BioFactors 19 (2003) 171–176
IOS Press

Short Communication

Dietary flavonoids and iodine metabolism

Janny P. Schröder-van der Elst[a,*], Jan W.A. Smit[a], Hans A. Romijn[a] and Daan van der Heide[b]
[a]*Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands*
[b]*Human and Animal Physiology Group, Wageningen University, Wageningen, The Netherlands*

Abstract. Flavonoids have inhibiting effects on the proliferation of cancer cells, including thyroidal ones. In the treatment of thyroid cancer the uptake of iodide is essential. Flavonoids are known to interfere with iodide organification *in vitro*, and to cause goiter. The influence of flavonoids on iodine metabolism was studied in a human thyroid cancer cell line (FTC-133) transfected with the human sodium/iodide transporter (NIS). All flavonoids inhibited growth, and iodide uptake was decreased in most cells. NIS mRNA expression was affected during the early hours after treatment, indicating that these flavonoids can act on NIS. Pendrin mRNA expression did not change after treatment. Only myricetin increased iodide uptake. Apeginin, luteolin, kaempferol and F21388 increased the efflux of iodide, leading to a decreased retention of iodide. Instead myricetin increased the retention of iodide; this could be of use in the radioiodide treatment of thyroid cancer.

Keywords: Flavonoids, thyroid cancer, iodide uptake

1. Introduction

Dietary flavonoids are plant pigments that are present in our daily food, for example in plants, fruit, grains, wine, tea and nuts. They are hydroxylated polyphenols and can be divided into different categories: flavonols (myricetin, quercetin, morin, kaempferol), flavonones (naringenin), flavones (luteolin, apigenin) and isoflavones (genistein). Flavonoids interfere with many enzyme systems in the body including thyroid hormone status [9]. The uptake of iodide is an essential step in thyroid hormone synthesis and is clinically important in the treatment of thyroid carcinoma with radioiodide. The uptake is mediated by the sodium/iodide symporter [Na^+/I^- symporter (NIS)]. Iodide transport is a process that is characterized by its dependency upon energy, temperature, sodium and especially TSH [1]. The NIS gene has been cloned [3,13], and antibodies have been used to characterize molecular form and distribution of the NIS protein [10].

Figure 1 shows a very simple scheme of the thyroid cell, depicting processes known to be involved in influx and efflux of iodide. At the left hand side of the cell, at the plasma membrane, the uptake of iodide is mediated by NIS, which is driven by the sodium concentration gradient over the cell membrane. This process consumes no energy, but excess Na^+ has to be pumped out of the cell by the Na^+/K^+-ATPase, counteracted by the influx of K^+, at the expense of ATP. The right hand (apical) side, is where the colloid

*Address for correspondence: Janny P. Schröder-van der Elst, Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands. Tel.: +31 71 5262173; Fax: +31 71 5248136; E-mail: J.P.van_der_Elst@LUMC.nl.

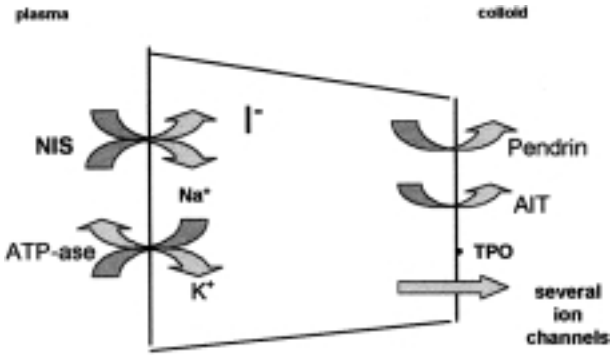

Fig. 1. A simple scheme of a thyroid cell. Iodide influx is mediated by the sodium/iodide symporter (NIS); iodide efflux can be mediated by pendrin, the apical iodide transporter (AIT) and ion (chloride) channels. Iodide organification occurs by thyroidperoxidase (TPO).

is stored, which is where the efflux of iodide for organification occurs. It is known that pendrin plays a role, perhaps just a minor one, while the recently cloned apical iodide transporter (AIT) might play a major role [11]. Apart from these transporters, several ion channels may be involved. The enzyme thyroid peroxidase (TPO) receives iodide directly for coupling onto thyroglobulin; which one of the transporters is responsible for this is not yet clear.

