to that found with pyrogallol (see [10]), prevent the *O*-methylation of not only DOPAC but also biogenic amines by COMT. However, it is unlikely that such high concentrations will be reached after 3-PPP treatment.

Rollema and Mastebroek [5] have suggested that the final product after the metabolism of 3-PPP first by the liver hydroxylating systems and then by COMT is 4-methoxy-3-PPP. However, since COMT generally methylates at the hydroxyl group *meta* to the substituent (although *para*-methylation has been found under certain conditions, see [13]), it is perhaps more likely that the final product is 3-(3-methoxy-4-hydroxyphenyl)-*N*-*n*-propylpiperidine. Needless to say, the position of the methylation can only be ascertained with certainty by isolating the product and comparing its chemical properties with chemically synthesized meta- and para-*O*-methylated 4-hydroxy-3-PPP.

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Protection against alloxan-induced diabetes in mice by the free radical scavenger butylated hydroxyanisole

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Recent research has clearly demonstrated the role played by free radicals in various important pathological processes [1, 2] and high-lighted the need for safe and effective inhibitors of free radical-induced tissue injury. Alloxan-induced damage to pancreatic β-cells, an example of this type of pathology [3, 4], can be inhibited *in vivo* by aliphatic hydroxyl radical (·OH) scavengers such as dimethylsulfoxide (DMSO)* [5], *N*,*N*′-dimethylurea (DMU) [6], thiourea [7], and the short chain aliphatic alcohols, e.g. *n*-butanol [7]. While such scavengers strongly indicate a role for ·OH in alloxan-induced diabetes, their general usefulness for preventing free radical-induced tissue injury is limited. They must, for example, be administered in large doses (g/kg) to give protection in this system [5–7] and others [8, 9]. Furthermore, in the process of scavenging ·OH some of these aliphatic compounds may form potentially damaging free radical by-products [10] or other toxic metabolites [11].

Butylated hydroxyanisole (BHA), a phenolic resonance-stabilised free radical scavenger [12], is sufficiently non-toxic to be used as a food additive [13]. Since phenols have a higher rate constant for reaction with ·OH than the above aliphatic substances [14, 15] and the free radical formed by BHA when it scavenges is not a reactive or damaging species [12], we predicted that BHA should be an effective low dose inhibitor of alloxan-induced diabetes.

* Abbreviations used: BHA, butylated hydroxyanisole; DMSO, dimethylsulfoxide; DMU, *N*,*N*′-dimethylurea.

Materials and methods

Male CBA mice between 8 and 10 weeks old and weighing 23–27 g were used. Alloxan monohydrate (Sigma Chemical Co., St. Louis, MO) was dissolved in normal saline at 6.25 mg/ml. BHA (Sigma Chemical Co.) was dissolved in pure olive oil (Faulding) at 15, 20, 25 and 30 mg/ml.

Mice were starved for 6 hr prior to receiving intraperitoneal (i.p.) injections of 0.1 ml saline, 0.1 ml olive oil or 0.1 ml olive oil containing 1.5, 2.0, 2.5 or 3.0 mg of BHA. One, two or three hours later intravenous (i.v.) injections of 0.2 ml saline or 0.2 ml saline containing 1.25 mg of alloxan monohydrate were given.

Except where otherwise stated, plasma from each mouse was assayed for glucose at the time of the first injection and 1, 24, 48, 72 and 96 hr afterwards. For each assay about 50 μl of blood was taken from the tail vein of each mouse into heparinised microtubes (Clay Adams). These were sealed and spun for 3 min in a microtube centrifuge (Clements) to collect plasma. Plasma glucose (mmol/l) was assayed using the GOD-Perid Test Combination (Boehrin-

ger Mannheim). Neither alloxan or BHA interfered with this assay (data not shown).

Results and discussion

High blood glucose levels competitively inhibit the capacity of alloxan to damage β-cells [16]. To avoid this complication [17], we first established that our treatments did not significantly alter plasma glucose (data not shown). Furthermore, neither saline nor olive oil given 1 hr before alloxan prevented subsequent hyperglycemia (not shown).

When given 1 hr before alloxan, 3 mg (120 mg/kg) BHA completely protected against diabetes, whereas the lower doses (of 2.5 and 2.0 mg) were less effective and (1.5 mg) did not protect (Fig. 1). Increasing the interval between administration of BHA (3 mg) and alloxan to 2 hr and 3 hr correspondingly diminished protection (Fig. 2). No protection was afforded when alloxan was given 2 hr or 3 hr after the lower doses (2.5 or 2.0 mg) of BHA (not shown).

The rate constants for the reaction of ·OH with phenol [15], and benzenes substituted with electron-donating groups in general [14, 15], are higher than those of aliphatic compounds such as DMSO, DMU or *n*-butanol [15]. This higher activity (a factor of 2–3) alone is not sufficient to explain the efficiency of BHA in blocking alloxan-induced diabetes (Table 1).

