and prednisolone were devoid of inhibitory potential. Amiodarone caused a reduction of T_3 uptake by MCT8 only at the highest concentrations used (44% at 50 μ M and 68% at 100 μ M), this effect was weaker than that produced by desethylamiodarone and dronedarone; buspirone resulted a potent inhibitor, reducing T_3 uptake even at low concentrations. L-carnitine inhibited T_3 uptake only at 500 mM and 1 M. Kinetic experiments revealed a noncompetitive mode of inhibition for all compounds. All drugs inhibiting T_3 uptake did not affect T_3 release. This study shows a novel effect of some common drugs, which is inhibition of T_3 transport mediated by MCT8. Specifically, dexamethasone, buspirone, desethylamiodarone, dronedarone behave as potent inhibitors of MCT8.

Introduction

Monocarboxylate transporter 8 (MCT8) is the first thyroid hormone (TH) transporter that has been linked to a human disease [1, 2].

Loss-of-function mutations in the MCT8 gene (SLC16A2) are the molecular cause of the Allan-Herndon-Dudley syndrome, a form of X-linked mental retardation and severe neurological impairment, associated with TH abnormalities, consisting of high 3,5,3'-triiodothyronine (T_3), low thyroxine (T_4) and 3,3,5'-triiodothyronine (rT_3) levels, and normal or slightly elevated concentration of the thyroid stimulating hormone (TSH) [3]. In man MCT8 is expressed at different levels in many tissues including liver, adrenal gland, brain, kidney, placenta, and thyroid [4–6].

MCT8 functions as a specific TH transmembrane transporter [7, 8], mediating the uptake as well as the efflux [9]. The precise mechanisms by which MCT8 facilitate TH transport are still unknown, although recently a model has been proposed [10]. It has been demonstrated that this process is Na^+ independent, not sensitive to pH and is a facilitated transport [11].

So far, only few drugs (i.e. desipramine and tyrosine kinase inhibitors) and natural compounds (silychristin) have been shown to interfere with MCT8 function through non-competitive and competitive inhibition, respectively [12–15].

Aiming at increasing the knowledge about inhibitors of MCT8 function, in the present work we examined whether some commonly used drugs are able to inhibit MCT8-mediated T_3 transport, and potentially interfere with TH action in target tissues. We selected drugs having a somewhat structural similarity with

TH and/or whose treatment is associated with thyroid function tests (TFTs) abnormalities, or which behave as antagonists of TH action. Figure 1 shows the chemical formulas of T_3 and selected drugs.

Overall, glucocorticoids, as other steroid hormones resemble TH in size, hydrophobic properties and mechanism of action through binding to members of the nuclear receptor superfamily, and some recent evidence suggest that also for steroids the cell-membrane passage might be carrier-mediated [16]. Moreover, the use of dexamethasone and other synthetic glucocorticoids in prenatal life and in childhood has been associated with adverse effects on the developing brain similar to those induced by hypothyroidism [17–19].

Amiodarone is a benzofuran frequently prescribed for the treatment of refractory cardiac arrhythmias, having a structural resemblance to TH. Besides hyper- and hypothyroidism, administration of amiodarone has been also associated with a low T_3 syndrome, which has been attributed to the inhibitory effect of amiodarone on type I and II 5'-deiodinase [20–21]. Furthermore, since a hypothyroidism-like condition of some tissues has been observed during treatment with amiodarone [22], it was demonstrated that this drug and its active metabolite, desethylamiodarone (DEA), block T_3 binding to nuclear receptors [23] and decrease expression of some TH-related genes such as alpha-myosin heavy chain and low-density lipoprotein receptor [24, 25]. DEA has been shown to competitively inhibit TR α 1 and to noncompetitively TR β 1 [26]. However, it is conceivable that the induction of the local hypothyroid state may be also due to the impaired TH uptake in tissues. Dronedarone is structurally related to amiodarone but lacks iodine moiety. As for amiodarone and DEA, dronedarone was shown to reduce *in vitro* binding of T_3 to TR α 1 without affecting TR β 1 receptors, while its metabolite debutyldronedarone, inhibited T_3 binding to both receptors [27].

Thyroid hormones influence mood [28] and there is evidence that some psychotropic medications can reduce TH uptake in brain [12]. Among these agents, we selected buspirone, a drug that, when used in major depression in augmentation with SSRIs or tricyclic antidepressants, shows to be less effective than T_3 in improving the response of clinical non responders [29, 30].

The use of carbamazepine and valproic acid is associated with TFTs abnormalities, the underlying mechanisms of which are not yet fully understood [31].

Lastly, basic and clinical studies performed in the late 1950s and mid-1960s, suggested that L-carnitine could contrast the effect of TH in both animals and humans [32, 33]. A more recent study on cultures of fibroblasts, hepatic and neuronal cells showed that L-carnitine dose-dependently inhibits the entry into the cell nuclei of T_4 and T_3 but not their binding to the cognate nuclear receptors, and caused a minimal additional decrease in whole-cell uptake of both hormones [34], suggesting that L-carnitine could be a naturally occurring inhibitor of TH action.

Herein, we show a significant inhibition of MCT8 by dexamethasone, buspirone, desethylamiodarone, dronedarone. Our findings can help to explain the side effects related to the use of these drugs.

Materials And Methods

Plasmid

Human MCT8 gene cloned in pcDNA3 expression vector used for transient transfection of COS-7 was kindly provided by Dr. Dumitrescu, University of Chicago, Chicago, Illinois (USA).

Drugs and concentrations

3,5,3'-Triiodo-L-thyronine, hydrocortisone, BSP, buspirone, dronedarone, amiodarone, desethylamiodarone, carbamazepine and valproic acid were obtained from Sigma-Aldrich; prednisone, prednisolone, dexamethasone acetate, L-carnitine were obtained from Caelo Caesar & Loretz. As solvents to prepare the stock solutions were used dimethylsulphoxide (DMSO) for Hydrocortisone, Amiodarone, Desethylamiodarone, Prednisone and Prednisolone; water for Bromsulphthalein (BSP), Valproic Acid, Buspirone and L-Carnitine; ethanol for Carbamazepine and Dexamethasone; 0.1M sodium hydroxide for T_3 .