Flavonoids interfere with processes in the thyroid. In the 1980s, Gaitan et al. [6] found that flavonoids could exert goitrogenic effects *in vivo* and inhibit the organification of iodide *in vitro*. In 1996, Sartelet et al. [12] found in an iodine deficient area in Guinea, (West Africa), that the prevalence of goiter was not explained by iodine deficiency alone. The daily diet of the population mainly consisted of pearl millet, especially *Digitaria exilis* (*fonio*). They found that this millet contains large amounts of apigenin and luteolin. They used extracts from this millet and their results showed that apigenin and luteolin are the most potent flavonoids capable of depressing both organification and secretory level of reconstituted thyroid follicles *in vitro*, similar to methylthiouracil [12]. The structure of luteolin is strikingly similar to the synthetic flavonoid F21388(3-methyl-4′,6-dihydroxy-3′,5′-dibromoflavone). Pregnant rats were treated with F21388 for 14 days. In Fig. 2, the radioiodide uptake is shown in the thyroids of dams and their fetusses, 24 hours after an injection with radioactive iodide. Iodide uptake, this is iodide influx and organification together, is essentially decreased after treatment with F21388, as well as for the maternal as for the fetal thyroid.

Cell culture studies show that flavonoids may have beneficial properties in the treatment of carcinoma by inhibiting cancer proliferation. Different mechanisms are involved. Flavonoids interfere with many enzymes, such as protein kinase C (PKC), many of the large number of protein tyrosine kinases(PTK) and DNA topoisomerase I and II, which are crucial to cellular function, gene expression and proliferation [9]. Yin et al. [16,17] showed that kaempferol, biochanin A, chrysin, genistein, apigenin and luteolin inhibit proliferation of thyroid follicular carcinoma cells. This was also found to be the case for papillary and anaplastic carcinoma cell lines.

Can these flavonoids be new compounds in the treatment of thyroid cancer? If these flavonoids can be used in the treatment of thyroid cancer, it is important to know whether this effect on cell proliferation is not accompanied by negative effects on iodide kinetics, leading to a lowered efficacy of radioiodide treatment. This was investigated using the *in vitro* model, FTC-133, a human follicular thyroid cancer cell line. This cell line does not take up iodide and does not contain TPO, so there is no organification of iodide to thyroglobulin. In this cell line iodide uptake increased considerably upon hNIS introduction

