Pre-clinical Testing of TMAZ Substance

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Zagreb, 6th of April 1998

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Introduction

The basic raw material used for preparation of TMAZ is a mineral from group of zeolite's with very high capacity of ionic exchange. We micronise the basic raw material by procedure of tribomechanical activation. The procedure increases specific surfaces, increases the capacity of liquid absorption, increases the electrostatic charge of damaged places on powder's surface, releases bound water crystals from volume of mineral structure and increases the capacity of ionic exchange. The attributes of raw material for producing TMAZ were described in patent report to European patent office (München, October, 1997). Until now, this technological process hasn't been used anywhere in the world. Zeolites are miscellaneous family of silicate minerals, colorless, or white and light red colored. The structure is Al – Na or Al – Ca – silicates that melt and lather on higher temperature, and that alkali metals could be exchanged by other ions. This crystal appears mostly in a form of "twins" or "quadrilles". The crystal structure is compound of te SiO_4 and AlO_4 tethraeders that make the basic structure of crystal. The crystals are saturated with small tubules and pores filled with ions (mostly cations, bound to tethraedars) exchangeable with those found in medium. Based on this characteristic, the zeolites were called natural ionic exchanger (permutites). The activation of ionic exchange can be obtained by gradual and slow heating up, which results in reversible separating of crystal water as well as other molecules as ammonium and hydro – sulfate. During the absorption and adsorption and ionic exchange, zeolites show extreme and continuous selectivity, so they are called "molecular sieves".

The knowledge about biological effects are obscure and limited to non - activated minerals. According to the literature available to us, the effects of tribomechanicaly activated zeolites weren't tested so far. The effects of natural or artificial zeolites can be summarized as follows: the protection from toxicological effects, protection from osteoporosis, and anti – microbial effects. It is known that Wa – zeolite A (silicium – dioxide and aluminum – chloride) increases the intensity of mineralization of bones in chicken and increases accumulation of Mn and Al (1). In the same model the therapeutical effect – speed up forming of bones, was seen as well (1). The same synthetic zeolite, treated with acid reduces activity of osteoclasts by three times in period of 24 h after treatment. Reduced activity of katepsin B was also demonstrated. The molecular structure of zeolite makes the base for such effects, although some activity is notable with partially damaged structure. The treatment with this zeolite increases bone formation by two processes: by inhibition of bone resorption and by stimulating the osteoblasts activity (2). Some zeolites show protecting activity against chemical and ra-

dioactive substances. For example, zeolites absorb toxin of cholera and endotoxin of E. coli, which are labile on higher temperatures. Positive effects were also seen in prevention of children's diarrhea (3). Zeolites can be used in protection from poisoning by organophosphorus substances. Zeolite – klinoptilolyte protects from toxic effect of organotiophosphate nerve gas VX. This poison inhibits activity of holinesterasis in erythrocytes, stomach, brain and liver when tested on Wistar rats. Used in concentration of 1g/kg of rat weight klinoptilolyte increased normal enzyme activity in erythrocytes and different organs (with exception of duodenum and large intestines) (4). It was also noted the increased activity of serum alcalic phosphatasis and glutanion of oxaloacetic transaminase, and lowered concentration of Fe at steers' (5). The protecting effect of zeolite on the accumulation of radioactive cesium 137 in sheep's was observed as well (6).

No data are available about effects of zeolites on tumor cells except one showing that the Si-zeolite, during the contact with the surface of Ehrlich's ascitic tumor cells, changes it's own surface chemistry (Al, Mg, Fe) as well as amount of Fe in tumor cells (7). Anti – microbial effect of silver zeolite is also noted. It inhibits the growth of Candidae albicans, Pseudomonas aeruginosa and Staphylococcus aureus (8).

Long lasting usage of zeolites has no toxic or mutagenic characteristics. The inhalation of klinoptilolytes in dosage of 30 or 60 mg/animal didn't increased the appearance of tumor in organs or tissues of Wistar rats (9). The zeolites from Pachensky and Chongurinsky added in food in amount of 5% of feeding mass didn't caused toxic effects on chicken. Growth, development, hematological or biochemical parameters weren't disturbed as well (10).