The non-bile acid organic anion BSP, a potent inhibitor of thyroid hormone transport on the basis of structural similarity [35], was used as positive control at 50 μ M concentration.

All the drugs and the concentrations used for the study are shown in Table 1.

Cell culture and transient transfection

COS-7 cells were cultured in 24-well dishes with Dulbecco's modified Eagle's medium supplemented with 10% FBS, 100 IU/mL penicillin, 100 mg/mL streptomycin, 2.5 mg/mL fungizone, and 1 mmol/L sodium pyruvate. For transient expression of hMCT8 gene, COS-7 cells were seeded at the concentration of 100,000 cells/well and subsequently were transfected in duplicate with 1 mg empty vector or pcDNA3-hMCT8 using Attractene Reagent (Qiagen).

Tripan blue dye exclusion

48 hours after transfection, cells were incubated for 1 hour or O/N at 37°C with different concentrations of the drugs to be tested (Table 1). Tripan blue dye exclusion test was used to determine the number of viable cells present in cell suspension as described previously [36].

T_3 uptake assay

In order to determine the kinetic characteristics of T_3 uptake in COS-7 cells, a time course experiment was performed. Forty-eight hours after transfection, cells were washed with Dulbecco's Phosphate Buffered Saline (D-PBS) medium and incubated for 5–60 min at 37°C with 150,000 cpm T_3 labelled with 125 I ([125 I]- T_3 PerkinElmer) in D-PBS medium plus 0.1% BSA (assay buffer). After incubation cells were quickly washed twice with ice-cold assay buffer and lysed with 0.1 M NaOH. Cell-associated radioactivity was

determined by counting the cell lysates in γ -counter and data were given in counts per minute (cpm). All subsequent uptake experiments were performed after 15 min incubation with radiolabelled T_3 .

Effect of drugs on T_3 uptake

To study the effect of the selected drugs on MCT8-mediated T_3 uptake, transfected cells were incubated at 37°C for 15' with 150,000 cpm [125 I] T_3 in the absence (basal value of uptake) or in the presence of different concentrations of each drug as indicated in Table 1. After incubation, cells were washed, lysed, and the accumulated radioactivity was counted as described above. Results were expressed as percent T_3 uptake with respect to value obtained in basal condition. Net uptake levels corrected for background observed in cells transfected with empty vector were used.

In order to obtain the IC_{50} values for each substance significantly diminishing [125 I] T_3 uptake, data were fitted by nonlinear regression using the GraphPad version 6.0.

Determination of the mode of inhibition

Transfected cells were incubated for 3 min with [125 I] T_3 different concentrations in the absence or presence of the IC_{50} of the different substances to test [13]. After two washes with D-PBS cells were lysed in 0.1 M NaOH, and cell-associated radioactivity was measured with a γ -counter.

To describe the T_3 transport across the cell membrane a Michaelis-Menten mechanism was assumed and kinetics data were plotted according to Eadie-Hofstee diagram. In this linearization of the Michaelis-Menten equation, the intersection with the ordinate axis represents the maximum velocity value (V_{max}), while the slope of the curve is the negative value of the Michaelis-Menten constant ($-K_m$). Parallel lines in the presence of the inhibitor imply that K_m is constant and there is no competition with substrate, while the decrease of V_{max} imply a reduction in active molecule concentration and a noncompetitive mode of inhibition.

Efflux of radiolabeled T_3

To determine the kinetics of T_3 efflux, COS-7 cells transiently transfected as above were preincubated for 30 minutes at 37°C with 150,000 cpm [125 I] T_3 diluted in assay buffer, washed with D-PBS medium plus 0.1% BSA and incubated for 1–30 minutes with fresh assay medium with or without 10 μ M unlabeled T_3 . After incubation, both medium and cells were collected and [125 I] T_3 associated radioactivity was quantified.

Effect of drugs on T_3 efflux

To evaluate the ability of the drugs having an effect on T_3 uptake to inhibit T_3 efflux, after 30 minutes preincubation with labelled T_3 , hydrocortisone, amiodarone, dronedarone, desethylamiodarone, dexamethasone, buspirone, L-carnitine, T_3 and BSP were added at their respective maximum inhibition

concentration for additional 15 minutes. Hereinafter the radioactive buffer was replaced with fresh assay buffer for 0-2-4-10-15 minutes, all supernatants were collected, cells were lysed after the last supernatant removal and medium and cell associated radioactivity was determined. Total radioactivity at the beginning of efflux (100%) was calculated by adding the radioactivity found in each supernatant to that of finally lysed cells. Data of T_3 efflux were expressed as percent of cell retained T_3 with respect to time.

Statistics

Variables were preliminarily tested for normal distribution and expressed as mean \pm SE. To evaluate the difference in T_3 uptake in presence of the different drugs tested compared to the basal uptake values, a one-way ANOVA with Dunnett's T_3 post-hoc test for multiple comparisons was used. A linear-mixed analysis for repeated measures was performed to evaluate the effect of different drugs tested on T_3 efflux across all time points. T_3 efflux values were log-transformed to correct for heterogeneity of variance and to produce approximately normally distributed data. Means were compared using Fisher's Least Square Difference post-hoc test. The two-tailed level of statistical significance was set at P less than 0.05. All statistical analyses were performed by using SPSS (IBM SPSS Statistics, version 25).

Results

Tripan blue dye exclusion

The selected drugs were tested at all concentrations showed in Table 1 and, also after an O/N incubation of transfected COS-7 cells with the substances, no cytotoxic effect was detected with a cell viability greater than 95% (Supplemental Table 1). Therefore all the concentrations were used in the following experiments.

Time course of T_3 accumulation in COS-7 cells

hMCT8 transfected COS-7 cells showed nearly 2.5-fold higher [125 I] T_3 uptake compared to cells transfected with empty vector.

In the former, [125 I] T_3 uptake increased linearly between 0 and 5 minutes. After 15 minutes incubation the plateau of the uptake was not yet achieved, and the curve continued to grow even if not linearly as in the first 5 minutes (Fig. 2). Inhibition studies were then performed at 15 min after coapplying to [125 I] T_3 increasing concentrations of each drug.

