

TMAZ nanoparticles as potential drugs influencing the cellular signal transduction pathways

Dr. Slavko Ivkovic, Dr. Thomas Baranek, Dr. Peter Bendzko, Prof. Dr. Jörg Schulz

KLINOMED GmbH
Institut für angewandte Nanotechnologie und Nanomedizin
Robert-Rössle-Str. 10, D-13125 Berlin, Germany

ABSTRACT

TMAZ, natural zeolite clinoptilolite with enhanced physicochemical properties, have been shown to possess antioxidant activity in humans. Modes of immunomodulation including superantigen-like action of ingested zeolite and perspectives of immunotherapeutic approaches for malignant and infectious diseases and autoimmune disorders are discussed.

Accumulating evidence from preclinical studies and from first human data suggests significant immunomodulatory effects of oral zeolite administration which may be of benefit for augmentation of medical treatments for a variety of diseases dependent on immunological changes.

TMAZ act as nonspecific immunostimulators similarly to superantigens (SAGs). SAGs bind as unprocessed proteins to particular motifs of the variable region of the β -chain ($V\beta$) of T-cell receptor (TcR) outside the antigen-binding groove and to invariant regions of major histocompatibility complex (MHC) class II molecules on the surface of antigen-presenting cells (APCs). As a consequence, SAGs stimulate at nano- to picogram concentrations up to 10 to 30% of host T-cell repertoire while only one in 10^5 - 10^6 T cells (0.01-0.0001%) are activated upon conventional antigenic peptide binding to TcR.

TMAZ can be used as an adjuvans or reborans in any standard therapy of malignant disease, with the aim to improve the general health condition of patients and help them to recover much easier and in a shorter period of time.

Keywords: klinoptilolith, nanoparticles, oxidative stress, antioxidants, cancer

1 INTRODUCTION

Zeolites are hydrated natural and synthetic microporous crystals with well-defined structures containing AlO_4 and SiO_4 tetrahedra linked through the common oxygen atoms. Clinoptilolite is a nontoxic natural zeolite with properties of an cation exchanger and adsorbent of proteins and small molecules such as glucose. Intake of TMAZ clinoptilolite by cancer patients has shown strong antioxidative properties. Finely ground natural clinoptilolite zeolite powder has also been shown to induce activation of $p21^{WAF1/CIP1}$. However, tissue culture experiments demonstrated that activated zeolite particles inhibit protein kinase B/akt, another kinase involved in antiapoptotic processes and cancer promotion.

2 TRIBOMECHANICAL ACTIVATION OF SOLIDS

Tribomechanical activation of solids implies the increasing of the reactivity of the active surface of the solid materials which undergo the mechanical processes of the surface straining through friction under dynamic processing conditions.

An increasing of reactive capacity of the material is a consequence of: damage of the material structure, an increase in dislocation and number of imperfections on the surface layer of the material, appearing of an excess of free and partially free ions (radicals), as well as an increase in potential for electronic donation.

As a result of the interactions between the solid materials during the processing of mineral raw materials, crystal grates of material at the surface of the particles and in the layers directly below the surface, are destroyed or damaged, and therefore partially transformed from a crystalline to an amorphous shape. This results in changes in the physico-chemical and energetic characteristics of the material.

3 INDUCTION OF PROGRAMMED CELL DEATH-APOPTOSIS

Here we provide evidence that TMAZ treatment to the animals increases levels of p21^{WAF1/CIP1} and p27^{KIP1} in tumor cell models. It is not yet clear whether inhibition of kinase B/akt is involved in regulation of expression of p21^{WAF1/CIP1} and p27^{KIP1} cell cycle inhibitors. Preliminary results also show that TMAZ adsorbs and deactivates nitric oxide and other oxidants. In addition, it has recently been reported that antioxidants stimulate the activation of cyclin inhibitor p21^{WAF1/CIP1}. This molecule is responsible for the arrest of cell growth, and its expression in adenocarcinomas of lung is positively correlated with optimistic survival prognosis [1].

