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COMMENTARY

INDUCTION OF CYTOTOXICITY IN MELANOMA CELLS THROUGH INHIBITION OF CATECHOL-*O*-METHYLTRANSFERASE

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Catechol-O-methyltransferase and S-adenosylmethionine

COMT† (EC 2.1.1.6) is a magnesium-requiring enzyme catalyzing the transfer of a methyl group to phenolic compounds with a catecholic (i.e. *ortho*-dihydroxyphenolic) structure [1]. During this reaction, an "active methionine," SAM, serves as a methyl group source. The transfer of the methyl group from SAM converts this methyl donor to SAH, a known COMT inhibitor. The regeneration of SAM includes SAH hydrolysis, the methylation of L-homocysteine to L-methionine by methionine synthase, and the condensation of L-methionine with ATP. The intracellular concentrations of SAM and SAH, often called the "methylation ratio," appear to play an important control part in COMT activity [2, 3].

Soluble and membrane-bound enzyme; localization

COMT is an intracellular enzyme that exists in vertebrates in two isozymic forms: a soluble form (S-COMT) and a membrane-bound form (MB-COMT) [4, 5]. The soluble form has two variants that differ in their molecular weight: COMT I has been shown to have a molecular weight of 24,000–26,000, and for COMT II a much higher molecular weight of around 48,000–66,000 has been reported [6, 7]. Although the molecular weight ratio suggests that COMT II could be a COMT I dimer, the two enzymes do not appear to be interconvertible upon their purification and isolation. Moreover, they have been shown to have distinct biochemical properties, and COMT II is not present in several tissues, including brain [8].

The relative proportion of S-COMT and MB-COMT in different tissues varies considerably. Although the activity of MB-COMT constitutes, in most cases, only a minor part of the total cellular COMT activity [4, 5], the K_m values of this isozyme show higher affinity for substrates. As a consequence of the differences in the K_m values for S- and MB-COMT, one can expect that the substrate concentration will greatly influence the metabolic contribution of the isoenzymic forms to overall *O*-methylation of substrate. At a low concentration of catechols, *O*-methylation by the MB-COMT (low K_m) will predominate, and only when this enzyme becomes saturated does the contribution of the S-COMT (high K_m) become significant [8].

COMT is a ubiquitous enzyme in nature, occurring in various plants, microorganisms, invertebrates and vertebrates. In mammals, the highest activities are in liver and kidney. COMT is also present in heart, lung, muscles, adipose tissue, blood cells and reproductive organs. In a recent study employing a highly specific antiserum prepared against recombinant rat COMT [9], the presence of a high amount of COMT protein was confirmed in many organs including the liver (hepatocytes), kidneys (proximal and distal tubules and collecting ducts), spleen (white pulp), and in the epithelial cells of the gastrointestinal tract. In addition, salivary glands and some of the pancreatic cells were strongly immunoreactive for COMT. In the brain the most intensely stained structures were the ventricular ependymal cells and the cells of the choroid plexus. As far as the eye structure is concerned, the ciliary body and the retinal ganglion cell layer with the nerve fiber layer expressed strong immuno-reactivity. The wide distribution of COMT in different anatomic structures supports the idea of the protective role of this enzyme against potentially toxic catecholic compounds of different origin.

COMT genetics

Recent progress in molecular biological techniques has opened many avenues for obtaining more information on the structure and regulation of the COMT gene and on the structural and functional features of the protein itself. In 1990, Salminen and co-workers described the cloning of COMT cDNA from rat liver [10], and 1 year later Lundström *et al.* [11] and Bertocci *et al.* [12] reported on the cloning of cDNA from human placenta and a hepatoma cell line. These experiments made it possible to determine the primary structure of the S-COMT protein. It was shown that the isozyme contained 221 amino acids with 80% overlap for the COMT preparations from rats and humans. Moreover, it was found that the DNA sequence preceding the S-COMT open reading frame in both species coded for a 43 (rat) and 50 (human) amino acid extension to the amino end