Effect of drugs on MCT8-mediated [125 I] T_3 uptake

Glucocorticoids

Exposure to each glucocorticoid gave different results. Hydrocortisone dose-dependently inhibited T_3 uptake. Low doses of hydrocortisone did not inhibit T_3 uptake. A non-significant inhibition with 100 μ M

(-21%; $P = 0.979$) and a significant inhibition at 500 and 1000 μM (-74% and - 77%, respectively) (Fig. 3A) was observed. Dexamethasone had a more pronounced effect on MCT8 transport. In particular, a non-significant inhibition of T_3 uptake was obtained at 0.1 and 1 μM (-21 and - 29%, respectively), while 10 and 100 μM significantly reduced T_3 uptake (-54 and - 65%, respectively; all $P < 0.001$) (Fig. 3A). Conversely, prednisone and prednisolone did not show inhibitory potential in the statistical modeling used in this study (Fig. 3A).

Antiarrhythmic drugs

Among the antiarrhythmic agents tested, amiodarone caused a significant reduction of MCT8-mediated T_3 uptake only at the highest concentrations: -43% at 50 μM and - 66% at 100 μM . This effect was weaker than that produced by desethylamiodarone and dronedarone. In fact, desethylamiodarone significantly inhibited T_3 uptake in COS-7 transfected with hMCT8 starting from 10 μM (-36.5%), and determined a 72% of inhibition at 100 μM ; dronedarone inhibited T_3 uptake in the same cells from - 38% at 1 μM concentration to -86% inhibition at 100 μM (Fig. 3B).

Anxiolytic drug

Buspirone resulted as a potent inhibitor, significantly reducing the uptake of T_3 in hMCT8-transfected cells even at low concentrations. After exposure to buspirone (1 to 125 μM), the reduction of MCT8-mediated T_3 uptake ranged between - 38 and - 86% (Fig. 3C).

Anticonvulsant agents

As shown in Fig. 3D, both carbamazepine and valproic acid did not show any inhibitory effect on MCT8-mediated T_3 uptake at all concentrations used (all $P > 0.05$).

L-carnitine

The widespread dietary supplement L-carnitine behaved as weak inhibitor of MCT8. In fact, when coapplied for 15 min, it significantly inhibited T_3 uptake in hMCT8 transfected COS-7 only at very high concentrations, that are 500 mM and 1 M (all $P < 0.001$). At these concentrations baseline T_3 uptake was reduced by 51% and 68%, respectively (Fig. 3E).

Mode of inhibition

For each inhibiting drug the concentration determining 50% reduction of T_3 uptake (IC_{50}) was evaluated, results are reported in Table 2. Thereafter, in order to determine the mode of inhibition, we titrated substrate concentrations in the presence or not of drugs (*i.e.* 635 μM hydrocortisone, 48.5 μM dexamethasone, 63 μM amiodarone, 53.1 μM desethylamiodarone, 35.7 μM buspirone, 503 mM L-carnitine or 50 μM BSP). Plotting the data according to Eadie-Hofstee diagram, for all drugs tested we observed an essentially parallel shift of the curve in the presence of each inhibitor (K_m values remained constant in the order to 10^{-2} μM , while a decrease of V_{max} values was observed), suggesting a predominantly noncompetitive mode of inhibition of MCT8-mediated T_3 transport by these substances

(Fig. 4A-G). For the positive control BSP a competitive mode of inhibition was observed as also described in literature (Fig. 4H).

Effects of drugs on T₃ efflux

Since MCT8 functions also as a T₃ exporter [7], we tested whether the drugs able to reduce T₃ uptake could inhibit T₃ efflux as well. None of these drugs affected T₃ release from cells transfected with hMCT8 at the respective maximal inhibitory concentration (Fig. 5A-B).

Discussion

Interfering effects of drugs on MCT8 function have been so far poorly investigated and proved for only few compounds.

Aiming at identifying new classes of inhibitors, we screened *in vitro* the effect on MCT8 of a set of common drugs, and we found inhibition of the T₃ transport by some of these drugs and at certain concentrations.

Among the glucocorticoids tested, hydrocortisone significantly inhibited T₃ uptake by MCT8 only at high concentrations, *i.e.* 500 and 1000 µM, with an IC₅₀ of 635 µM. These values are far from the therapeutic plasma levels of the drug reached during the replacement therapy of adrenal insufficiency, the disease where it is usually used. In fact, after an oral dose of 20 mg/day of hydrocortisone the plasma levels of drug are around 8 µM [37], making unlikely an *in vivo* effect on MCT8, unless hydrocortisone can exert partial inhibition in some organs during long-term treatment or can accumulate in some tissue. Concentrations lower than those having an inhibiting effect in our *in vitro* system are also reached after intravenous administration of 100–300 mg/day used for anti-inflammatory purposes [38]. Prednisone and prednisolone were devoid of inhibitory potential on T₃ uptake by MCT8 in the statistical modeling used in this study. In contrast, dexamethasone behaved as a more potent inhibitor. Indeed, it could significantly inhibit T₃ transport into hMCT8-transfected COS-7 cells already at 10 µM concentration and showed an IC₅₀ of 48.5 µM. These findings make more likely an *in vivo* effect of the drug on MCT8.

