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Topical antirheumatic agents as hydroxyl radical scavengers

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Abstract

Camphor, capsaicin, ketoprofen, lavender oil, cineole, β-pinene and methyl nicotinate which are used in topical formulations for the relief of pain associated with rheumatic and musculo-skeletal disorders were assessed for hydroxyl radical scavenging properties. The compounds tested were irradiated with simulated sunlight in a model aqueous system containing dequalinium chloride and hydrogen peroxide. The rate of degradation of the dequalinium chloride by the photogenerated hydroxyl radicals was measured and found to follow second-order kinetics. Ketoprofen, a clinically used antirheumatic agent, gave the optimal results. The hydroxyl radical scavenging properties of the other compounds are discussed in terms of their chemical structure and possible reactivity.

Keywords: Photochemical; Hydroxyl radical; Topical; Rubefacient

1. Introduction

Martindale's Extra Pharmacopoeia (Reynolds, 1993) describes several preparations which are marketed for the topical treatment of rheumatic conditions and musculo-skeletal disorders. These preparations are often formulated as creams and applied by rubbing into the skin around the painful affected area and contain non-steroidal antirheumatic agents, volatile oils and/or compounds which exert a rubefacient activity. Sakurai et al. (1993) have patented a formulation in which antirheumatic drugs are incorporated into microspheres and these are suspended in a hydrogel base. The preparation is claimed to act by the release of the medication by the action of hydroxyl radicals generated in the inflamed area to which the preparation has been applied. Parkash et al. (1993) have shown that some non-steroidal anti-inflammatory agents are effective as hydroxyl radical scavengers by measuring the rate constant of the decomposition of dequalinium chloride in aqueous media by photogenerated hydroxyl radicals derived from hydrogen peroxide according to the method of Patel and Sugden (1992). The objective of the present work was to examine the active ingredients commonly used in topical antirheumatic preparations and to measure their hydroxyl radical scavenging properties at 38 ± 1°C.

2. Materials and methods

2.1. Materials

The following materials were obtained from the sources indicated: camphor (BDH), capsaicin

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(Sigma Chemical Co.), cineole (Aldrich Chemical Co.), dequalinium chloride (Aldrich Chemical Co), ethanol (Burroughs Ltd), hydrogen peroxide (Boots plc), ketoprofen (Sigma Chemical Co.), lavender oil (Bush, Boake Allen), mannitol (Aldrich Chemical Co.), methyl nicotinate (Aldrich Chemical Co.) and β-pinene (Aldrich Chemical Co.).

2.2. Apparatus

A Cecil 273 spectrophotometer with matched silica cells of 1 cm path length was used.

2.3. Methods

A calibration graph was constructed using a series of dilutions of dequalinium chloride in deionised water (2.0, 4.0, 8.0, 10.0, 12.0, 16.0 mg/100 ml). The linear regression analysis, based on three replicates, gave a regression coefficient of 0.9997 ($p = 0.001$). A stock solution of dequalinium chloride in deionised water (384 μg/ml) was prepared and the container wrapped in aluminium foil to exclude light. Aliquots (220 ml) of the solution were prepared in which the following materials were added individually in stoichiometric amounts with respect to the dequalinium chloride:- camphor (24.38 mg), capsaicin (48.9 mg), cineole (0.1 ml), ketoprofen (40.72 mg), lavender oil (0.1 ml), mannitol (66 mg), menthol (25.03 mg) and methyl nicotinate (21.96 mg). A second series in which the solutions were made up with the addition of ethanol (4 ml) and a third series in which the solutions were made up with hydrogen peroxide (30% v/v, 4 ml) were prepared. A fourth series was prepared in which both ethanol and hydrogen peroxide were added using the same quantities as listed above. Each of the solutions listed was irradiated by the method of Evans et al. (1975) for a period of 5 h with absorbance readings taken at 326 nm at time zero and every 0.5 h thereafter. The appropriate blanks were used in each case. The data recorded represent the mean of three readings.

2.4. Treatment of results

The reaction order and rate constant were determined by taking linear regression analyses

Table 1
Photodegradation results of dequalinium chloride (DC)(384 μg/ml) with additives