To evaluate whether the inhibition of cell growth by TMAZ is due to programmed cell death, i.e., apoptosis, an attempt was made to isolate small DNA fragments. Large amount of small (degraded) DNA fragments in DNA isolate would indicate that TMAZ induces programmed cell death in treated cells. DNA isolated from TMAZ treated cells exhibited significant degradation in comparison to DNA from untreated cells. The DNA degradation in TMAZ treated cells is most probably due to induced programmed cell death (apoptosis).

TMAZ induced translocation of p65 to the nucleus of RFM mice spleen cells. This result suggests that TMAZ acts as an immunoactivator, activating NFκB, and therefore inducing transcription of genes regulated with NFκB.

A decreased amount of p50 and increased amount of RelB proteins in treated mice compared to control mice could be due to a changed number and /or ratio of B- and T-lymphocytes.

4 ANIMAL DATA

4.1 Methods

C57B1/6 mice with B 16 metastasis were used for the study. CBA/HZgr and RFM mice were used for evaluation of oxidative stress parameters and for datacollection of cellular immune-response measurement.

In the study 15x10⁴ melanoma cells were injected into mice treated with TMAZ and into control group, the number of lung metastases was reduced from 36.05 ± 13.09 (in the control group consisting of five animals) to 21.0 ± 4.96 for treated mice (consisting of five animals).

4.2 TMAZ effects in animals

The effects of Doxorubicin and TMAZ, given in combination, shows a significant reduction of the metastases count, compared to the control group (p<0.0001) and to the group treated by Doxorubicin only (p=0.001). The same results were shown by treatment with Endoxan.

Doxorubicin and Endoxan – induced oxidative stress (lipid-peroxidation within tumor). TMAZ did not influence the effect of Doxorubicin and Endoxan – it induced oxidative stress (HNE- protein conjugates) in malignant cells (p > 0.1), while it prevented the formation of HNE-protein conjugates in tumor stroma (p = 0.005)completely.

After i.p. application of TMAZ, the number of peritoneal macrophages, as well as their production of superoxide anion, increased. At the same time translocation of p65 (NFκB subunit) and activated T-cell immunological (cellular) response that could be involved in an anticancer effect of TMAZ in vivo [2].

5 HUMAN DATA

5.1 Methods

5.1.1 The Randox Total Antioxidant Status (TAS)

The TAS test is a two-reagent assay, which can be performed using serum or plasma samples. Additionally, the assay may be used to measure the antioxidant potential of (suitably solubilised) food and drug samples [3].

Incubation of ABTS® with a peroxidase (metmyoglobin) and hydrogen peroxide results in the production of the radical cation ABTS⁺. ABTS⁺ can be detected at 600 nm. Antioxidants in patient serum or plasma samples inhibit the reaction. The degree of inhibition is proportional to the concentration of antioxidants in the patient sample.

The Antioxidant effect of TMAZ was shown a compared study with 114 cancer patients, healthy volunteers and a control group.

Results are shown in Figure 1. The improvement of TAS (mmol/L) in healthy volunteers -1- are compared to 114 malignant patients -2- treated with TMAZ (30day – 16g/d) and the controll group, respectively. The blue columns represent the TAS values (mmol/l) before treatment, the red columns after treatment, respectively [4].

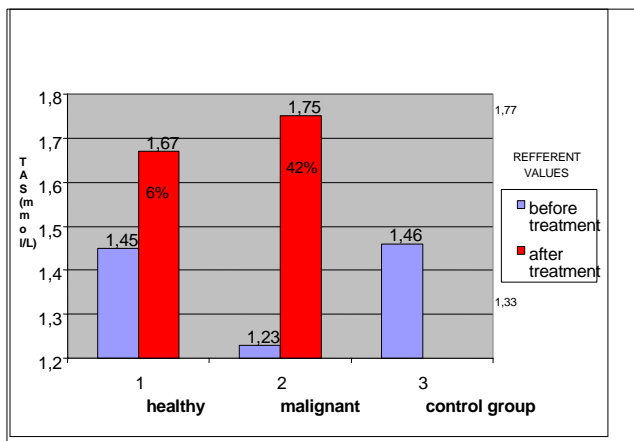


Figure 1: Antioxidant effect of TMAZ in 114 malignant patients

5.1.2 The Free Radical Analytical System (FRAS)

The free radical effects can be measured by using the free radical analytical system (FRAS) assessing the derivatives of reactive oxygen metabolites(D-ROMs).FRAS is the only system available in the world which is able to dose all types of hydrperoxide present in a biological sample via a simple, rapid, reliable and repeatable method using a drop of blood.