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† Abbreviations: COMT, catechol-*O*-methyltransferase; MB-COMT, membrane-bound COMT; S-COMT, soluble COMT; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; L-DOPA, L-3,4-dihydroxyphenylalanine; 5,6DHI, 5,6-dihydroxyindole; and 5,6DHI2C, 5,6-dihydroxyindole-2-carboxylic acid.

of the polypeptide. The extension contained at that terminus a stretch of 18–24 hydrophobic amino acids, suggesting that this part of the molecule might play a role in transmembrane anchoring or as a signal for membrane insertion of the MB-COMT [11]. A hydrophobic segment of 21 amino acids was also found for the deduced structure of COMT from the human hepatoma cell line [12].

As far as the secondary and tertiary structures of COMT are concerned, these have been described recently with a 2.0 Å resolution sensitivity [13]. Furthermore, the authors showed that the co-enzyme binding domain was very similar to that of SAM-dependent DNA-methylase. As one would expect, the substrate binding parts of the two enzymes were different.

In 1986, Brahe and co-workers reported the assignment of a COMT locus to chromosome 22 [14]. In the following years, utilization of primers derived from known cDNA sequences made it possible to analyze the genomic localization and organization in more detail [10, 11]. The cloning experiments provided evidence that there was only one COMT gene in humans and other mammals. Winqvist *et al.* [15] localized the human COMT gene in band q11.2 of the 22nd chromosome. In the next year, Grossman and co-workers came to a similar conclusion [16]. They mapped the chromosomal position of the COMT gene to 22q11.1 → q11.2, a region to which several anonymous DNA sequences, but thus far no structural genes have been assigned. A detailed structure of the human COMT gene with its promoter regions has been described recently [17].

The level of COMT activity in different tissues appears to be under the influence of a common genetic polymorphism [18]. Some studies have shown that the gene frequencies of the alleles for low and high COMT activity in red blood cells (which correlates with the COMT activity in other tissues) are nearly equal in a randomly selected population sample. Thus, approximately 25% of individuals are homozygous for the traits of low and 25% for high COMT activity, and roughly 50% are heterozygous and express intermediate enzymic activity. Theoretically, these interindividual differences in COMT activity can have important pharmacological and (patho)physiological consequences.

Variations in COMT activities have been described in connection with certain diseases. Low erythrocyte COMT activities were reported for patients with major or recurrent and bipolar depression [19], and a low *in vivo* COMT activity was suggested in relation to Huntington's disease because low levels of *O*-methylated dopamine metabolites were found in the midcaudate and midputamen [20]. In contrast, in children with Down's syndrome, significantly increased COMT activity has been observed [21].

There are only a few reports dealing with the ethnic differences in COMT activity. Earlier work by Rivera-Calimlim and Reilly [22] showed a higher COMT activity in erythrocytes of the oriental population when compared with white individuals. The recently published comparison of the COMT activity from red blood cells in African-Americans and in the white population revealed that the first group had significantly higher COMT activity [23]. In a similar study from Norway [24], the Saami (Lapps) population was found to express significantly lower COMT activity in erythrocytes than Caucasians do. Whether all these ethnic differences have some implications on the metabolism in general and on pigment metabolism specifically remains to be seen.

COMT as a detoxifying enzyme

O-Methylation catalyzed by COMT is an important step in the biotransformation (inactivation, detoxification) of different endogenous and exogenous catechols. The enzyme itself has a broad substrate specificity. Its physiologic substrates include L-DOPA and catecholamines, but also different acids and alcohols of a catecholic nature. In pigment-producing cells (melanocytes), 5,6DHI and 5,6DHI2C belong to the important COMT substrates. *O*-Methylation of catecholestrogens provides a major route for the rapid metabolic clearance of these steroids. One of the very interesting but rare examples of non-catecholic COMT substrates is L-ascorbic acid [25], whose 3-keto-L-gulonic acid form resembles the catechol configuration. Whether this particular methylation process has any (patho)physiological significance is not known.