Dexamethasone is a synthetic glucocorticoid used as long-term treatment of adults affected by congenital adrenal hyperplasia (CAH). Furthermore, it can also be used throughout pregnancy in the management of fetuses with CAH or administered during early pregnancy if a fetal CAH is suspected. Finally, it is indicated for the management of women at risk of preterm birth, since it promotes the rapid maturation of underdeveloped organs in the fetus [25, 26]. However, the use of this drug as that of other synthetic glucocorticoids, especially if administered as multiple courses, has been associated with adverse effect on the developing brain [38–40]. A study performed on mice showed reduced plasticity and a lower number of proliferating cells in the hippocampus, impaired cognitive function and reduced lifespan after a single dose of dexamethasone administered prenatally [41]. In humans, dose-dependent associations are found between repeated administration of dexamethasone prenatally and the incidence

of periventricular leukomalacia and neurodevelopmental abnormalities [42], attention deficits in children of two years of age [43], and poorer cognitive performance at 6 years of age [44]. All these evidences, together with the potential teratogenicity and long-term effects of prenatal treatment with dexamethasone, prompted the authors of the 2018 Endocrine Society's guidelines on CAH to do not recommend specific prenatal treatment protocols and to advise that clinicians continue to regard prenatal therapy with dexamethasone as experimental [45]. In contrast, evaluation of treatment with hydrocortisone of premature infants found no difference in cognitive function, incidence of cerebral palsy, motor function or occurrence of brain lesions on MRI [46]. These differences are usually explained by pharmacological differences between dexamethasone and hydrocortisone [17], but our study shows that other factors might contribute to these disparities such as the different effect of these drugs on MCT8-mediated T_3 transport. The adverse cerebral effects of synthetic glucocorticoids are attributed to impaired neuronal glucose uptake, decreased excitability, atrophy of dendrites, altered development of myelin producing oligodendrocytes, and perturbation of important structures involved in axonal transport evoked by genomic and not genomic action of these drugs [17–18]. However, the findings herein reported suggest that some of the detrimental neurological effects of dexamethasone can also be due or enhanced by the reduced TH entry into brain caused by the inhibition of MCT8-mediated transport; indeed, it is well known the association between hypothyroidism and neurological and psychiatric impairment [47–48]. As the mode of inhibition in our cellular system was noncompetitive, dexamethasone likewise blocks the substrate channel of MCT8 through direct binding to the transporter, a propriety that it share only partially with hydrocortisone, but not with the other glucocorticoids tested in the present study.

The antiarrhythmic agents amiodarone, its main metabolite DEA, and the derivative dronedarone were the other drugs tested in our heterologous expression system for interfering effects on MCT8. Only few previous studies in rat cell lines, performed before the identification of the different classes of TH transporters, have showed some inhibiting effects of amiodarone on TH transport into hepatocytes and pituitary cells [49–50]. In COS-7 cells transiently transfected, we found that amiodarone caused a significant reduction of T_3 uptake by MCT8 only at 50 and 100 μ M with an inhibition of 44 and 68 %, respectively of baseline values. Its metabolite DEA produced a stronger effect, reducing the T_3 uptake already at 10 μ M. Serum levels of amiodarone in humans receiving long-term treatment vary from 1 to 6 μ M [51], which are therefore lower than the inhibiting concentrations observed in the present study. However, amiodarone is concentrated in certain tissues over time, and it may conceivably accumulate at levels that would allow it to work as inhibitor of MCT8 transport. Indeed, autopsy studies have revealed that liver, lung, and heart preferentially accumulate high concentrations of the drug, containing between 2,600 mg/kg and 200 mg/kg of amiodarone and desethylamiodarone, while brain and thyroid gland accumulate 60 mg/kg [51]. In light of the foregoing, amiodarone might produce variable *in vivo* effects on different tissues based on varying concentrations within tissue and the type of TH transmembrane transporters expressed. The mode of inhibition we found for both compounds was noncompetitive, therefore it might be postulated that amiodarone and its metabolite bind to MCT8 and hamper T_3 uptake by steric hindrance or conformational changes, but this hypothesis remains to be proven. Dronedarone

exhibited the strongest effect inhibiting T_3 uptake by MCT8 starting from 1 μ M concentration, and also in a noncompetitive manner. *In vivo*, relatively high doses of dronedarone (100 mg/kg/day) are required to reduce plasma concentrations of TSH, T_3 , and T_4 significantly [27]. Although it has little effect on plasma thyroid hormones, our results suggest that it can alter TH status in some tissues by reducing transmembrane transport mediated by MCT8.

Similarly to dronedarone and dexamethasone, in our *in vitro* system the psychotropic agent buspirone acted as a potent inhibitor of MCT8, significantly reducing the uptake of T_3 by MCT8 even at low concentrations. Therapeutic plasma levels of buspirone range between 1,6 – 4,8 μ M, values which are in the same order of our inhibiting concentration in *vitro*. Buspirone is an anxiolytic medication that acts as a partial serotonin 1A receptor agonist. It is also used in major depression in augmentation with SSRIs or tricyclic antidepressants, showing, however, to be less effective than T_3 in improving the response of clinical non responders [29, 30]. It is well known that thyroid hormones influence mood [28]. Since previous studies have suggested a possible inhibition of antidepressant DMI on T_3 uptake in neurons [12] our data show that it should be safe to avoid the association of these two drugs for a possible additive effect on T_3 transport in brain.

In contrast with buspirone and the above described drugs, the anticonvulsants carbamazepine and valproic acid did not show any inhibitory effect on MCT8-mediated T_3 uptake at all concentrations used. We decided to test these drugs because TFTs abnormalities have been reported during their use [31]. Specifically, a reduction in serum T_4 and less frequently in T_3 , associated with unchanged TSH levels, have been found in children using carbamazepine. Although the results are controversial, studies report alteration in TFTs also during treatment with valproic acid, being subclinical hypothyroidism the more frequent feature found [31]. Carbamazepine seems to increase the metabolism of the TH in the liver and the peripheral conversion of T_4 to T_3 ; furthermore it competitively binds to thyroxin-binding globulin [52–54]. For valproic acid the mechanisms are only hypothesized and not clearly established [55]. Our study seems to exclude for both drugs an inhibitory effect on MCT8 function as the pathogenetic mechanism of the associated TFTs.

Lastly, we tested the effect of L-carnitine on T_3 transport mediated by MCT8. In our *in vitro* system L-carnitine behaved as a weak inhibitor of T_3 transport by MCT8. In fact, T_3 uptake was reduced only at very high concentrations of L-carnitine (500 mM and 1 M), values not reached even during oral administration of pharmacological doses of L-carnitine (*i.e.* in the order of grams) [56, 57]. Plasma carnitine concentrations are approximately 0.05 mM, which are 20-to 100-times less than in tissues, being the highest tissue concentration around 100 mM [58]. Therefore, it can be hypothesized that L-carnitine could exert its inhibitory effect on transmembrane cell transporters other than MCT8 and, as previously shown, mainly on a still unknown nuclear membrane TH transporter [34].