Considering the way of preparing the substance which is most likely the key of TMAZ action, it's a unique substance, with still unexplored effects on living organisms. It belongs to zeolites family of mostly aluminum and silicate structure. Various zeolites of similar but also of different structure, have been tested for toxicity. Although different results have been obtained, it seems that zeolites of the same composition as TMAZ are not toxic for living organisms. We can't relate this knowledge completely to TMAZ, because it's the way of preparation of zeolite, which is important for its eventual effect on living organism.

Pre – clinical toxicology will be led according to standards and regulations of OECD, which are accepted from large number of European countries and USA. The study includes testing of acute, subchronic and chronic toxicity, which are always carried out on at least on specie of rodents and dogs or monkeys. Testing is usually approached by setting the "limit"

test - applying the high dosage of substance by once, usually 5g/kg orally, or 2g/kg dermal. The dosage can be increased 10 – 100 times, for eventual human's usage. After that, follows so called "up and down" test with 2 – 4 doses, lower than the limit dose and selected to produce a range of effects. If a range of mortality is seen, these data can be used to calculate LD_{50} and $LD_{50/10}$, as well as ED_{50}. Duration of "limit" test as well as "up and down" test is 15 days. In addition to mortality the animals should be monitored for eventual morphological changes and changes in behavior. At the end of the experiment, pathohystologycal analysis of all organs should be done on euthanatized animals as well on those found dead or in moribund condition during the course of the study. The last test is "Range – finding study" which is similar to the previous one. It lasts 4 weeks, and the animals are treated with three different dosages of substance. In this case, except pathohystlohical study, important clinical – laboratory and hematological parameters should be defined. These were test of acute toxicity. The experiments of subchronic and chronic toxicity are carried like the experiments of acute toxicity on both sexes of animals, separately, and last 3 and 12 month. The animals are usually treated with at least 3 dosages of substance. Every month clinical – laboratory and hematological parameters should be determined in addition to hystopathological analysis of all organs. The control group of animals should always be included in the studies; their treatment is designed towards nature and way of applying the substance which is tested.

The toxicity on reproductive system is especially important and should also be tested. These experiments are carried out on animals with short gestation period, but larger number of progeny. For such experiments, mice are appropriate.

The toxicity on gonads is especially examined, as well as toxicity on mice's fetus and his pre- or postnatal growth. It is also important to determine the effect of substance on lactation.

In conditions when TMAZ is applied locally, it's necessary to examine local tolerance and hypersensitivity of organism. For that purpose, different hysto– or cytochemical and enzyme methods are available.

In addition to afore mentioned toxicology studies pharmacokinetic studies should be done as well.

Literature

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tection, 60:358-362, 1997; 4.Mojzis J. and coo.: Veterinary Medicine, 39:443-449, 1994; 5:Bacham S.E. and coo.: Journal of Animal Science, 70:3125-3132, 1992; 6.Jandl J. and Novosad J.: Veterinary Medicine, 40:237-241,1995; 7.Seal s. and coo.: Biological Sciences, 263:943-951, 1996; Matsunra T. and coo.: Journal of Dentistry, 25:373.377, 1997: 9.Tatrai E. i Ungvary G.: Journal of Occupational Medicine and Environmental Health, 6:27-34, 1993; 10.Sorokina E. and coo.: Voprosy Pitaniia, 3:16-18, 1995.

Common goal

To create the base for routine pre–clinical testing of TMAZ and to examine the possible therapeutic effects and to study entire toxicology.

The purpose of suggested research

To resolve the question of TMAZ toxicity: at the level of the whole organism, but also on separate organic systems. To find the doses at which TMAZ can be used without harmful effects on organism. It is especially important to evaluate toxicity of TMAZ on reproductive system of mice, as well as farmacochinetic of TMAZ. It is also very important to show the level of tolerance and sensitivity of organism on TMAZ. All this knowledge will be used for testing of TMAZ as potential regenerating, antiinflammatory, antiallergenic and anticancer substance. Special attention will be put on testing the effectiveness of TMAZ in regenerating processes .