The recognition of the important role of COMT in the inactivation of catecholic neurotransmitters has contributed significantly to the prompting of COMT investigation in different areas. Since the discovery of dopaminergic cell loss accompanied by the decrease of striatal dopamine in Parkinson's disease, and the introduction of L-DOPA as an effective treatment for this serious illness, there has been increasing attention directed towards COMT functioning and the possibilities of inhibiting COMT activity to prolong the half-life and, thus, the therapeutic effect of L-DOPA [for review, see Refs. 26–28].

One of the characteristic reactions of catecholic compounds is their dehydrogenation (oxidation) to *o*-quinones, which can be again reconverted to the original catechols. This redox cycling process is known to generate potentially hazardous free radicals that can cause damage to DNA and other macromolecules. The existence of the redox cycling has been suggested in the case of catechol oestrogens that are considered as potential carcinogens [29]. In a recently published study where a group of hamsters was treated with oestradiol, all the animals developed kidney tumours [30]. When oestradiol was given together with quercetin (COMT inhibitor), the number of large tumour nodules and the incidence of abdominal metastases increased significantly. The authors ascribe the augmented tumorigenic effect to 4-hydroxyoestradiol, which was protected by quercetin against methylation and which may undergo metabolic redox cycling with generation of potentially mutagenic radicals.

Quercetin itself is a flavonoid containing a catechol moiety that can be methylated rapidly by COMT. When assayed by various bacterial mutagenicity tests, this substance was found to be highly mutagenic [31]. Surprisingly, in the animal experiments no carcinogenic potential could be detected. The reason for the loss of the carcinogenic behaviour of quercetin in the animal experiments is apparently the rapid *O*-methylation of this compound by COMT. The methylation rate of quercetin was shown to be higher by up to three orders of magnitude than those of endogenous catechols, such as catechol oestrogens and catecholamines [32].