Interestingly, none of the substances able to reduce uptake by MCT8 affected efflux of T_3 . The current mechanism of membrane traversing for TH by MCT8 is that of the two banana – shaped 6 –

transmembrane –helix bundle tilting around a central TH-interacting cavity, with alternating extra-and intracellular accessibility [10]. Starting from this model, the possible explanation for the lack of effect on efflux of the tested compounds may be the conformational change induced by the binding of these drugs to some sites of TH-interacting cavity, that while blocking extracellular TH entrance lead to an enlargement of the intracellular entrance. A such phenomenon has been already described associated to mutations of *MCT8* gene [10].

In conclusion in this study we show *in vitro* a novel effect of some commonly used drugs, which is the inhibition of T₃ transport into cells mediated by MCT8. Specifically, hydrocortisone, amiodarone and L-carnitine modestly inhibit T₃ uptake whereas dexamethasone, desethylamiodarone, dronedarone, and buspirone behave as potent inhibitors. Treatment with these substances may interfere with T₃ delivery and action in the tissues where MCT8 represents the main mediator of transmembrane passage of TH. Perturbation of tissue TH concentrations may explain some of the side effects related to the use of these drugs.

Declarations

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Author contributions

C.D.C. designed, performed and interpreted the data and wrote the manuscript. G.D.M., E.F, A.D., design of the work and interpretation of data. P.A interpreted the data and revised the manuscript. P.F. analyzed the data. S.B, P.V, M.T revised the manuscript.

Compliance with ethical standards

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this article.

Disclosure Statement

The authors have nothing to disclose.

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Tables

Table 1

Tested substances and used concentrations.

CLASS	DRUG	CONCENTRATION
GLUCOCORTICOIDS	Hydrocortisone	0,1 - 1 - 10 - 50 - 100 - 500 - 1000 [μ M]
	Dexamethasone	0,1 - 1 - 10 - 100 [μ M]
	Prednisone	10 - 100 - 1000 [μ M]
	Prednisolone	10 - 100 - 1000 [μ M]
ANTIARRHYTHMIC DRUGS	Amiodarone	0,1 - 1 - 10 - 50 - 100 [μ M]
	Desethylamiodarone	1 - 10 - 50 - 100 [μ M]
	Dronedarone	0,1 - 1 - 50 - 100 [μ M]
ANXIOLYTIC DRUG	Buspirone	0,1 - 1 - 10 - 25 - 50 - 125 [μ M]
ANTICONVULSANT AGENTS	Carbamazepine	25- 50 – 100 - 1000 [μ M]
	Valproic acid	50 - 100 [μ M]
DIETARY SUPPLEMENT	L- Carnitine	0,01 - 1 - 100 - 500 - 1000 mM
POSITIVE CONTROL	BSP	50 [μ M]

Table 2

IC₅₀ of substances inhibiting T₃ uptake mediated by MCT8.

Class	Drug	IC ₅₀
GLUCOCORTICOIDS	Hydrocortisone	635 [μM]
	Dexamethasone	48,5 [μM]
ANTIARRHYTHMIC DRUGS	Amiodarone	63 [μM]
	Desethylamiodarone	53,1 [μM]
	Dronedarone	35,7 [μM]
ANXIOLYTIC DRUG	Buspirone	30 [μM]
DIETARY SUPPLEMENT	L- Carnitine	503 [mM]

