International Organization for Chemical Sciences in Development

Working Group on Plant Chemistry

CHEMISTRY, BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF AFRICAN MEDICINAL PLANTS

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African traditional healer and *Harpagophytum procumbens* (Pedaliaceae)

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21. Hypoxoside: a putative, non-toxic prodrug for the possible treatment of certain malignancies, HIV-infection and inflammatory conditions

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Introduction

One of the best studied phytochemicals from an African plant is hypoxoside ((*E*)-1,5-bis(4'-β-D-glucopyranosyloxy-3'-hydroxyphenyl)pent-4-en-1-yne). This unique norlignan diglucoside was first described in the early eighties (Marini-Bettolo *et al.* 1982; Drewes *et al.* 1984). It is the first phytochemical found to contain a pent-1-en-4-yne structure. Hypoxoside is a major component of the corms of Hypoxidaceae and can readily be converted to a more lipophylic aglucone, rooperol, by β-glucosidase deconjugation (Fig. 21.1.) (Theron *et al.* 1994)

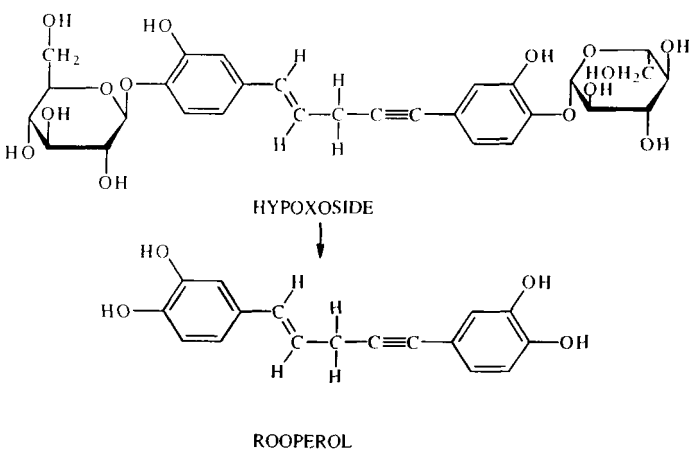

Fig. 21.1. The inactive diglucoside, hypoxoside, is deconjugated by β-glucosidase to form the lipophylic biologically active aglucone, rooperol.

Of considerable interest over the past 14 years has been the question whether hypoxoside has any therapeutic properties and if so, what the molecular mode(s) of action could be. According to reports, extracts of various members of the Hypoxidaceae have been used *inter alia* as a tonic, rejuvenator, treatment for testicular tumors, internal cancers, hypertrophy of the prostate and urinary diseases (Nicoletti *et al.* 1992). Indeed patents were granted for the use of extracts of the family Hypoxidaceae and rooperol and derivatives for the treatment of cancer (Drewes and Liebenberg 1987a, 1987b).

Research conducted over the past 14 years has shown that rooperol has potent pharmacological activity whereas hypoxoside is mainly inert. Nevertheless a hypothesis has been formulated that hypoxoside could act as a non-toxic, multi-functional prodrug (Albrecht *et al.* 1995a).

Pharmacological properties of rooperol

Rooperol is a potent inhibitor of lipoxygenase in the synthesis of leukotrienes at concentrations as low as 1 μM (Van der Merwe *et al.* 1993). It is also active against a wide panel of rodent and human cancer cells lines giving 50% growth inhibition at about 10 μg/ml. Some cell lines such as the NCI-H522 and ATCC HTB 53; A-427 derived from human non-small-cell-lung cancers, were found to be 10 times more sensitive, suggesting specific inhibitory mechanisms (Albrecht *et al.* 1995a). Recent work in our laboratory has clearly shown that rooperol can induce apoptosis in HL60 human promyelocytic leukemia cells (Theron, E., University of Stellenbosch, unpublished data). We have also found that rooperol can actively inhibit mutagenesis in the Ames test and scavenge free radicals 10 times more actively than ascorbate (Albrecht, C. and Bester, C., University of Stellenbosch, unpublished data). Using a cell line transfected with HIV-LTR-Luc-reporter gene (Israel *et al.* 1992) we also found that rooperol can inhibit the phorbol ester induction of this gene (Theron, E. and Albrecht, C., University of Stellenbosch, unpublished data).

Hypoxoside as a prodrug

When hypoxoside is taken orally by man it is not absorbed as such, but is first deconjugated by colonic bacterial β-glucosidase to form rooperol, which can be found in the faeces (Kruger *et al.* 1994). Subsequently, rooperol metabolites are found in the serum and urine. These metabolites were shown to be mainly glucuronides, sulfates and mixed glucuronide/sulfates (Kruger *et al.* 1994). A similar situation is found in the mouse except that the metabolites are only detected in the bile and not in the serum or urine (Albrecht *et al.* 1995a). Direct injection of rooperol into the bloodstream of baboons showed that it was conjugated to form metabolites within a few minutes (Coetzee *et al.*, *Drug*

Research, in press). Injection of hypoxoside showed no evidence of any metabolism.