The above-mentioned data suggest that the catecholic

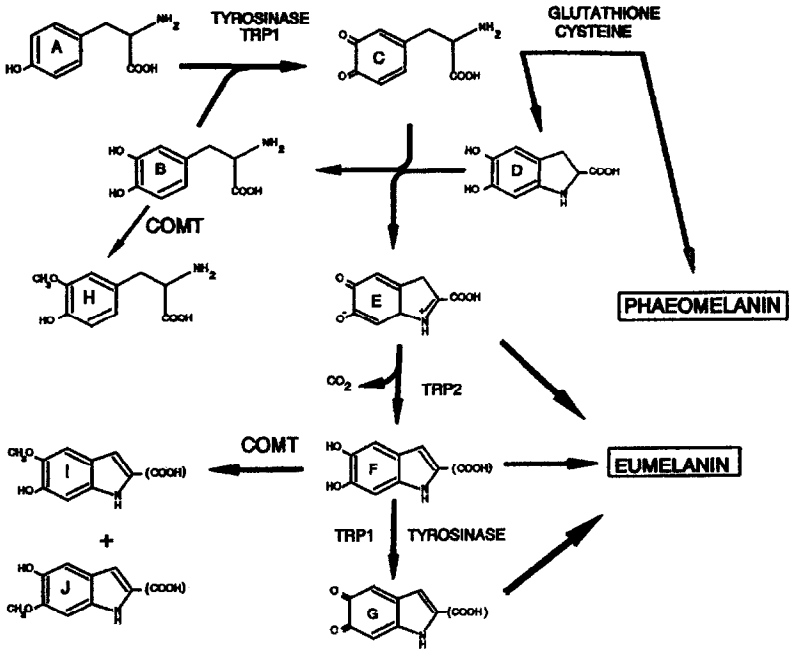

Fig. 1. Scheme of the melanogenic pathway leading to the synthesis of phaeomelanin (yellow/red pigment) or eumelanin (brown/black pigment). (A) L-tyrosine, (B) L-DOPA, (C) dopaquinone, (D) leucodopachrome, (E) dopachrome, (F) 5,6-dihydroxyindole (-2-carboxylic acid), (G) indole-5,6-quinone(-2-carboxylic acid), (H) 3-*O*-methoxytyrosine, (I) 6-hydroxy-5-methoxyindole(-2-carboxylic acid), and (J) 5-hydroxy-6-methoxyindole(-2-carboxylic acid). TRP 1 and 2: tyrosinase-related proteins 1 and 2.

moieties of many compounds can be held responsible for their mutagenic activities because these structures can easily undergo metabolic redox cycling, causing the generation of potentially mutagenic free radicals. The *O*-methylation of catechols may efficiently decrease the potential for redox cycling and thus protect the cells against internal free radical damage. Moreover, the increased lipophilicity of *O*-methyl derivatives might speed up their transmembrane transport out of the cell. From this point of view, COMT can be seen as a protective intracellular enzyme, and that view is reflected by its wide distribution in the tissues. The importance of this protective mechanism for the cell also can be inferred from the fact that the cell must provide energy of one ATP (for SAM synthesis) for one "simple methylation reaction. As shown below, in pigment-forming cells, COMT enzyme has acquired an additional protective task.

COMT in the skin and its role in melanogenesis

Knowing that COMT has a wide tissue distribution, it is not surprising that COMT activity is also detectable in skin. As far back as 1964, Bamshad and co-workers [33] demonstrated the presence of COMT activity in human, rat, mouse and frog skin. They also examined COMT activities in normally pigmented and apigmented (vitiliginous) Caucasian skin, but they were not able to find any differences. In a subsequent work [34], Bamshad focused on the measurement of COMT activity in epidermis and dermis separated from each other by keratoderm. He showed that COMT activity was much higher in epidermis than in dermis.

In a later study by Le Poole *et al.* [35], COMT activity was determined in the epidermis of vitiligo patients and control individuals. The authors found that epidermal homogenates from vitiligo lesions expressed higher activity than those from healthy controls. Whether these differences are relevant with regard to pathogenic mechanisms is still unclear. Melanocytes make up only 3–5% of the epidermal cell population. In keratinocytes, COMT can be involved in the catabolism of epinephrine. Schallreuter and co-workers [36] recently reported that epidermal keratinocytes are able to synthesize epinephrine. However, it is not completely clear whether the epinephrine formation and the presence of COMT in one cell should only be regarded as an expression of a humoral autocrine system of keratinocytes or if it has another function. Nevertheless, increased levels of *O*-methylated catecholamine metabolites, homovanillic acid and vanilmandelic acid have been reported during the active phase of vitiligo, and the production of catecholamines in the skin was suggested to be involved in the etiopathogenesis of this pigment disease [37].

Melanocytes are responsible for pigment (melanin) production in several organs, including skin. Melanin is synthesized intracellularly in small particles called melanosomes. Melanosomes are highly organized spherical or ellipsoidal particles consisting of a membrane and structural matrix on which melanin is deposited. Each melanocyte transfers the melanosomes to surrounding keratinocytes. The degree of melanization, and the size and distribution of melanosomes in the whole epidermis determine the constitutional and facultative skin colour.