Figures

Figure 1

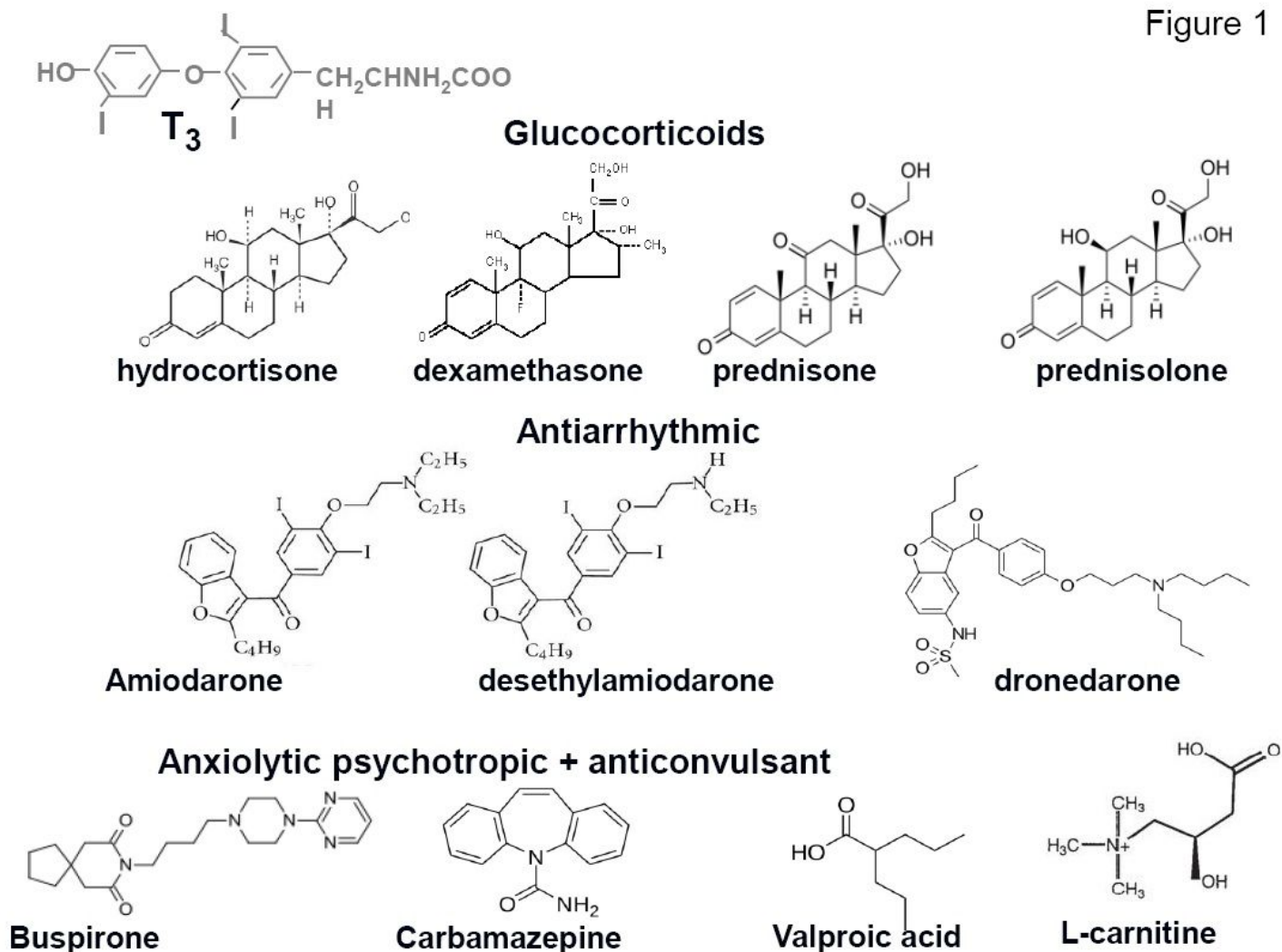


Figure 1

Chemical structure of the tested compounds and of T3.

Figure 2

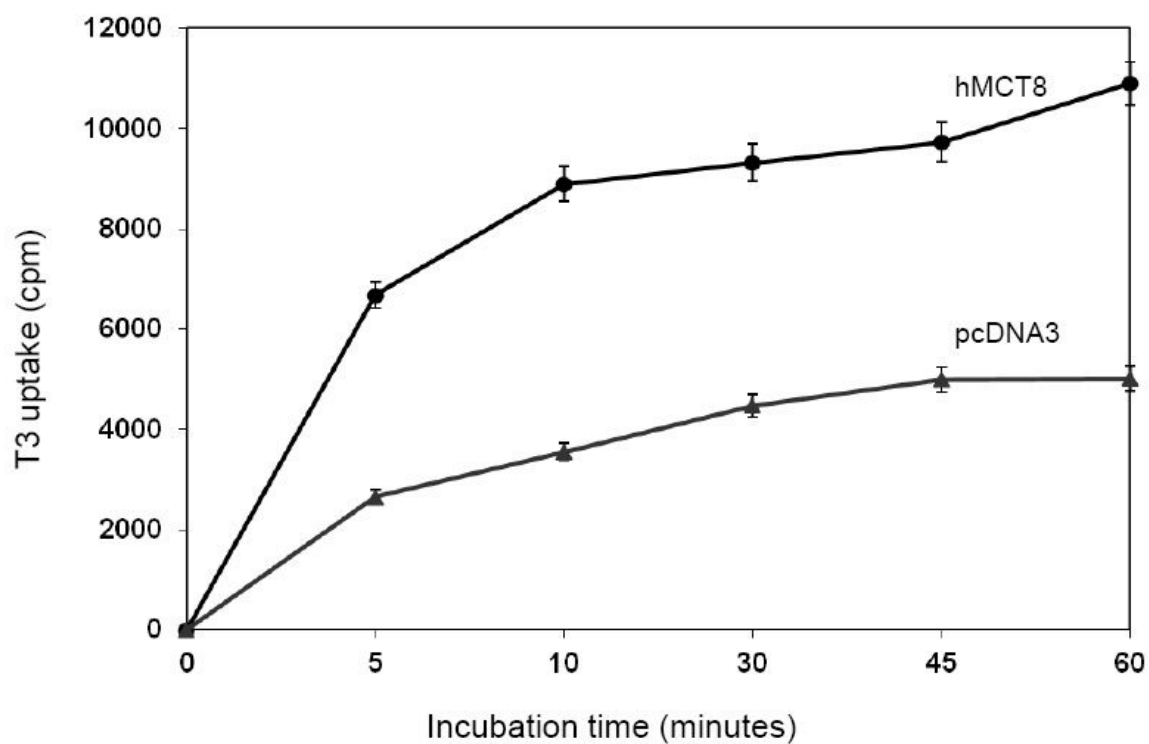


Figure 2

Time course of [125I]T3 accumulation in COS-7 cells transfected with the empty vector (pcDNA3) and with hMCT8. Data are expressed as mean \pm SE. All experiments were performed in triplicate.