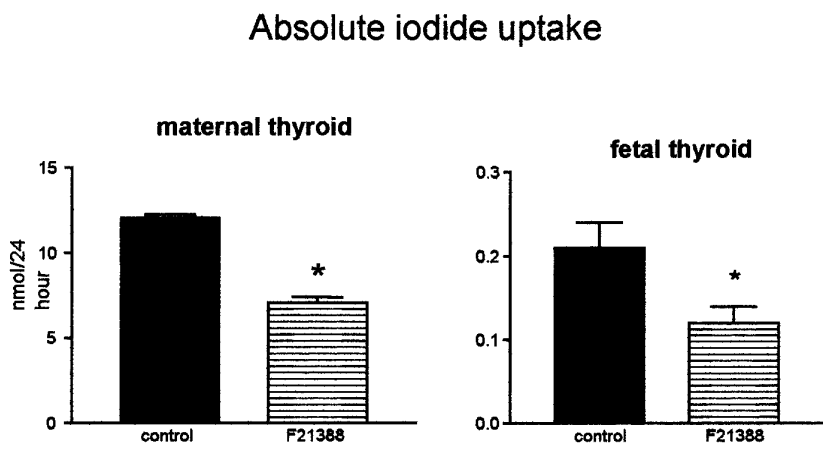

Fig. 2. The absolute iodide uptake by thyroids of dams and their fetusses. At day 20 of pregnancy, dams ($n = 6$ for each group) were injected i.v. with 10 μCi $Na^{125}I$. After 24 hours the thyroids of dams and fetusses (mean is $n = 10$ per dam) were taken out and counted. Absolute amounts of iodide was calculated using the % uptake and the specific activity of the 24 hours urine. Treatment was 20 μmol F21388/rat/day for 14 days. Values are mean $\pm$ SD; *At least $p < 0.05$.

Table 1
The effects of 50 μM flavonoids on iodide uptake

Control	100 ± 4
Myricetin	142 ± 8
Quercetin	$73 \pm 4^*$
Morin	92 ± 5
Naringenin	$70 \pm 3^*$
Apigenin	$37 \pm 2^*$
Kaempferol	$35 \pm 3^*$
Luteolin	$10 \pm 1^*$
Genistein	$33 \pm 2^*$
Fisetin	$54 \pm 4^*$
F21388	$14 \pm 2^*$

Iodide uptake was measured after 4 days of incubation with 50 μM flavonoids. Iodide uptake is expressed per μg DNA and depicted as % of control, so this is corrected for the inhibition of growth. Only morin did not affect iodide uptake. All other flavonoids decreased iodide uptake significantly. In contrast, only myricetin increased iodide uptake. We found the same when cells were incubated for 2 or 6 days with the flavonoids. $n = 6$; * at least $p < 0.05$.

through transfection [14,15]. When perchlorate was added, the uptake was inhibited for more than 90%, implying that the functionality of the hNIS transporter was exactly the same as *in vivo*. In this, the model differs from that of Gaitan and Sartelet, in which organification was the end-point of measurement, and Gaitan and Sartelet related this to TPO activity [6,12]. In our model, the influx and efflux of iodide is actual influx and efflux of inorganic iodide. These cells were incubated with flavonoids.

Incubation for 2, 4 and 6 days with 50 μM flavonoids showed similar effects on proliferation as

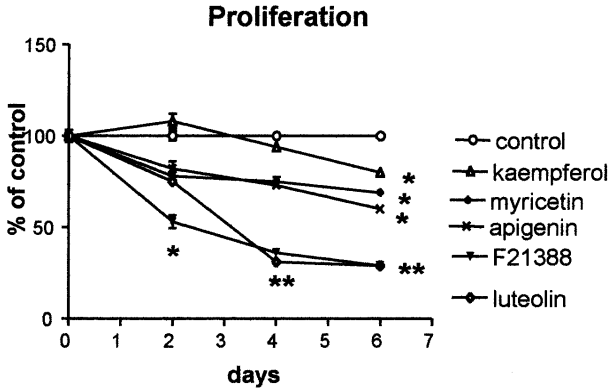

Fig. 3. Effects of flavonoids on proliferation of hNIS transfected FTC-133 cell line. DNA was measured and expressed as % of control of the same day. Control cells showed an increase in DNA, from 14 μg DNA per well (day 2) to 34 μg DNA (day 6). Kaempferol, myricetin, apigenin, F21388 and luteolin inhibited the increase in DNA.