Pigment production (melanogenesis) represents a sequence of reactions starting from tyrosine and leading to

the biosynthesis of the polymer melanin (see Fig. 1). Tyrosine is normally metabolized via *p*-hydroxyphenylpyruvate to link up with the intermediary metabolism of the cell. Melanogenesis is thus a special branch of tyrosine metabolism dependent on the presence of the enzyme tyrosinase that bears responsibility for the catalysis of (at least) two initial reactions. These two steps include hydroxylation of L-tyrosine to L-DOPA, which is instantly converted to dopaquinone—a point from which the biosynthesis of two types of melanin, eumelanin and phaeomelanin, proceeds separately. Eumelanin synthesis includes formation of indolic compounds (5,6DHI and 5,6DH12C), which can be easily oxidized to corresponding quinones. From the toxicologic point of view, the polymerization of reactive melanogenic intermediates can be considered as a detoxification process. Moreover, the compartmentalization of melanin production in melanosomes has probably developed to protect the rest of the cellular environment against the reactive intermediates.

In spite of the above-mentioned cellular precautions, some of the unpolymerized molecules escape from the melanogenic compartments, and they may endanger important cellular structures (including DNA) by different mechanisms [38, 39]. Hence, the additional protective role of COMT in pigment-producing cells is based on the methylation of the *o*-dihydroxyphenolic and *o*-dihydroxyindolic melanogens inside the melanocytes. Our studies on the melanoma cell cultures brought clear evidence that intracellular methylation of indolic compounds indeed takes place [40]. In 1963, Axelrod and Lerner [41] showed that the indoles 5,6DHI and 5,6DHI2C could serve as substrates for COMT from rat liver and hamster melanoma, and the suggestion was made that *O*-methylation of L-DOPA (see Fig. 1) could serve as a regulatory step in melanogenesis.

Several reports have described changes in COMT activities during the oestrous cycle and pregnancy, which are under the control of steroid hormones such as progesterone and oestradiol [42–44]. Subjects with elevated serum oestrogen concentrations, as during pregnancy, may develop spotty cutaneous hyperpigmentations (melasma). Therefore it is interesting to know that human melanocytes contain oestrogen receptors that may mediate the effect of these hormones on tyrosinase activity and melanin production [45, 46].

The mentioned data indicate that neurotransmitters and steroid hormones, which might affect COMT activity, can also influence skin pigmentation. Next to this, the ethnic differences described for COMT activities [22–24] also suggest that the enzyme may be involved in the regulation of melanogenesis. Additional research is necessary to shed more light on this point. It is important to note, however, that in pigment cells tyrosinase may potentially threaten its own cells by production of reactive intermediates, whereas the role of COMT is a protective one. These considerations have prompted us to explore the role of COMT in the pigment cells more closely.