We evaluate the FRAS values in malignant patient before (423) and after (311) treatment with TMAZ compared to the control group (healthy volunteers). The results shows a significant reduction of free radicals in patients suffering from cancer.

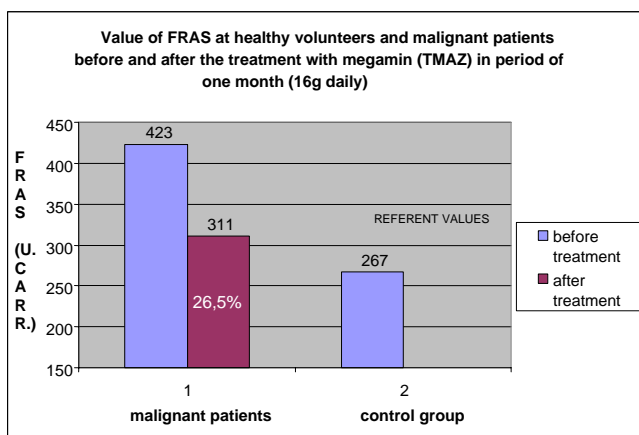


Figure 2: Value of FRAS in malignant patients and healthy volunteers

5.2 TMAZ effects in humans

Our results indicate that tribomechanically activated zeolite clinoptilolite, is potentially a new antioxidant which seems to have bigger capacity than already known antioxidants.

TMAZ has strong adjuvant and in some cases direct anti tumour effect in treatment of some solid tumours in animals. TMAZ induces p21^{Ci1/Waf1} and p27^{Kip1} induction followed by programmed cell death of some human tumour cells. TMAZ significantly increases TAS values of patients with malignant diseases as well as healthy volunteers. Daily dose of patients with malignant diseases should be 8 gram per day.

We collected data of 21 cerebral malignant patients and of 40 patients with SCLC(small cell lung cancer), by using the Karnowski-Index, so that we can summaries that patients with malignant diseases who were treated with TMAZ more than 8 weeks, have improved their general health condition, additionally.

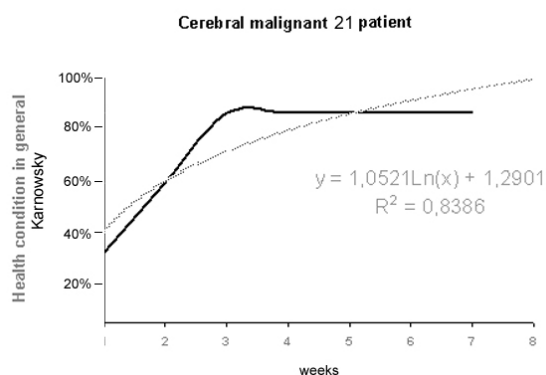


Figure 3: Karnowski-Index progress of 21 cerebral malignant patients treted with TMAZ

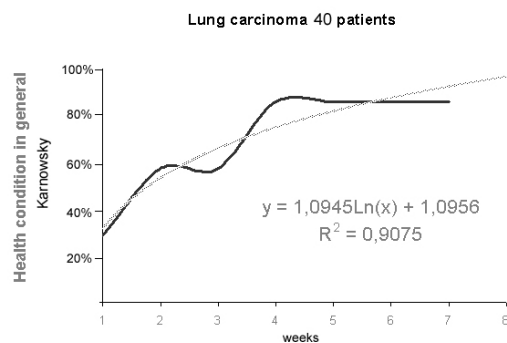


Figure 4: Karnowski-Index progress of 40 lung carcinoma patients treated with TMAZ

Both figures shows that all patients (cerebral malignancy and SCLC), treated with TMAZ, were under significantly better general health conditions after at latest 4 weeks.