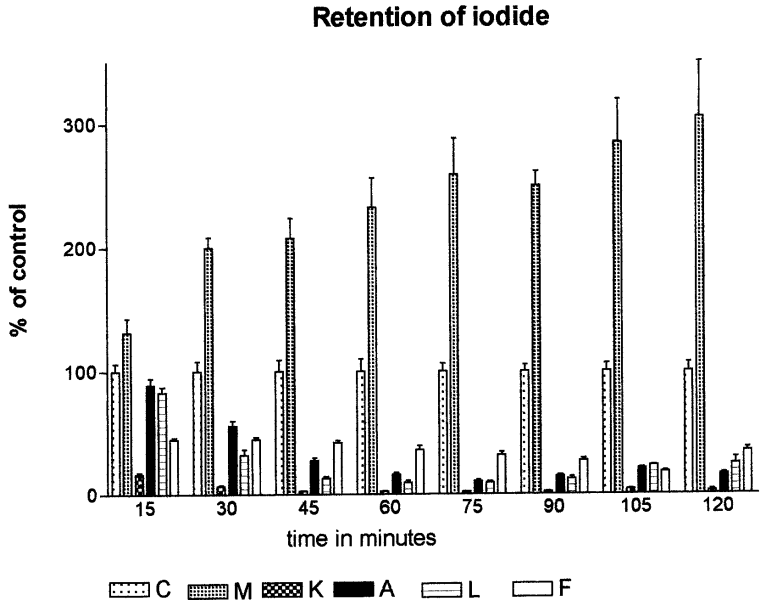

Fig. 4. The retention of iodide in the hNIS transfected FTC-133 cell line. After 4 days of incubation in the presence of 50 μM of myricetin (M), kaempferol (K), apigenin (A), luteolin (L) and F21388, cells were preloaded with radioiodide for 1 hour. Cells were washed. After several time points as indicated, amounts of radioiodide present in the cells were calculated. Compared to control, A, K, F and L decreased cellular iodide retention significantly. In contrast, M increased the retention significantly. $N = 4$ for each time point.

described as by Yin [16]. Figure 3 shows that kaempferol, myricetin, apigenin, F21388 and luteolin, inhibited the increase in DNA. When iodide uptake was measured at these time points, most flavonoids decreased this iodide uptake significantly. Table 1 shows this for day 4. Only morin did not affect iodide uptake; in contrast, only myricetin increased iodide uptake. The same was found when cells were incubated for 2 or 6 days with these flavonoids. To evaluate if this inhibition of iodide uptake could be caused by effects on influx (NIS), expression levels of hNIS mRNA were measured at different time points. Kaempferol, apigenin, luteolin and F21388 decreased NIS mRNA expression significantly after 1,

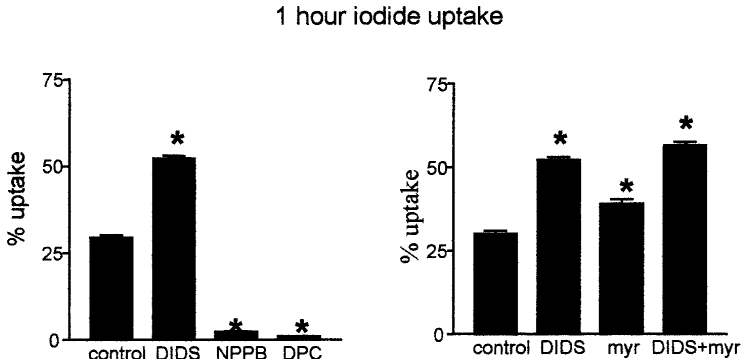

Fig. 5. The effects of DIDS, NPPB and DPC on iodide uptake of the hNIS transfected cell line. 50 μM DIDS, DPC or NPPB was added together with $Na^{125}I$ in the incubation medium. After 1 hour radioiodide content of the cells was measured. DIDS increased iodide uptake; DPC and NPPB decreased iodide uptake. In the experiment on the right hand side, cells were incubated with 50 μM DIDS and/or 50 μM myricetin ($n = 8$). Values are mean $\pm$ SD. *At least $p < 0.05$.