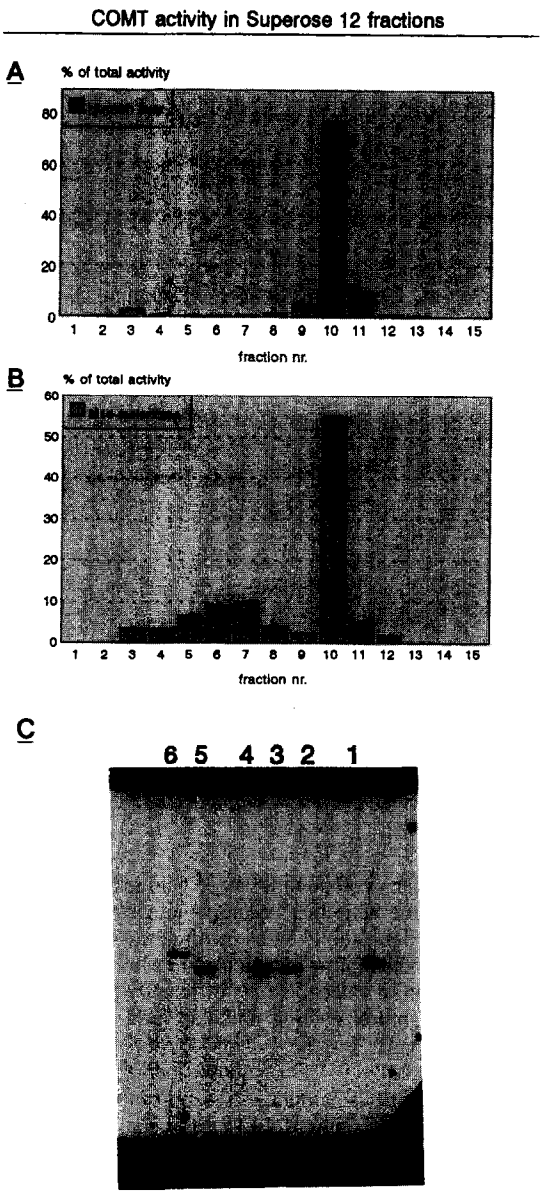

Fig. 2. COMT purification using fast-protein liquid chromatography with gel filtration on a Superose 12 column and western blotting. The distribution of total COMT activity as measured in the fractions from a crude homogenate of human liver (A) and M14-melanoma cell extract (B) is shown. Western blotting of several purified samples (C) was carried out by Dr. C. Tilgmann. The samples were run on a 12.5% SDS-polyacrylamide gel, and the proteins were transported to PVDF-membranes. COMT proteins were detected with a guinea pig anti-COMT antiserum [50]. In lane 1, purified human S-COMT (Dr. C. Tilgmann) with a molecular weight of 26,000 can be seen. This S-COMT band is also present in the chromatofocussing (lane 2, human liver, and lane 3, M14-melanoma) and anion exchange chromatography fractions (lane 4, human liver) containing optimal COMT activity. In the Superose 12 fractions from the M14-melanoma (B), S-COMT was present in fractions 9–11 (lane 5) and MB-COMT (30 kDa) in fractions 5–7 (lane 6).

Studies on COMT in relation to malignant melanoma, activity measurement and localization

To study the role of COMT in melanogenesis and especially its detoxifying potential in malignant melanoma cells, the enzymatic conversion of 5,6DHI2C in melanocytes and melanoma cells was investigated using high-performance liquid chromatography combined with fluorometric detection [47]. It was shown that COMT from bovine liver had a lower K_m value for 5,6DHI2C (44 μM) than for 3,4-dihydroxybenzoic acid (152 μM).

In the case of COMT from cultured M14 melanoma cells, an even higher affinity for 5,6DHI2C (K_m value: 3 μM) was found. During these experiments the natural occurrence of the *O*-methylated products (5H6MI2C and 6H5MI2C) in melanoma cell extracts was confirmed.

In another set of experiments, we were able to show that the subcellular localization of COMT activity in Greene's hamster melanomas differed from that of tyrosinase, and therefore a regulatory role of COMT in melanosomes or coated vesicles (preventing the polymerization of melanin precursors by *O*-methylation) was considered unlikely [48]. Using gradient ultracentrifugation and immunoblotting, we were also able to demonstrate that a substantial part of COMT activity in cultured human melanocytes and melanoma cells (IGR-1) was represented by the membrane-bound enzyme. Furthermore, fractionation of cells by gradient ultracentrifugation and electron microscopic investigation indicated that the MB-COMT was preferentially localized in the endoplasmic reticulum [49, 50].