Specific goals of research

To determine the effect of TMAZ on mice, rats and dogs, during the acute subchronic and chronic apply. To determine the dosage of TMAZ which causes LD_{50}, $LD_{50/10}$ and ED_{50}. To determine the effects (if any) of TMAZ on all organic systems and organs (pathohystology). To determine effects of TMAZ on different protein – enzymatic systems of organism. To determine effect of TMAZ on hematological parameters. To determine whether TMAZ has antiinflammatory, antiallergic and anticancer effects. To determine the protective role of TMAZ in UV irradiation, as well as it potential in speeding up the regeneration processes (wounds healing).

Progress Report

We investigated the effects of TMAZ on human tumor cell lines in vitro as well as on the in vivo growth of melanoma B16BL6 tumor in mice. We also investigated the effectiveness of TMAZ in late diabetes mellitus complications. Preliminary acute toxicology study on mice and rats did not show any toxic effects of the substance. Some interesting results have been obtained on cancer patients (TMAZ capsules included in therapy regimen) in United States and Croatia.

According to our preliminary results and experience there are several indications for TMAZ - application: different types of tumor (including terminal cases), different neurological disorders, kahexia, Krone's disease, some viral diseases, paradentosis, etc. To enter into double blinded clinical trial it is necessary to finish acute and subacute toxicology on mice, rats and dogs.

Effect on experimental diabetes mellitus and blood parameters

Diabetes mellitus is a severe metabolic disorder affecting 2-5% of people in Croatia. Although current treatment of primary symptoms of disorder has some success the main obstacle in therapy are so called late complications. From this point of view searching for effective drug in late complications treatment is more than justified. The preliminary experiments in testing the effectiveness of TMAZ in late diabetes mellitus complications gave some very encouraged results. In short - diabetic mice (alloxan 75 mg/kg body weight) were feed during 14 days (starting 7 days after diabetes development) with 50, 100 and 200 mg/animal of TMAZ. The treatment resulted in recovery of diabetic animals body weight (in average, the treated mice were 4g heavier then control, diabetic, non TMAZ treated mice) as well as in improvement of glucose tolerance. After i.v. injection of 1g of glucose per kg of body weight, the blood glucose level normalized in only two hours. TMAZ was also found to be effective in correcting the number of peripheral blood leukocytes lowered dramatically by stress caused by the way of feeding. None of the tested doses of TMAZ caused the hypoglycemic effects. The effectiveness of TMAZ was also tested in another mice experimental model - spontaneous diabetes mellitus which develops as result of autoimmune processes. In this model glucose tolerance test is more like as one in classical diabetes mellitus, although hyperinsulinemia and hyperglycemia are pronounced. In addition TMAZ was also effective in alleviating the clinical signs of autoimmune process.

Effect on experimental tumors

1. *In vitro systems*. Over the past three months TMAZ has been tested for inhibition of MiaPaCa2 (human pancreatic carcinoma), HeLa (human cervical carcinoma) and Hep2 (human laryngeal carcinoma) cell growth *in vitro*.

The preliminary results, showed that TMAZ has growth inhibitory effect on tumor cells [HeLa, MiaPaCa2 and CaCo2 (human colon carcinoma)], while almost none on control (normal) cells [Hef522 (normal human fibroblasts)].

We used several technics for cell number (cell growth) evaluation; 1. MTT test, 2. cell counting and 3. determination of protein content.

Using MTT test and cell counting we noticed pronounced cell growth inhibition of all cell lines tested in drug concentrations of 0.5-50 mg/ml. However, we found both methods inappropriate for in vitro TMAZ testing, because of specific physical and chemical properties of substance. Since it is insoluble and effective in rather high concentrations (0.5-50 mg/ml) it could physically damage cells by scraping them from the plate on which they are growing.