System	Order of reaction	Rate constant
DC (control)	no photodegradation in 5 h	
DC + H_2O_2 (control)	1st	38.89×10^{-2}
Dc + H_2O_2 + mannitol(control)	1st	2.48×10^{-2}
DC + H_2O_2 + ethanol(control)	2nd	4.144×10^{-2}
DC + H_2O_2 + mannitol + ethanol	1st	14.30×10^{-2}
DC + H_2O_2 + camphor + ethanol	2nd	2.89×10^{-3}
DC + H_2O_2 + camphor + mannitol + ethanol	2nd	2.67×10^{-3}
DC + H_2O_2 + capsaicin + ethanol	2nd	1.91×10^{-3}
DC + H_2O_2 + cineole + ethanol	2nd	3.88×10^{-3}
DC + H_2O_2 + cineole	1st	2.16×10^{-2}
DC + H_2O_2 + cineole + mannitol + ethanol	2nd	2.64×10^{-3}
DC + H_2O_2 + ketoprofen + ethanol	2nd	1.42×10^{-3}
DC + H_2O_2 + ketoprofen + mannitol + ethanol	2nd	1.66×10^{-3}
DC + H_2O_2 + lavender oil	2nd	3.77×10^{-3}
DC + H_2O_2 + lavender oil + mannitol	2nd	2.44×10^{-3}
DC + H_2O_2 + menthol + ethanol	2nd	2.15×10^{-3}
DC + H_2O_2 + methyl nicotinate + ethanol	2nd	2.15×10^{-3}
DC + H_2O_2 + methyl nicotinate + ethanol + mannitol	2nd	2.22×10^{-3}
DC + H_2O_2 + β-pinene	2nd	3.88×10^{-3}
DC + H_2O_2 + β-pinene + ethanol	2nd	2.66×10^{-3}
DC + H_2O_2 + β-pinene + mannitol + ethanol	2nd	1.73×10^{-3}

of plots of the percentage residual dequalinium chloride, the log of the percentage of dequalinium chloride remaining and the reciprocal of the percentage dequalinium chloride remaining vs time. The plot yielding the best straight line as determined by the method of Patel and Sugden (1992) was deemed to represent the order of the reaction. The rate constants were calculated from the slopes of the graphs.

3. Results and discussion

The results of the experiments are shown in Table 1.

All systems which did not contain hydrogen peroxide did not exhibit any photodegradation of the dequalinium chloride within the time span of 5 h.

Perusal of Table 1 shows that dequalinium chloride undergoes photodegradation in light in the presence of hydrogen peroxide and the rate constant for this first-order reaction is markedly reduced when mannitol is added in stoichiometric amounts, indicating that the reaction is hydroxyl radical mediated as described by Patel and Sugden (1992). The addition of ethanol and mannitol to the reaction mixture in this work gave a first-order reaction which was unusual in the light of the work of Parkash et al. (1993), since ethanol is also a hydroxyl radical scavenger.

Sakurai et al (1993) based their patent claim on the concept that inflammatory reactions were due to the liberation of hydroxyl radicals at the site of injury. Irrespective of the validity of this claim, there is a wealth of anecdotal evidence to support the topical use of volatile oils, esters and some established non-steroidal anti-inflammatory agents to treat the pain caused by musculo-skeletal injuries by topical application of creams which are absorbed by the tissues and act at the site of the damage.

Lavender oil which contains β-pinene and cineole has been used in dermatological preparations and consequently these two compounds were assessed for hydroxyl radical scavenging activity. Camphor, menthol and methyl nicotinate have been used as rubefacients and as components of

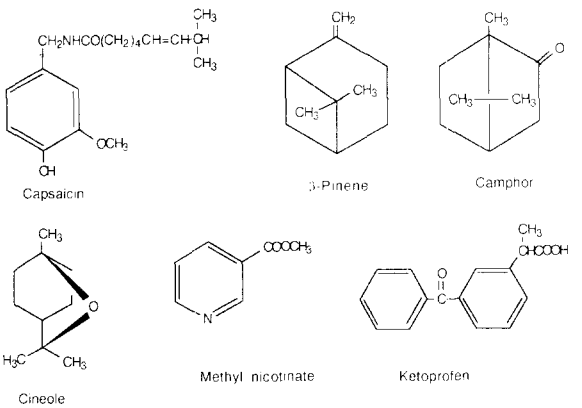

Fig. 1. Chemical structures of compounds tested.

topical creams for the alleviation of pain associated with musculo-skeletal disorders. Capsicum oleoresin has been recommended as a counter-irritant and is a component in some topical applications (Reynolds, 1993) and its hydroxyl radical scavenging properties were assessed with this use in mind. The pure material, capsaicin, was used in the present work.

Examination of Table 1 shows that dequalinium chloride in aqueous solution is subject to photochemical degradation in the presence of hydrogen peroxide and that this reaction is retarded by the addition of mannitol, a known hydroxyl radical scavenger. In the series of experiments in which ethanol was used to solubilise the test material in an aqueous solution the control standard is the reaction undertaken with ethanol which followed second-order kinetics with a rate constant of 4.14×10^{-3}. Perusal of Fig. 1 shows the structures of the compounds tested and indicates the possible sites for reaction with hydroxyl radicals.