According to our experience, in (malignant) melanocytes a considerable amount of MB-COMT can be found. Fast-protein liquid chromatographic purification of human liver COMT and COMT from a human melanoma cell line UCLA-SO-M14 (M14) was performed using either gel filtration on Superose 12, ion-exchange chromatography (mono Q) or chromatofocussing (mono P). In the case of human liver, mainly one peak of activity was found when any of the mentioned purifications was used, whereas in M14 and cultured melanocytes the enzymatic activity was also detected in other fractions. Figure 2 demonstrates activity measurements in the Superose 12 fractions obtained from human liver and M14 cell extract. In the first case, 92% of all activity was recovered in fractions 9–11. In M14 cells, only 62% of total activity was found in these fractions, whereas remaining activity was present in earlier fractions 5–7. Further analysis by western blotting (performed by Dr. C. Tilgmann, Orion-Farmos, Orion Research Centre, Helsinki, Finland) with an anti-COMT guinea pig serum revealed that COMT present in fractions 9–11 was S-COMT, whereas fractions 5–7 contained MB-COMT. As already referred to [8], K_m values for most of the catechol substrates of MB-COMT were approximately 100 times lower than those of the soluble enzyme. This could indicate that the contribution to total activity, as far as the high-affinity MB-COMT form is concerned, is likely to predominate at low (physiologic) substrate concentrations. Accordingly, in an *in vitro* kinetic model for brain dopamine and norepinephrine metabolism [8, 51], it was shown that the contribution of MB-COMT was higher than that of S-COMT at concentrations lower than 20 and 300 μM, respectively. Our COMT incubations in the fractions after the gel filtration on Superose 12 were performed under saturating conditions for the substrate 5,6DHI2C [47]. It is very possible that the actual contribution of MB-COMT to the total activity could have been much higher at lower concentrations. With analogy to catecholamines [8, 51], in melanocytes, under physiologic concentrations of *o*-dihydroxyindoles, MB-COMT may be more prevalent and functionally much more significant than S-COMT.

Induction of cytotoxicity in (malignant) melanocytes

Due to the generation of reactive *o*-quinone intermediates, it has long been recognized that melanogenesis constitutes a potential hazard to melanocytes [33, 52, 53]. Some earlier studies dealing with experimental malignant melanoma treatment investigated the utilization of the specific property of melanin production within these cells and showed that the intermediates of melanin metabolism were indeed cytotoxic [54, 55]. Several reports also indicated that exogenous phenolic compounds may be noxious for melanocytes because they can be converted into toxic products by tyrosinase [54–56]. To select not only the most effective but also specifically acting toxic substances for melanoma cells, the tyrosinase-dependent toxicity towards epithelial cells (CNCM-I-221) using a large series of substituted phenols was studied [57, 58]. The metabolism of monophenolic compounds proceeds preferentially via ring hydroxylation and is in accord with the commonly recognized phase I step of drug metabolism. Our analytical study indeed demonstrated that the metabolism of 4-hydroxyanisol (a known antimelanoma and depigmenting agent) proceeds by way of hydroxylation to 3,4-dihydroxyanisole, which can also function as a substrate (competitive inhibitor) for COMT (Fig. 3) [59]. In our approach, we used 4-hydroxyanisol as a standard for comparison of its toxic effects with those of our examined substances. In another *in vitro* study [49], we tested twelve potential COMT inhibitors for their (tyrosinase-dependent) toxicity towards CNCM-I-221-cells and their direct toxicity towards M14-melanoma cells. In melanoma cells, most of the agents showed an equal or higher inhibition of thymidine incorporation at 10^{-4} M when compared with 4-hydroxyanisole.

Männistö *et al.* [60] recently reviewed COMT inhib-

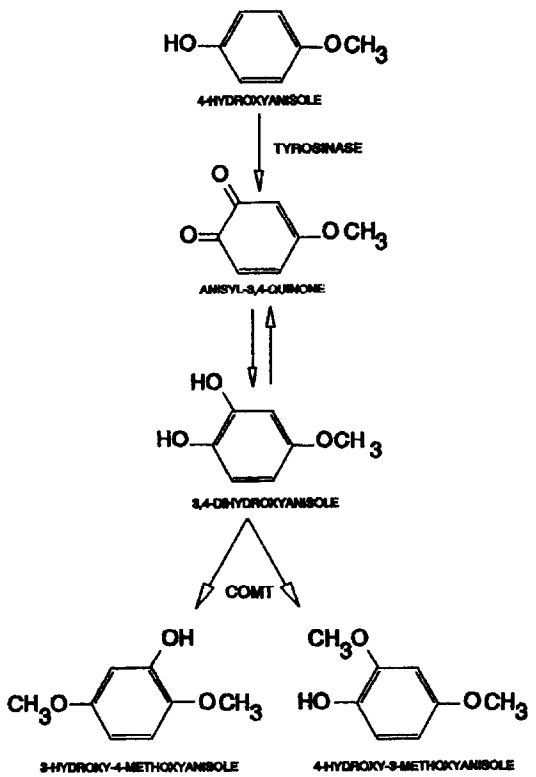

Fig. 3. Scheme of 4-hydroxyanisole metabolism, showing the generation of a potentially toxic 3,4-dihydroxyanisole and its detoxification by COMT [59].