To solve this problem we pretreated the growth medium with different amounts of TMAZ (18 hours/shaking). After that we pelleted the substance by centrifugation, and incubated the cells with such pretreated medium for 72 hours. The results showed the inhibition of cell growth in a dose-dependent manner, being the most effective at the concentration of 50 mg/ml. The strongest inhibition was seen on MiaPaCa2 cells (almost 50% of inhibition), while the growth of Hef522 cells was slightly stimulated (Fig. 1 and 2).

2. In vivo drug evaluation system. Melanoma B16 cells, $1x10^6$, were inoculated i.m. in C57BL mice on day zero. For the next five days the mice were treated with TMAZ, orally, twice a day. Tumor volume was recorded. TMAZ decreased tumor volume and lifespan of mice (Fig. 3).

Effect of TMAZ on spontaneous tumors on dogs

Case # *1, pinch, age 7, male*

Diagnosis: salivary gland tumor, August 1997.

The dog was very calm, had difficulties with swallowing.

Different types of antibiotics, chich were given to him, had no effect.

The application of TMAZ started on January 1998.

Only a week after beginning of tretment, tumor reduced more than 30% and became softer. Four weeks latter, there was no sign of tumor at all.

On latest examination dog shows no signs of disease.

Case #2, bobtail, age 4, female

Diagnosis: autoimune hemolytic anemia.

Very calm (inactive), anemic, had difficulties with breathing.

The cortisone medicaments and cytostatics that were given to her had no effect.

The application of TMAZ started in February 1998.

Two days after beginning of treatment dog started to take food, and the next day acts normally as there is no any disease. At that time the lab - tests weren't show signs of improvement.

Two weeks later, the number of erythroctytes and concentration of hemoglobin were improved.

Case # 3, snautzer, age 8, male

Diagnosis: prostate tumor

Inactive, depressive, refuses food, unable to urinate normally.

Receives antibiotic therapy, but with no effect. Permanent catheter inserted.

The application of TMAZ started in January 1998.

After one week tumor size reduced 30%. Catheter removed.

After one month prostate was normalized, and dog doesn't show any signs of sickness.

Case # 4, poodle, age 11, male

Diagnosis: prostatic and testicular tumor.

Except increased size of testicle (the size of the goose's egg) there are no other visible signs of disease.

The owner refuses operation because of dog's age.

The TMAZ treatment started in March 1998.

A week after beginning of therapy, the testicle is getting smaller, so is the prostate, what is visible on x - ray.

The owner claim that dog is more active now.

Case # 5, half blood, age 13, female

Diagnosis: 5 tumors located on mammary glands (till size of hazelnut)

Dog doesn't show any other signs of disease, except visible tumors.

Surgeon refused operation because of inside tumor progress and the age of animal.

The TMAZ treatment started on December 1997.

Ten days after beginning the treatment, all tumors have disappeared.

Three months later, there are no more visible changes on location glands.

Case studies of cancer patients in United States using TMAZ capsules in their treatment regimen

1. Case #1 - 70 year old male subject with prostate cancer.

Diagnosed with prostate cancer in April of 1997.

His prostate specific antigen (PSA) count was 23 in April 1997 and increased to 40 in October of 1997.

Received an implant in his stomach (hormone therapy) in February 1998 to prepare him for radiation therapy.

Began taking TMAZ in November of 1997. His PSA levels continued to drop monthly after beginning the TMAZ therapy.

His latest PSA count taken in mid-March shows a level of 2.4. Patient has made a decision to not take radiation treatment.

His general condition is very good. He has put on weight and reports that he feels better now than he has for two years.

2. Case # 2 - 53 year old male subject with cancer of the liver and kidneys.

Cancer in the kidney was detected 1.5 years ago during a surgery for kidney stones.

After surgery it was discovered through a biopsy that the cancer has migrated to the liver.

Doctors determined that there was no possibility of using chemotherapy treatment.

Subject's condition deteriorated further. He lost 35 pounds. He was unable to walk more than just ten feet without having to sit down.

Began taking TMAZ capsules in November (8 per day). Within one week he noticed that his skin color was no longer a yellowish color and had returned to its normal color.