Summarising these results we are able to say that TMAZ can be used as an adjuvans or reborans in any standard therapy of malignant disease, with the aim to improve the general health condition of patients and help them to recover much easier and in a shorter period of time.

6 CONCLUSION

Accumulating evidence from preclinical studies and from first human data suggests significant immunomodulatory effects of oral TMAZ administration which may be of benefit for augmentation of medical treatments for a variety of diseases dependent on immunological changes.

Increased efforts in basic research are needed to clarify proposed mechanisms of action, and randomized controlled trials should be carried out to confirm promising results from pilot studies

Anticancer therapeutic protocols based on specific combination of various antioxidants are accepted. Here we have a new potential antioxidant- tribomechanically activated zeolite (TMAZ).

TMAZ significantly increases total antioxidant status (TAS) of patients with malignant diseases as well as healthy volunteers.

In addition, further, direct interactions of silicate particles with alveolar cells have been observed and may alternatively translate to the understanding of immunostimulation by TMAZ after oral administration.

It seems that mineral particles can trigger alterations in gene expression by initiating signaling events upstream of gene transactivation [5].

Exposure of alveolar macrophages to silicate particles has also been shown to lead to activation of mitogen-activated protein kinases (MAPK), stress-activated protein kinase (SAPK), and protein kinase C (PKC) [6].

Important transcription factors such as activator protein 1 and NF κ B are also activated, and expression of proinflammatory cytokines such as interleukin 1 α , interleukin 6, and TNF- α is enhanced [7].

Macrophage activation and subsequent initiation of intracellular signaling pathways together with the observation of polyclonal human T-lymphocyte activation in vitro led to the hypothesis of silicate particles acting as superantigens (SAGs), which has been discussed in more detail in the introduction of this article. If this hypothesis can be confirmed, new perspectives in the treatment of autoimmune disorders, infectious and malignant diseases, the pathogenesis of which is linked to the action of SAGs,

may arise from the dietary supplementation with TMAZ. Further antitumor effects of TMAZ have been demonstrated in vitro and in animal studies and may add to the assumption of benefits of zeolite administration as an augmentation of cancer therapy.

Results of prospective, observed clinical trials in different indications (e.g. psoriasis, neurodermatitis, malignant melanoma, small cell lung cancer, HIV and Diabetes) are pending.

REFERENCES

- [1] Ueki A, Yamaguchi M, Ueki H, Watanabe Y, Ohsawa G, Kinugawa K, Kawakimi Y, Hyodoh F (1994) Polyclonale Aktivierung der T-Zellen durch Silicate in Vitro. *Immunologie*: 82:3332-335.
- [2] Cefali EA, Nolan JC, McConnell WR, Walters DL: Pharmacokinetic study of zeolite A, sodium aluminosilicate, magnesium silicate and aluminum hydroxide in dogs. *Pharm. Res.* 12 (1995), pp. 270–274.
- [3] Ivkovic S, Zabcic D. Antioxidative Therapy: Nanotechnology product TMA-Zeolite reduces oxidative stress in cancer and diabetic patients. *Free Radic Biol Med* 2002;33(Suppl 2):331.
- [4] Ivkovic S, Zabcic D. The effect of tribomechanically activated zeolite (TMAZ) on total antioxidant status of healthy individuals and patients with malignant disease. *Free Radic Biol Med* 2002;33(Suppl 1):172
- [5] Tsuda T, Morimoto Y, Yamato H, Nakamura H, Hori H, Nagata N, et al. Effects of mineral fibers on the expression of genes whose product may play a role in fiber pathogenesis. *Environ Health Perspect* 1997;105(Suppl 5):1173-1178.
- [6] Lim Y, Kim SH, Kim KA, Oh MW, Lee KH. Involvement of protein kinase C, phospholipase C, and protein tyrosine kinase pathways in oxygen radical generation by asbestos-stimulated alveolar macrophage. *Environ Health Perspect* 1997;105(Suppl 5):1325-1327.
- [7] Simeonova PP, Toriumi W, Kommineni C, Erkan M, Munson AE, Rom WN, et al. Molecular regulation of IL-6 activation by asbestos in lung epithelial cells: role of reactive oxygen species. *J Immunol* 1997;159:3921-3928.