itors. Most of the twelve compounds tested in our study [49] belonged to the generation of COMT inhibitors already dealt with in the earlier review on COMT by Guldberg and Marsden [4]. Compounds like tropolone and pyrogallol are probably the most widely studied COMT inhibitors, but they are only valuable for research purposes since their high toxicity does not allow their utilization in clinical studies. Different authors have reported recently on the development of a new generation of very potent and selective COMT inhibitors [60–64]. In most cases, these inhibitors contain a nitrocatechol moiety with the nitro group substitution at the fifth position. From these inhibitors, OR-462 and OR-486 were both reported to reduce the formation of 3-*O*-methyl-DOPA more effectively than L-DOPA/carbidopa (DOPA decarboxylase inhibitor) after oral administration. OR-486 had some effect on striatal COMT activity in contrast to OR-462, which mainly acts as a peripheral COMT inhibitor. Together with entacapone (OR-611), the aforementioned nitecapone (OR-462) has been the subject of a large number of studies, first in Parkinson's disease model systems and recently in patients and healthy volunteers ([65], see also the review in Ref. 60). Next to this, Ro 41-0960 and Ro-40-7592 also were shown to be selective COMT inhibitors that differ from OR-462 and OR-611 in having more central effects in the brain [60, 66]. Another new compound, CGP 28014, which is a pyridine derivative, does not inhibit COMT *in vitro*. *In vivo* it behaves, however, as an efficient inhibitor preventing the formation of homovanillic acid and 3-methoxytyramine in the brain [64, 67]. Some of the new COMT inhibitors like nitecapone (OR-462), OR-486 and Ro-41-0960 were also included in our study [49]. In our hands, OR-462 was quite effective, whereas OR-486 exerted only medium inhibitory activity, and the effect of RO-41-0960 was not better than that of 4-hydroxyanisole.

As can be inferred from the just mentioned data, the experience with L-DOPA treatment of patients with Parkinson's disease may be of great interest for investigations concerning the treatment of melanoma through induction of intracellular toxicity. The use of L-DOPA itself was considered to be of limited value owing to its toxic side-effects on the neural and cardiovascular system [55, 68]. The use of slow release L-DOPA preparations with appropriate (peripherally acting) COMT inhibitors could result in the desired selective toxicity in melanoma tumours. Other combinations, such as the use of COMT inhibitors with the most effective phenolic compounds described above, may prove even more successful for induction of cytotoxicity in melanoma cells. Whether both S- and MB-COMT have to be inhibited to produce a sufficient induction of cytotoxicity in melanoma cells should be part of a future investigation.

Concluding remarks

COMT is a widely distributed enzyme that fulfills an important task: to protect cells against potentially harmful exogenous and endogenous catechol substances. The enzyme is also present in the skin, chiefly in its upper parts (epidermis). In pigment-producing cells (melanocytes), COMT has another important function, namely to protect the cells against their own reactive compounds generated during melanogenesis. This "double task" makes this enzyme an important but also vulnerable element in cellular metabolism. COMT inhibition in pigment cells can induce internal cytotoxicity. Recent *in vitro* studies have shown that COMT inhibitors may indeed selectively damage melanoma cells. In designing these experiments, advantage has been taken of the growing knowledge from another medical field, i.e. the utilization of COMT inhibitors in the treatment of Parkinson's disease.

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