Figure 3A

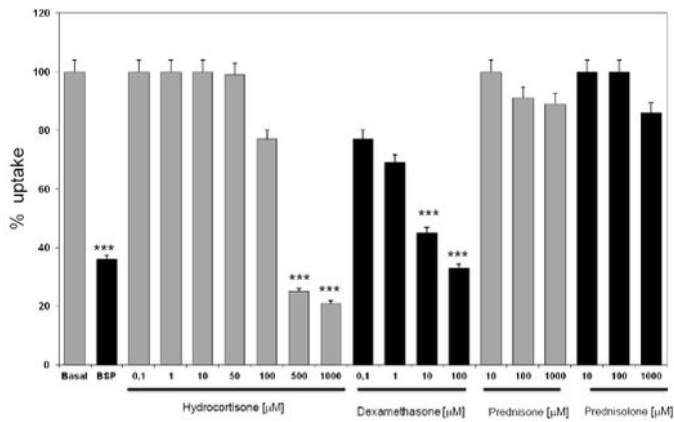


Figure 3B

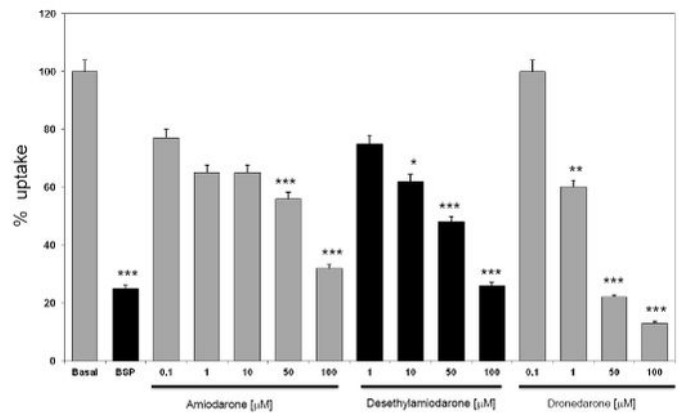


Figure 3C

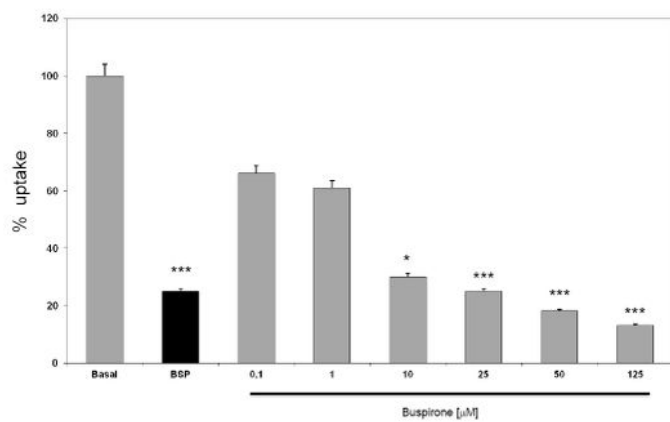


Figure 3D

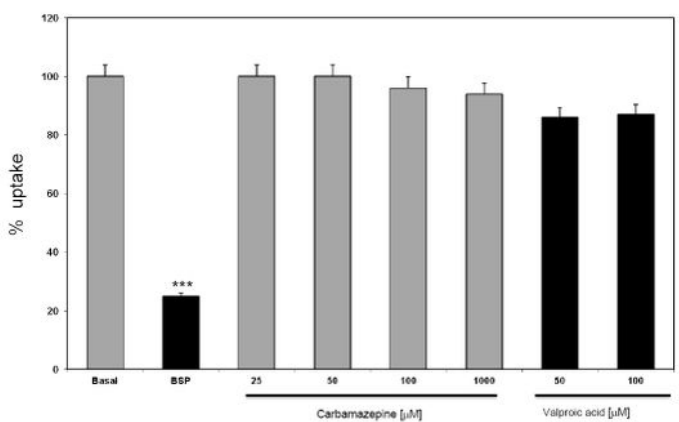


Figure 3E

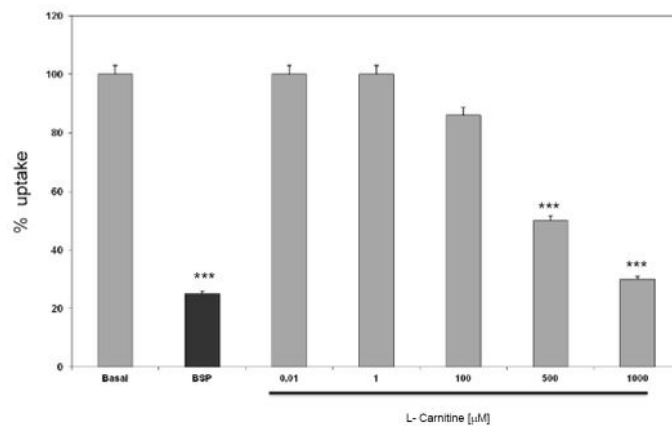


Figure 3

Effects of the selected drugs on MCT8 mediated $[^{125}\text{I}]\text{T}_3$ uptake. (A) Glucocorticoids, (B) Antiarrhythmic drugs, (C) Anxiolytic drug (Buspirone), (D) Anticonvulsant agents, (E) L-carnitine. Results are the means \pm SE of three independent experiments and are expressed as percent $[^{125}\text{I}]\text{T}_3$ uptake with respect to values obtained without inhibitor (basal condition, set to 100%). $[^{125}\text{I}]\text{T}_3$ uptake of cells transfected with empty vector was considered as background and subtracted. * $P < 0.05$; ** $P < 0.001$ *** $P < 0.0001$.

