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PRELIMINARY COMMUNICATION

1-METHYLNICOTINAMIDE: A POTENT ANTI-INFLAMMATORY AGENT OF VITAMIN ORIGIN

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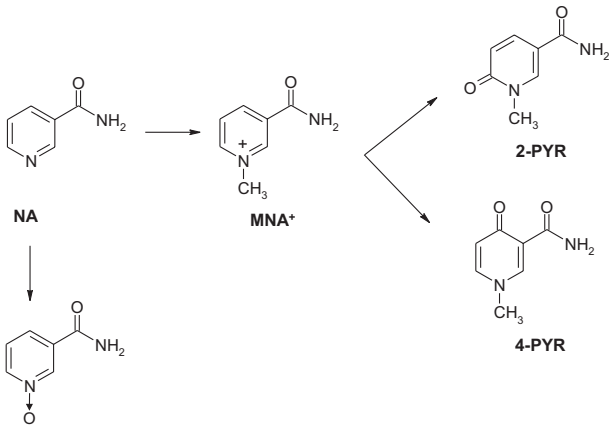
It has been found that 1-methylnicotinamide (MNA^+), a metabolite of nicotinamide, possesses significant anti-inflammatory properties. MNA^+ is chemically stable, non-toxic and well tolerated. MNA^+ can be used to treat wide variety of diseases and disorders and the use of this compound provides certain advantages over the use of nicotinamide.

Key words: *nicotinamide metabolite, skin diseases, inflammation, reactive oxygen species*

INTRODUCTION

1-Methylnicotinamide (MNA^+) is one of the two major primary metabolites of nicotinamide (NA) next to nicotinamide-N-oxide (N-OX). It is further metabolized to 1-methyl-2-pyridone-5-carboxamide (2-PYR) and 1-methyl-4-pyridone-5-carboxamide (4-PYR) (Scheme 1). All four metabolites are excreted with urine [8, 11].

It is well known that NA possesses remarkable anti-inflammatory properties [2, 5, 9]. MNA^+ similar to NA is chemically stable, non-toxic and well tolerated. The therapeutic function of MNA^+ has not been considered to date. Here we show that MNA^+ possesses significant anti-inflammatory pro-



Scheme 1. Major metabolites of nicotinamide (NA)

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perties. It can be used to treat a wide variety of diseases and disorders and the use of this compound provides certain advantages over the use of NA.

MATERIALS and METHODS

Materials

MNA^+ was synthesized according to the known procedure [10] and its purity established by HPLC exceeded 99.7%. NA and other chemicals were purchased from Sigma (USA).

Formulations used in clinical tests

The gel formulation was based on polyacrylic acid and the ointment consisted of a mixture of eucerine and glycerol (1:2). The concentration of MNA^+ varied from 0.1 to 0.5%.

Clinical observations

The therapeutic function of MNA^+ was verified in clinical observations carried out against placebo treatment using a double-blind protocol. Ninety patients with mild to moderate inflammatory acne vulgaris and 85 patients with acute, short-lasting exogenous eczema as a form of allergic contact dermatitis were enrolled in the studies. Therapeutic potential of MNA^+ treatment of the first degree burns caused by heat or UV-light was also estimated. Twenty two patients participating in the open pilot studies had shallow burns on face or limbs. MNA^+ was applied topically twice daily and clinical effects were evaluated during and after the treatment lasting from 7 to 21 days. The studies were done under the permission of the Ethical Commission.

Studies of scavenging properties

The rate constants of the reactions with hydroxyl radical and superoxide radical anion were measured by means of pulse radiolysis. Details about pulse radiolysis system can be found elsewhere [6]. The reaction with hydroxyl radical was investigated in N_2O saturated aqueous solution monitoring the rate of product build-up (for NA) and in O_2 saturated solution by the competition kinetics with SCN^- (for MNA^+). The reaction with superoxide radical was investigated in an O_2 saturated sodium formate solution, and monitored the superoxide radical anion decay.

RESULTS and DISCUSSION

It has, surprisingly, been found that MNA^+ can be used to treat a wide variety of skin diseases and that the use of this compound also provides certain advantages over the use of NA, in particular an increased efficacy at a specified dose and/or a reduction in undesirable side effects. In particular, topical treatment of skin diseases such as acnes with a MNA^+-containing gel has been shown to produce at least a similar therapeutic effect at concentrations approximately 100 times lower than the corresponding NA treatment and with no appreciable side effects. The therapeutic function of MNA^+ has also been verified in clinical observations carried out against placebo treatment using a double-blind protocol and the obtained results are presented in Figure 1. It is clearly seen that MNA^+ is beneficial in treatment of these dermatoses.