Subject has put back all of the weight he lost and his overall health continues to improve. He can now walk over one mile without a problem with fatigue.

Subject was being treated by the Veteran's Administration Medical Services. He is now going to visit the Mayo Clinic for final tests to be certain the cancer is in remission. He now feels like he is in perfect health.

3. Case # 3 - 55 year old male subject with lung cancer.

Patient was diagnosed with lung cancer approximately six (6) months ago. Has been a cigarette smoker for many years.

Doctors put subject on chemotherapy treatment and his body had very bad reactions to the chemotherapy. He decided to stop the chemotherapy treatment in February, 1998.

Patient was in a very weak condition as a result of the chemotherapy plus his body had lost substantial weight.

Began taking capsules approximately one (1) month ago (February, 1998).

Immediately he noticed that his facial color improved and his overall health improved.

Subject continues to take the capsules and will have a complete examination (physical) in next two weeks with a scan to determine the status of the cancer.

4. Case # 4 - 50 year old male subject with Krone's Disease.

Patient was operated in 1974 (age 26) for short bowel syndrome (at Mayo Clinic). An illeostomy was performed at that time. Krone's disease and chronic fistulas were diagnosed.

Has suffered with this disease for many years.

Recently was hospitalized for treatment of prostatitis (February 1998). Doctors examined his bowel during this hospitalization and discovered that there was no evidence of the Krone's disease in the bowel. Subject has been taking TMAZ capsules since December, 1998 and the doctor's attribute the absence of the Krone's Disease to the TMAZ.

5. Case # 5 - 68 year old male subject with colon and lung cancer.

Cancer was first discovered in November of 1996 during a colonoscopy operation. The surgeons removed 6 inches of colon and a large cancerous growth.

In December of 1996 it was determined through diagnosis that the cancer had spread to the liver and lungs.

Started chemotherapy treatment in January of 1997 and this continued through 1997 with very little improvement according to CAT scans performed.

Began taking TMAZ in November of 1997. CAT scan on December 2 showed no sign of the cancer. His physical condition has also improved greatly since taking the TMAZ.

Case studies of cancer patients in Croatia using TMAZ capsules in their treatment regimen

Case #1 - 77 year old female subject with renal carcinoma (hypernephroma lat. sin)

Patient was diagnosed with large expansive tumor in upper sinister part of kidney (75x62 mm). Tumor penetrated renal capsule and infiltrated into vagina (3.5x1.5 cm). patient is not able to seat. She does not allow surgery and chemotherapy. In very bad mental and physical condition, unable to walk. Began taking TMAZ capsules in March 3 (3x2 daily 400 mg increasing than 4x3). Within tow days she was able to walk and seat. She feel much better Her general condition is very good. She has put on weight and reports that she feels better since taking the TMAZ.

Case # 2 - 34 year old male (lymphosarcoma femoris l. sin, multiple metastases)

Patient was diagnosed in December 12. 1997. CT tumor localized in basis of thorax, paravertebral region (8 cm) with multiple metastases in abdomen (16 cm), near kidney and spleen. Relatively big tumor mass in bladder (5 cm). Patient was in very weak condition as a result of chemotherapy and irradiation. He decided to stop the chemotherapy. He was unable to walk Began taking TMAZ 20. February 1998. (3x1 capsule). Doses was increased daily. Today he is taking 6 capsules every 3 hours. Immediately he noticed improvement, he has put on weight, his facial color improved and his overall health improved. 10 days upon he could walk from tumor mass was reduced dramatically. His blood and biochemical parameter were improved.

Case #3 - 55 year old female

Surgically removed (1996) cervical carcinoma. Recidive of tumor with multiple bone metastases. Tumor prolaps (~ 9x9 cm) from vagina.

After 2. cycles of chemotherapy she began taking TMAZ (20.01.1998.) capsule and powder directly. Immediately 03.02.1998. tumor mass reduced 50%. She feels pain relieve in abdomen. She now feels much better. Her general condition has also improved greatly since taking the TMAZ. Today she insists to take TMAZ 4 capsules every 2 hours.