Figure 4A

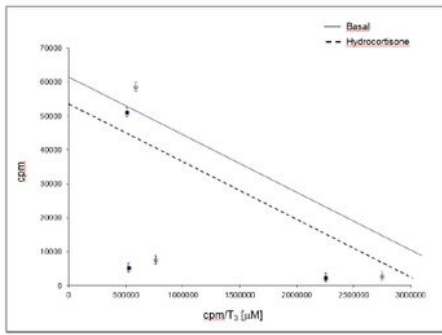


Figure 4B

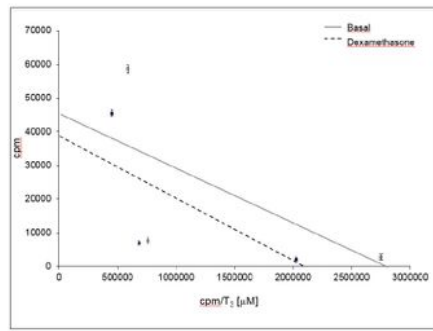


Figure 4C

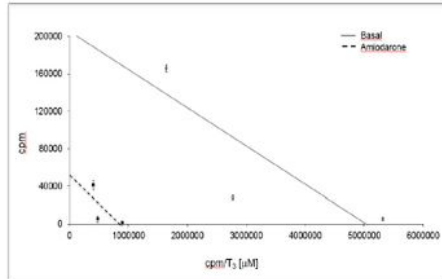


Figure 4D

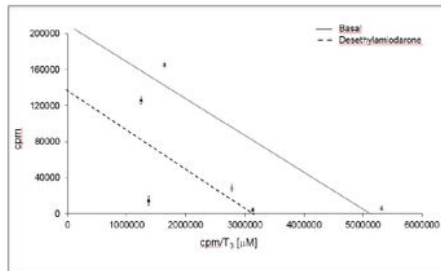


Figure 4E

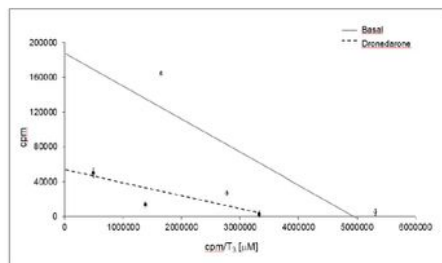


Figure 4F

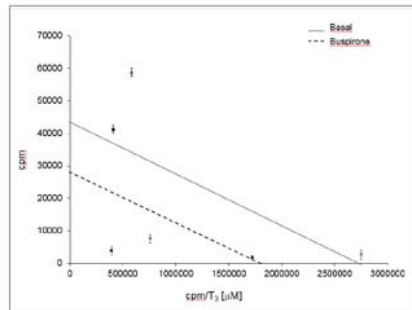


Figure 4G

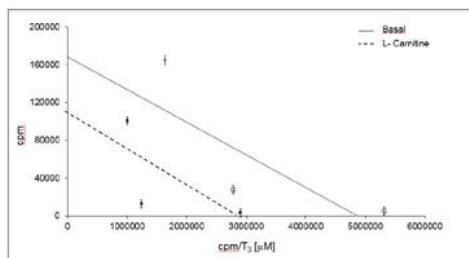


Figure 4H

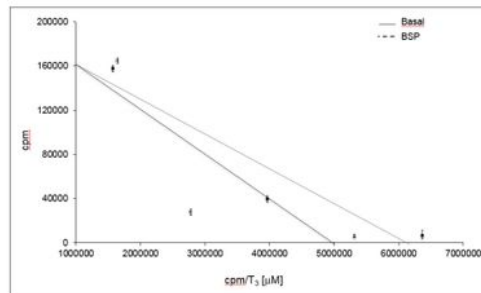


Figure 4

Eadie-Hofstee plot of labeled T3 uptake in the presence of the concentration of each substance corresponding to the IC₅₀ value. (A) Hydrocortisone, (B) Dexamethasone, (C) Amiodarone, (D) Desethylamiodarone, (E) Dronedrone, (F) Buspirone, (G) L- Carnitine, (H) BSP positive control.

Figure 5A

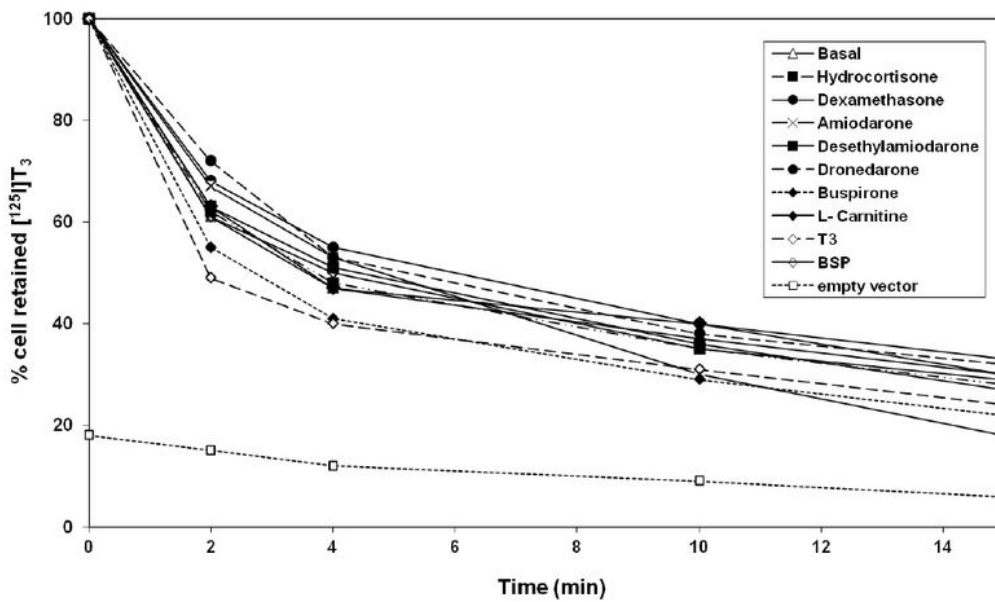


Figure 5B

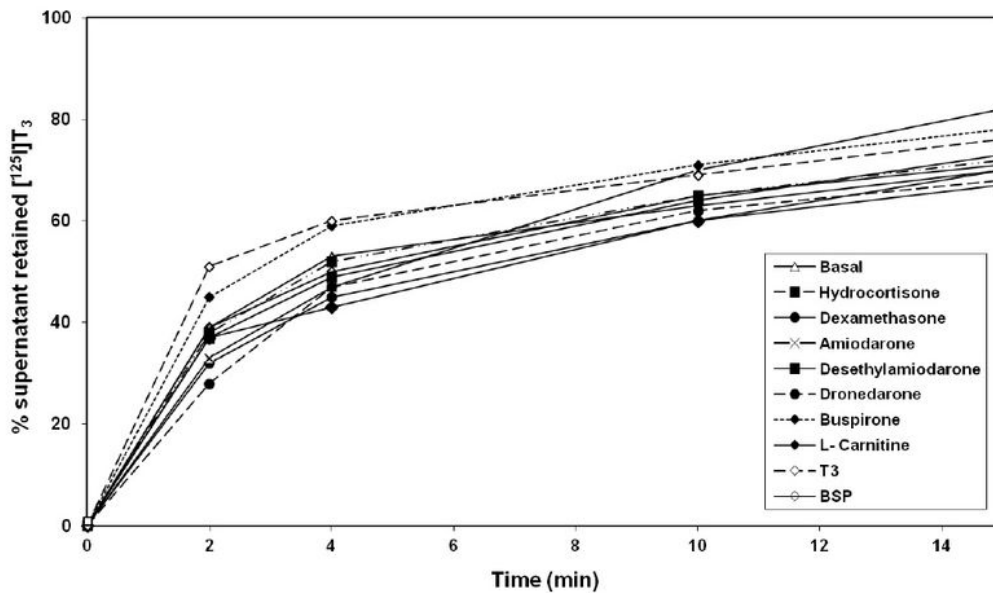


Figure 5

Effect of drugs on $[^{125}\text{I}]\text{T}_3$ efflux. Efflux was measured before (basal) and after exposure to drugs having an effect on T_3 uptake. Results are the means of three independent experiments and are expressed as percent of cell (A) and supernatant (B) retained $[^{125}\text{I}]\text{T}_3$ with respect to time.

Supplementary Files

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