Aromatic free radical scavengers will scavenge not only highly reactive species, such as ·OH, but also secondary radical species, for example, peroxy radicals [18]. Aliphatic scavengers will not do this, and must be present in high concentrations to prevent ·OH-initiated free radical chain reactions in tissues. In addition scavengers (which are not themselves radicals) form secondary radicals in the process of scavenging. For example, ·OH can react with DMSO to give the methyl radical (·CH_3) [10], which is less reactive toward biomolecules than ·OH [10], but potentially dangerous. In contrast, the resonance stabilised free radical

Table 1. Comparison of protective effects of free radical scavengers against alloxan-induced diabetes

Scavengers	Protective dose of scavenger (g/kg)	(mmoles/kg)	Alloxan (mg/kg)
DMSO[2]	7.3	93.6	50
DMU[3]	4.0	45.5	75
Thiourea[4]	3.0	39.5	50
N-Butanol[4]	0.8	10.8	50
BHA	0.12	0.67	50

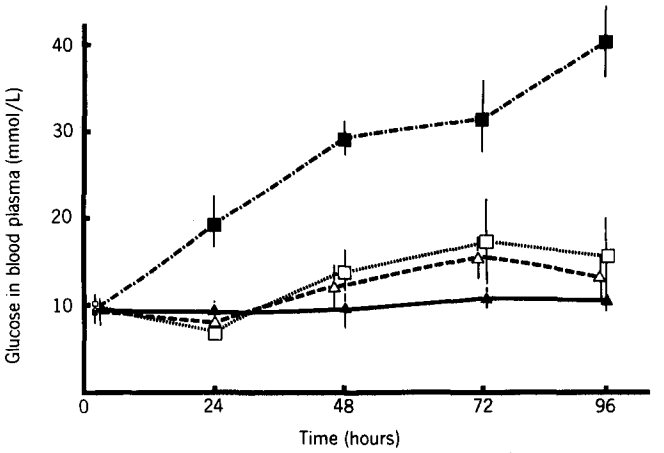

Fig. 1. Blood glucose levels after i.p. BHA 60 mg/kg (■); 80 mg/kg (□), 100 mg/kg (△) and 120 mg/kg (▲) followed by alloxan 50 mg/kg (i.v.) 1 hr later.

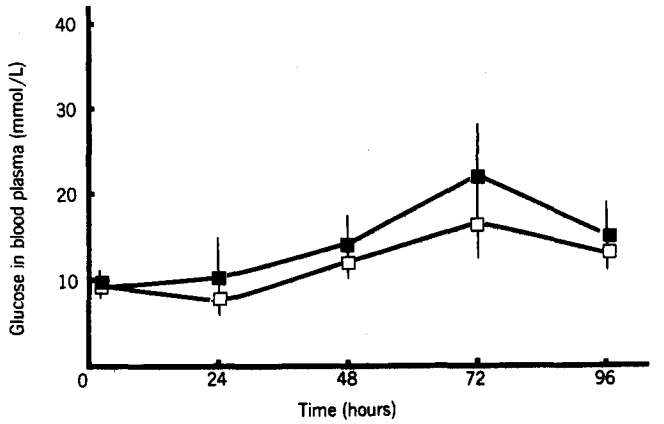

Fig. 2. Blood glucose levels after i.p. BHA 120 mg/kg followed by alloxan 50 mg/kg (i.v.) 2 hr (□) and 3 hr (■) later.

formed from BHA is unreactive [12] and such radicals only react with another free radical, or oxygen [18], thereby increasing overall scavenging efficiency.

In summary, the resonance-stabilised free radical scavenger butylated hydroxyanisole inhibits alloxan-induced diabetes in mice in a dose and time dependent manner. It is considerably more effective on a molar basis than the conventionally used aliphatic hydroxyl radical scavengers such as dimethylsulfoxide (DMSO), *N,N'*-dimethylurea (DMU), thiourea, or the short chain aliphatic alcohols.

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Activity of benzimidazole carbamates against L1210 mouse leukaemia cells: Correlation with *in vitro* tubulin polymerization assay

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The use of mammalian tubulin as a screen for potential anti-cancer drugs is well documented. Numerous reports on the qualitative correlation between the *in vitro* inhibition of polymerization of tubulin to form microtubules and the anti-cancer activity of vinblastine [1], maytansine [2, 3], podophyllotoxin [4–6], steganacin [6, 7], colchicine [5, 8–10] and their respective analogues have been published. However, this relationship does not extend to the biological actions of all such derivatives, as the analogues of podophyllotoxin, etoposide and teniposide, which do not inhibit the polymerization of tubulin [11] are potent anti-cancer agents, and the colchicine analogues isocolchicine, colchiceine and β-lumicolchicine, have been shown to inhibit purine uptake in cells by a mechanism considered to be microtubule-independent [12].

The activity of benzimidazole carbamates (Fig. 1) as inhibitors of tubulin polymerization *in vitro* has been the subject of a number of reports in the literature [13–15]. This mechanism is thought to account for their wide range of pharmacological actions as antifungal, anthelmintic and antineoplastic agents. In particular, the antitumour activity of nocodazole (Table 1, 21) gives rise to the question of whether the entire class of benzimidazole carbamates possesses similar activity [16, 17].

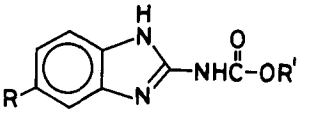

Fig. 1. Structure of benzimidazole carbamates.

Based on the results of a previous study of the structure–activity relationships of benzimidazole carbamates as inhibitors of tubulin polymerization [15], a number of 5(6)-substituted methyl benzimidazole carbamate analogues were tested against L1210 mouse leukaemia cells to determine whether qualitative and quantitative correlation existed between the two assays.

Experimental

Compounds 1–10 and 15 (Table 1) were prepared as described previously [15]. Compounds 11 and 12 were gifts from Hoechst (Australia) and Wellcome (Australia), respectively. Compounds 14 and 21 were gifts from Janssen