Case #4 - 77 year old male. Subject with pancreatic cancer and diabetes mellitus.

Patient was diagnosed in September 1997, stent implanted 6. January 1998. After treatment of diabetes he was released from the hospital without any specific anticancer therapy.

During his stay in hospital patient began taking TMAZ 20. January 1998. 3x2 capsules increasing dose every day. Today he is taking 5 capsules each 2 hours. Upon release from hospital he feels much better, he walks. His general condition is very good.

Case #5 - 45 year old male subject with disseminated tumor in retroperitoneum, ileum, duodenum, head of pancreas and diffuse liver and peritoneal metastases. He received 6 cycles of chemotherapy.

He is taking 3x3 capsules and feels much better upon chemotherapy. After increases of doses 4x4 he stated with physical activities. His overall health continues to improve.

Hypothesis

We assume that tested substance, TMAZ, will not show any toxic effects. In respect to we this expect eventual therapeutic effects on inflammations and tissue regeneration.

It is reasonable to assume that after oral usage TMAZ does not enter the organism, but is simply excreted by feces. However, considering TMAZ particle's size (between 1 and 5μ) it is possible that is absorbed partially in intestines, enters the portal blood and further the other body compartments. If enters to blood circulation, we can expect to be ingested by macrophages in liver (phagocytosis), and perhaps in other organs as well. Considering that TMAZ is inorganic particle, it's possible that after entering the cell, it stays inside without disposing out of body.

Methods

TMAZ application: per as and intraperitoneally. Resection of animals – pathohystology (adrenal glands, brain, esophagus, heart, kidneys, small intestine, large intestine, liver, lungs, lymph nodes, ovaries, pancreas, prostate, salivary glands, spleen, stomach, groins, timus, thyroid gland, parathyroid glands, larynx, bladder, uterus, fetus in experiment with toxicity on reproductive system).

The analysis of clinical – laboratory parameters from blood and serum (alkaline phosphatase, alanine aminotransferase, aspartate, aminotransferase, gamma-glutamiltransferase, glucose, creatinine, bilirubine, urea, total proteins, albumine, electrolite). Determination of hematological parameters in peripheral blood (number of erythrocytes, hematocryte, number of leukocytes, differential blood picture, blood coagulation) and organs that hematopoietic organs (bone marrow and spleen).

Hysto- and cytochemical methods for monitoring circumference and type of tissue damage (irritation, inflammation, allergy, and transformation). Measuring of HO with help of antibodies, or determination of mRNA for this enzyme.) Isolation and testing of kB (NFkB) nuclear factor and his inhibitor (IkB). Testing of interleukine presence: IL1, IL6 and TNF alpha (antibodies / ELISA test). Testing of protein synthesis in liver. protein purification - A

sepharosis. Cytochemical test of feritine on lymphocytes as measure for lymphocytes activity. The inhibition of migration of macrophages or testing for presence of MIF (migration inhibiting factor), testing of late sensitivity (DTH) or test with SRBC. Tests for anticancer effect of TMAZ; appearance of tumor induced by chemical cancerogene in the presence or absence of TMAZ.

Tracing the number of offsprings and their lethality in perinatal and postnatal period. TMAZ will be applied to mice by probe or by mixing with food. Urine and feces samples collection by keeping the animals in metabolic cages.

Nuclear microanalysis of substance in particular organs, feces, urine etc. will be done by Van der Graaf ‘s accelerator.

Protocol and research plan

All research of common toxicity will be tested on two sorts of rodents: mice and rats, also on dogs, both sexes separately. The substance will be given to the animals by probe (per os), and intraperitonealy. Two grams per mouse will be given orally, one-time (limit test) and appropriate dosages will be given to dogs and rats. In i.p. appliance the dosage will be 2 mg by mouse and appropriate dosage for dogs and rats. In this case viability of animals will be monitored. The following will be "up and down" test and "range finding study" which last 4 weeks. The dosages will be determined according the result of "limit" test. If the dose, used in test is non – toxic, following dosages: 100, 150, 200 mg per mouse (appropriate for rats and dogs), will be applied orally 4 times daily. Following criteria will be monitored:

a) viability
b) variation of weight (comparing the weight before treatment and during the treatment by every week measuring)
c) variation of clinical – laboratory and hematological parameters (at the end of fourth week)
d) patohystological changes on different organs (at the end of 4th week)
e) biochemical and morphological changes of urine.