and 2 days of incubation. After 4 days NIS mRNA expression is normalized, except for cells treated with kaempferol. This means that only for a short period of exposure to flavonoids, the diminished amount of NIS can cause a diminished influx of iodide. Myricetin did not change NIS mRNA expression. None of the flavonoids used, affected pendrin mRNA expression. After 4 days of incubation in the presence of 50 μM of flavonoids, the efflux of iodide was measured. This was done by measuring the rate of disappearance of radioactive iodide from the cells, preloaded with radioiodide, ^{125}I, during 2 hours. Compared to control, apigenin, kaempferol, F21388 and luteolin increased the efflux, leading to a decrease in retention of iodide in the cells (Fig. 4), which is indeed not the effect wanted in radioiodide treatment of thyroid cancer. In contrast, only myricetin decreased the efflux, leading to a threefold increase in iodide retention. Together with the decrease of cell growth, this could mean that myricetin may be benificial for the efficacy of radioiodide treatment of thyroid carcinoma.

As incubation with flavonoids did not affect pendrin mRNA expression, other mechanisms, transporters or ion channels could be involved in the changes in efflux. For example, Cl^--channels that are also capable to transport iodide. One of these types of Cl^--channels is CFTR: cystic fibrosis transmembrane conductor regulator. A mutation in the gene of CFTR leads to defective Cl^--secretion and -absorption across the epithelium. This mutation causes cystic fibrosis [2]. In patients this can be accompanied by goiter and sub-clinical hypothyroidism [4]. Devuyst et al. [5] showed that CFTR is expressed in the human thyroid. In our cell line, CFTR was also present. Illek and Fischer [8] showed that certain flavonoids (genistein, apigenin, kaempferol and quercetin) stimulate Cl^--conductance by activating CFTR of human airway epithelium *in vitro* and *in vivo*. The increase was dose-dependent; N-phenylantranilic acid (DPC), a Cl^-- channel inhibitor blocked the Cl^--conductance.

There is a large family of Cl^--channels. The Cl^--channels can be blocked by several drugs, such as DIDS, (4,4-diisothiocyano-2, 2′-stilbene disulphonate), NPPB [5-nitro-2-(3-phenylpropylamino)benzoic acid] and DPC. Iodide uptake was measured in this hNIS-transfected cell line in the presence of these Cl^--channel inhibitors. Figure 5 shows that the effects of DIDS, NPPB and DPC on iodide uptake were very different. DIDS increased iodide uptake in a time and dose dependent manner. DPC and NPPB decreased iodide uptake dramatically as was shown earlier [7]. This was attributed to a direct effect on NIS, nevertheless, additional effects on efflux by Cl^--channels cannot be excluded. The increase by DIDS is most likely to be caused by efflux inhibition. The combination of myricetin and DIDS seems to have additional effects on iodide uptake.

In conclusion: Most flavonoids inhibit the growth of thyroid cancer cells, but they also decrease radioiodide uptake. Of the investigated flavonoids, only myricetin is the flavonoid of choice for treatment of thyroid cancer because it inhibits growth and it increases iodide uptake, thus increasing the retention of iodide. But for the population in general, with apigenin and luteolin or other flavonoids in mega-dose intake, it has to be taken into account that in normal thyroids the iodide uptake is lowered and goiter will develop.

References

[1] N. Carrasco, Thyroid hormone synthesis: thyroid iodide transport: the Na^+/I^- symporter (NIS), in: *Werner & Ingbar. The Thyroid, a fundamental and clinical text*, (8th ed.), L.E. Braverman and R.D. Utiger, eds, Lippincott Williams & Wilkins, Philadelphia, USA, 2000.

[2] S.H. Cheng, R.J. Gregory, J. Marshall, S. Paul, D.W. Souza, G.A. White, C.R. O'Riordan and A.E. Smith, Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis, *Cell* **63** (1990), 827–834.

[3] G. Dai, O. Levy and N. Carrasco, Cloning and characterization of the thyroid iodide transporter, *Nature* **379** (1996), 458–460.

[4] F. Deluca, F. Trimarchi, C. Sferlazzas, S. Benvenga, G. Constante, C. Mami, G. DiPasquale and G. Magazzu, Thyroid function in children with cystic fibrosis, *European Journal of Pediatrics* **138** (1982), 327–330.