When rooperol metabolites (220 µg/ml) were added to cancer cells in culture no inhibition of growth was found. However when β-glucuronidase (100 µg/ml) was added, some of the metabolites were deconjugated to form rooperol and the cells were killed (Albrecht *et al.* 1995a). Furthermore when rooperol metabolites were incubated with activated macrophages for a day, it was found that rooperol formed (Gabrielse *et al.*, University of Stellenbosch, unpublished data). These data lead to the hypothesis that rooperol metabolites could act as prodrugs which are activated by enzymatic deconjugation to form pharmacologically active rooperol. Such conversion of the prodrug to the active drug could conceivably take place in pathological tissue where the lysosomal deconjugases such as β-glucuronidase were present outside the cells. It is known that many tumors contain necrotic areas in which lysosomal enzymes are present. Furthermore release of such enzymes also occurs in inflammatory sites due to macrophage activation.

These considerations motivated us to conduct clinical studies of cancer patients and patients with HIV. Motivation for the later was based on the premise that HIV-infected lymph nodes could also contain active macrophages, releasing enzymes that could cause rooperol to form and that the rooperol could inhibit the expression of the HIV genome. It was also found that the di-sulfate metabolite of rooperol inhibited the *in vitro* proliferation of HIV-1 (Albrecht, C. and Kruger, P., University of Stellenbosch, unpublished data).

Clinical evaluation of hypoxoside as a prodrug

Ethical permission was granted by the University of Stellenbosch and the South African Medicines Control Council to conduct Phase 1 studies with hypoxoside for the putative treatment of cancer patients (for whom no other therapy was indicated) and patients with HIV.

Patients received 1200 -3200 mg of dried methanolic extract of the dried corms of *Hypoxis rooperi* in capsule form (200 mg/capsule), in three daily doses. Total rooperol metabolite concentrations were in the order of 100 µg/ml during these studies. All patients were subjected to frequent and extensive laboratory and clinical examinations including computed tomography of the cancer patients.

Nineteen non-small-cell lung cancer patients on hypoxoside therapy survived for an average of 4 months with progression of their primary tumors and metastases, while 5 survived for more than one year and one survived for 5 years before dying of tuberculosis. Histological examination of a lesion in the lung showed that it only contained connective tissue and no cancer cells.

No toxic effects, in clinical examinations or biochemical or hematological measurements, were found that could be ascribed to hypoxoside (Smit *et al.* 1995).

A further Phase 2 clinical study was conducted on more than 100 cancer patients for whom no further conventional therapy was available. No toxic effects

were detected in these patients and it was found that the median survival of a cohort of 16 pancreas patients was increased from an expected 3 months to 10 months.(Smit, B. and Albrecht, C., University of Stellenbosch, unpublished data).

Extensive pharmacokinetic analyses of these patients were conducted. Main findings were the absence of hypoxoside or rooperol in the serum and the presence of the rooperol metabolites which had half lives ranging from 20 to 50 hours following first order kinetics. (Albrecht *et al.* 1995b).

Patients with HIV have taken the methanolic extract of *Hypoxis rooperi* corms now for more than two years and their CD4 lymphocyte counts have remained remarkably stable while a decrease in serum p24 HIV antigen has been found and a decreased expression of the HLA-DR CD8 lymphocyte activation marker (Bouic, P., University of Stellenbosch, unpublished data).

Hypoxoside and NDGA

During our studies we became aware of similar studies on the phytochemical nordihydroguaiaretic acid (NDGA) derived from the creosote bush (*Larrea* sp.). This molecule is very similar to rooperol, *i.e.* containing two catechols suspended on a carbon bridge, however, NDGA lacks the pent-1-en-4-yne configuration. NDGA has been shown to inhibit leukotriene synthesis (Van der Merwe *et al.* 1993), act as an anti-mutagen (Wang *et al.* 1991), inhibit the transcription of the HIV gene (Gnabre *et al.* 1995) and induce apoptosis in HL60 cells (Theron, E., University of Stellenbosch, unpublished data).

Conclusions

These studies have demonstrated that rooperol has potent, diverse and important pharmacological properties relevant to cancer, inflammation and HIV. Furthermore extensive clinical experience has shown that oral dosing of hypoxoside in man is safe. The reason for this, is the rapid metabolism of rooperol to form conjugated metabolites that are inert. It is postulated that these metabolites can act as prodrugs and can be activated to form rooperol in targeted, pathological sites where lysosomal deconjugases are present. Although not conclusive, some of our clinical data suggests that such a process may be occurring *in vivo*. Direct proof of this is now required.

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