The same experiment will be repeated for intraperitoneal usage of TMAZ, but with different dosages: 0.5, 1 and 2 mg per mouse (also appropriate for dogs and rats), 4 times daily. The experiments of subchronic and chronic toxicity will be designated in same way, but these parameters (except weight variation) will be monitored, every month. Animals treated with saline and also with quarcite sand (non – zeolite source, but manufactured same as TMAZ) will be used for control experiments.

During the examination of toxicity, TMAZ will be applied also locally (on skin and eyes). TMAZ (in form of powder) or TMAZ mixed with neutral ointments and oils will be applied on shaved and non – damaged skin of animal (standard procedure). One side of the animal's body (or one eye) will be used for testing TMAZ and the other side, for control. The same tests should be done on mice, rats and/or rabbits and dogs. The duration of appliance will be set predicted time according to treatment on humans, but no longer than 4 weeks. The efficiency of one-time, high dosage of TMAZ will be tested. Possible changes after one local application could be none, redness of skin, swell, desquamation and inflammation (infective or non- infective). After repeated appliance might appear atrophy, benign or malignant tissue

swell, pigmentation (mostly because of photosensitivity or phototoxicity), hypersensitivity (allergic reactions), alopetia (hair falling on treated place). Since the function of this substance is not known, we need to take in to account all the possibilities and possible effects in order to make appropriate treatments. During this research, the TMAZ will be tested in combination with familiar local allergens, carcinogens and by exposing to UV–light and ordinary light (artificial) in order to examine antiallergic, antiinflammatory and anticancer capacity of TMAZ. Further, we want to examine how it works in a process of tissue regeneration (wounds healing) and photosensitivity.

To test the effect of TMAZ on reproduction, mice of both sexes will be fed with TMAZ 60 and 15 days before conception, depending on sex, and during next 42 days. During experiments, certain number of animals will be sacrificed for determination of eventual gametotoxicity, toxicity during organogenesis (fetal period). Some of the animals will be sacrificed in postnatal period in order to trace eventual toxicity during the postnatal period.

For experiments which concern dog reproduction, the TMAZ treatment will be similar as previous. The substance will be applied once per day (orally) in dosage from 1g/kg during 15 days, or 4 weeks, for examination of acute toxicity, during 3 month for examination of subchronic toxicity and during a year for examination of chronic toxicity.

Expected results

We expect that TMAZ is non-toxic, even completely non- harmful, in shorter or longer period of its appliance.

Significance

This research is very important, because, it will show if the appliance of TMAZ is possible without harmful effects on organism and if TMAZ causes inflammations and hyper-sensitivity. The examinations will give the answer if TMAZ regenerates tissue, or if heals wounds. By solving these questions we will create base for other pre – clinical research.

Application of research

The research has direct clinical appliance.

The leader of project:
Prof. dr. Krešimir Pavelić

Fig. 1.