In the case of camphor the rate constant was reduced to 2.89×10^{-3} and in the presence of a stoichiometric amount of mannitol the rate fell to 2.67×10^{-3}. This was an indication of camphor having a hydroxyl radical scavenging effect in the system tested. However, the effect of the addition of mannitol in this system was less significant than in the system where mannitol was the sole hydroxyl radical scavenger added. This suggests that there is a possibility of condensation be-

tween camphor and mannitol in a similar manner to that between acetone and mannitol yielding, in this case, a much less effective hydroxyl radical scavenger. Capsaicin exhibited a more potent effect, having a rate constant of 1.91×10^{-3}. However, the latter effect can be explained by consideration of the structure of capsaicin which has a phenolic group in the *para* position of a benzene ring and this structure could easily undergo reactions with a hydroxyl radical, leading to the eventual substitution of the ring with a hydroxyl group as has been demonstrated with phenylephrine (Al Tai et al., 1982). With the addition of mannitol the reaction ceased, suggesting a possible synergistic action of these two compounds acting together. Cineole, on the other hand, was unusual in that the reaction followed first-order kinetics. It would be reasonable to expect that this ether would undergo oxidation by hydroxyl radicals yielding products as is the case of oxidation of cineole with aqueous potassium permanganate solution. However, on the addition of mannitol to the system the reaction followed second-order kinetics and gave a rate constant of 2.64×10^{-3}. Ketoprofen, the only clinically used non-steroidal anti-inflammatory agent tested, followed second-order kinetics with a rate constant of 1.42×10^{-3} but on the addition of mannitol in stoichiometric quantity the rate constant was raised slightly to 1.66×10^{-3}. This small effect suggested that this result could be due to a number of possibilities; firstly the mannitol may have interfered with the hydroxyl radical scavenging of ketoprofen by reaction with the ketone group of ketoprofen. Such a reaction would be subject to considerable steric hindrance, since the keto group is flanked by aromatic rings so that rate and extent of the reaction would be low. An alternative explanation is that the results are within the scope of experimental error. Ibuprofen, which is closely related to ketoprofen, has been reported to have hydroxyl radical scavenging properties (Hamberger and McCay 1990), adding support to the suggestion that ketoprofen is acting as a hydroxyl radical scavenger.

The addition of lavender oil gave a reaction which followed second-order kinetics and had a rate constant of 3.77×10^{-3} whilst the addition of mannitol reduced this rate constant to 2.44×10^{-3}. The component substances in lavender oil which were assessed with ethanol, cineole and β-pinene gave rate constants of 3.88 and 2.66×10^{-3}, respectively, and followed second-order kinetics.

β-Pinene with ethanol and mannitol gave a rate constant of 1.73×10^{-3} which supported the suggestion that this reaction was hydroxyl radical mediated. In the case of β-pinene, the double bond would be likely to follow a similar reaction pathway to that of cyclohexene on reaction with hydroxyl radicals derived from Fenton's reagent, yielding the corresponding *cis*-diol (Le Roux et al., 1967). This diol could be expected to exert some hydroxyl radical scavenging effects of its own due to hydrogen abstraction by hydroxyl radicals from the α-position of the two alcoholic hydroxyl groups (Anbar et al., 1966) and this may explain the potent scavenging effect of this additive. Menthol, a cyclic secondary alcohol, gave a rate constant of 2.66×10^{-3} and followed second-order kinetics whilst the addition of mannitol completely stopped the photochemical degradation of the dequalinium chloride. Menthol would be expected to undergo oxidation with hydroxyl radicals and thus act as a scavenger. The observation that the addition of mannitol completely stopped the reaction would lend some support to the suggestion that if menthol undergoes the normal oxidation to a ketone then this compound, menthone, does not readily condense with mannitol to yield a less effective hydroxyl radical scavenger. Methyl nicotinate, a rubefacient, on iradiation in ethanol solution in the model system gave a rate constant of 2.15×10^{-3} and on the addition of mannitol this was raised to 2.22×10^{-3}, both reactions following second-order kinetics. Steenken and O'Neill (1978) reported that pyridine rings react with hydroxyl radicals by undergoing hydroxylation at positions 3, 2 and 4. However, in the case of methyl nicotinate, position 3 of the pyridine ring is blocked and thus reaction can take place only at positions 2 and 4. The reducing properties of the intermediate hydroxypyridine radicals are regarded as being in the order of $3 > 2 > 4$. The modest hydroxyl radical scavenging activity of methyl nicoti-

nate suggests that the main therapeutic affect of this substance may well be the increase in blood flow in the affected area due to the rubefacient activity, rather than a specific hydroxyl radical scavenging action.

The results of this work show that there is some evidence of hydroxyl scavenging by the compounds tested and that it is possible that this property may be a feature of their therapeutic value as active ingredients of topic applications for inflammation associated with musculo-skeletal disorders. This does support a rationale for topical creams for this purpose provided that the base is so formulated as to permit rapid penetration of the active ingredient into the affected area.

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