Particularly interesting results were obtained for treatment of burns. The positive therapeutic effects demonstrated by fast regression of inflammation symptoms within first days of the treatment were observed for all patients participating in the study. Substantially accelerated healing processes observed in the treated areas of the burned skin point to angiogenic potency of MNA^+. Such a property of MNA^+ has already been suggested [7]. MNA^+ indeed appears to be an efficient topical remedy to treat mild burns.

It has to be stressed that the tolerance of dermatological formulations containing MNA^+ was very good, comparable with vehiculum (placebo).

Since inflammatory process is often associated with excessive formation of free radicals (reactive

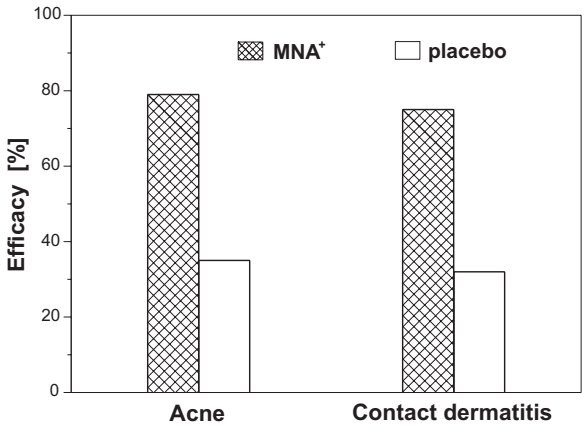

Fig. 1. Efficacy of topical MNA^+ application at a concentration of 0.3% tested against placebo (statistical significance $p < 0.005$)

oxygen species) [4, 13], we decided to estimate MNA^+ function as a potential scavenger of reactive forms of oxygen, in particular superoxide radical anion ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$). Since anti-inflammatory properties of NA are known and they are sometimes linked to its scavenging properties [12], we decided to compare, as is shown in Table 1, scavenging properties of MNA^+ in comparison with NA.

Based on the results presented above superior anti-inflammatory function of MNA^+ over that of NA cannot be linked to scavenging properties of MNA^+. As shown in Table 1, both MNA^+ and NA cannot be regarded as effective scavengers of $O_2^{\bullet-}$ and scavenging properties against $^{\bullet}OH$ are rather medium for both compounds, however, NA is clearly more effective than MNA^+. Thus, it seems unlikely that anti-inflammatory properties of MNA^+ can be associated with its scavenging properties.

Table 1. Scavenging rate constants

Compound	Reactive oxygen species	
	$k_{O_2^{\bullet-}}$ [M^{-1} s^{-1}]	$k_{^{\bullet}OH}$ [M^{-1} s^{-1}]
MNA^+	$< 5 \cdot 10^3$	$6 \cdot 10^7$
NA	$< 5 \cdot 10^3$	$8 \cdot 10^8$

It is known that MNA^+ can be a substrate of xanthine oxidase and the rate of enzymatic oxidation is a function of pH [1, 3]. However, we have found that this process is not very effective at neutral value of pH, and K_m measured under these conditions was found to be 30 mM. Therefore, MNA^+ at the concentrations applied in the experiments described above cannot contribute to a remarkable production of superoxide in the xanthine oxidase-mediated process.

It seems to us that the superior therapeutic properties of MNA^+ over NA can be associated with ionic character of MNA^+. We have found that MNA^+, in contrast to NA, can be bound to glycosaminoglycans. For example, 52% of MNA^+ was bound from the solution ($2 \cdot 10^{-5}$ M; pH = 7) to Sepharose-immobilized heparin (0.25 g/10 ml). Under similar conditions no binding of NA was seen. It can be expected that MNA^+ when introduced into blood circulation will interact with glycosaminoglycans located on a surface of vascular endothelium cells without effective penetration inside the tissue, thus increasing its local concentration on the surface.

In principle, MNA^+ can exert its therapeutic function through multiple mechanisms, however, it seems likely that MNA^+ anti-inflammatory properties can be associated with its ability to reduce adherence of pro-inflammatory cells and molecules to a surface of vascular endothelium. Some work has been initiated to substantiate this view. Also conclusion concerning a scope of MNA^+ therapeutic application needs to be substantiated by more extensive clinical observations.

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