[5] O. Devuyst, P.E. Golstein, M.V. Sanches, K. Piontek, P.D. Wilson, W.B. Guggino, J.E. Dumont and R. Beauwens, Expression of CFTR in human and bovine thyroid epithelium, *American Journal of Physiology* **272** (1977), C1299–C1308.

[6] E. Gaitan, R.H. Lindsay, R.D. Reichert, S.H. Ingbar, C. Cooksey, J. Legan, E.F. Meydrech, J. Hill and K. Kubota, Antithyroid and goitrogenic effects of millet: Role of C-glycosylflavones, *Journal of Clinical Endocrinology and Metabolism* **68** (1989), 707–714.

[7] C. Gerard, V. Rigot and C. Penel, Chloride channel blockers inhibit the Na^+/I^- symporter in thyroid follicles in culture, *Biochemical Biophysical Research Communications* **204** (1994), 1265–1271.

[8] B. Illek and H. Fischer, Flavonoids stimulate Cl conductance of human airway epithelium *in vitro* and *in vivo*, *American Journal of Physiology 275* (*Lung Cellular Molecular Physiology*) (1998), L902–L910.

[9] E. Middleton, C. Kandaswami and T.C. Theoharides, The effects of plant flavonoids on cells: implications for inflammation, heart disease, and cancer, *Pharmacological Reviews* **52** (2000), 673–751.

[10] A. Paire, F. Bernier-Valentin, S. Selmi-Ruby and B. Rousset, Characterization of the rat thyroid iodide transporter using anti-peptide antibodies, Relationship between its expression and activity, *Journal of Biological Chemistry* **272** (1997), 18245–18249.

[11] A. Rodriguez, B. Perron, L. Lacroix, B. Caillou, G. Leblanc, M. Schlumberger, J. Bidart and T. Pourcher, Identification and characterization of a putative human iodide transporter located at the apical membrane of thyrocytes, *Journal of Clinical Endocrinology and Metabolism* **87** (2002), 3500–3503.

[12] H. Sartelet, S. Serghat, A. Lobstein, Y. Ingenbleek, R. Anton, E. Petitfrère, G. Aguie-Aguie, L. Martiny and B. Haye, Flavonoids extracted from fonio millet (Digitaria exilis) reveal potent antithyroid properties, *Nutrition* **12** (1996), 100–106.

[13] P.A. Smanik, Q. Liu, T.L. Furminger, K. Ryu, S. Xing, E.L. Mazzaferri and S.M. Jhiang, Cloning of the human sodium iodide symporter, *Biochemical and Biophysical Research Communications* **226** (1996), 339–345.

[14] J.W.A. Smit, J.P. Schröder-van der Elst, M. Karperien, I. Que, G. van der Pluijm, B. Goslings, J.A. Romijn and D. van der Heide, Reestablishment of *in vitro* and *in vivo* iodide uptake by transfection of the human sodium symporter (hNIS) in a hNIS defective human thyroid carcinoma cell line, *Thyroid* **10** (2000), 939–943.

[15] J.W.A. Smit, J.P. Schröder-van der Elst, M. Karperien, I. Que, J.A. Romijn and D. van der Heide, Expression of the human sodium/iodide symporter (hNIS) in xenotransported human thyroid carcinoma, *Experimental and Clinical Endocrinology and Diabetes* **109** (2001), 52–55.

[16] F. Yin, A.E. Giuliano and A.J. Van Herle, Growth inhibitory effects of flavonoids in human thyroid cancer cell lines, *Thyroid* **9** (1999), 369–376.

[17] F. Yin, A.E. Giuliano and A.J. Van Herle, Signal pathway involved in apigenin inhibition of growth and induction of apoptosis of human anaplastic thyroid cancer cell lines (ARO), *Anticancer Research* **19** (1999), 4297–4303.