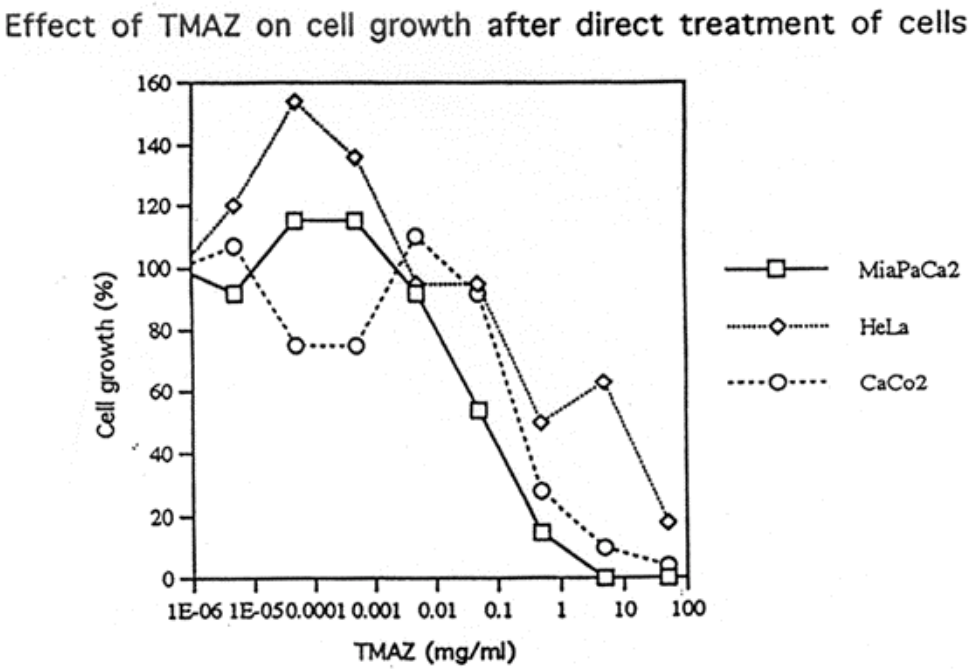
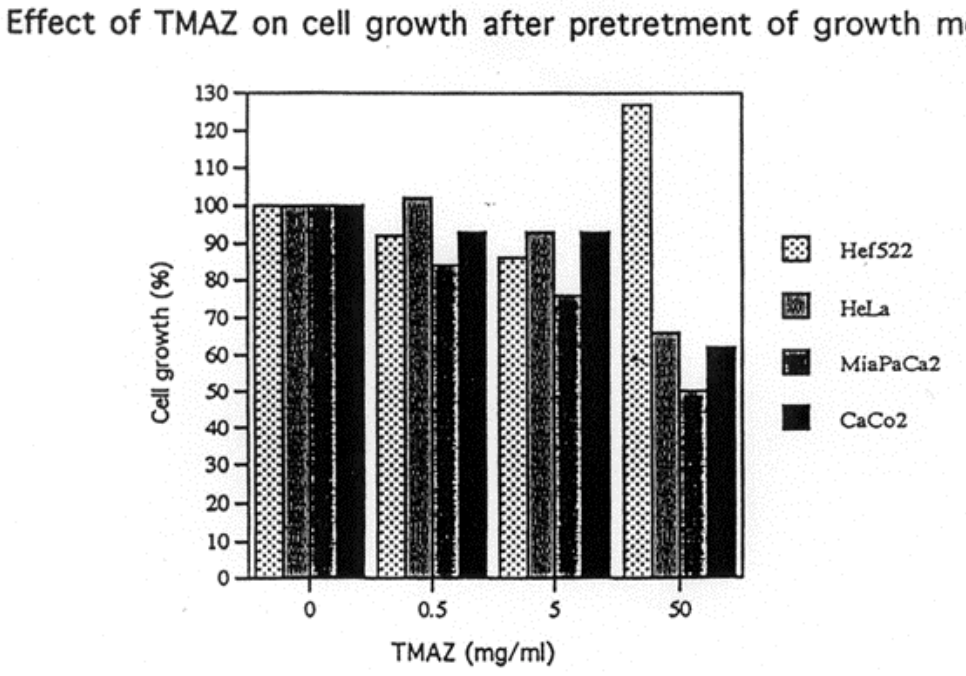

Effect of TMAZ on cell growth after direct treatment of cells
Cell growth (%)
160
140
120
100
80
60
40
20
0
1E-06 1E-05 0.0001 0.001 0.01 0.1 1 10 100
TMAZ (mg/ml)
MiaPaCa2
HeLa
CaCo2

Fig. 2.


Effect of TMAZ on cell growth after pretretment of growth medium
Cell growth (%)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
0.5
5
50
TMAZ (mg/ml)
Hef522
HeLa
MiaPaCa2
CaCo2

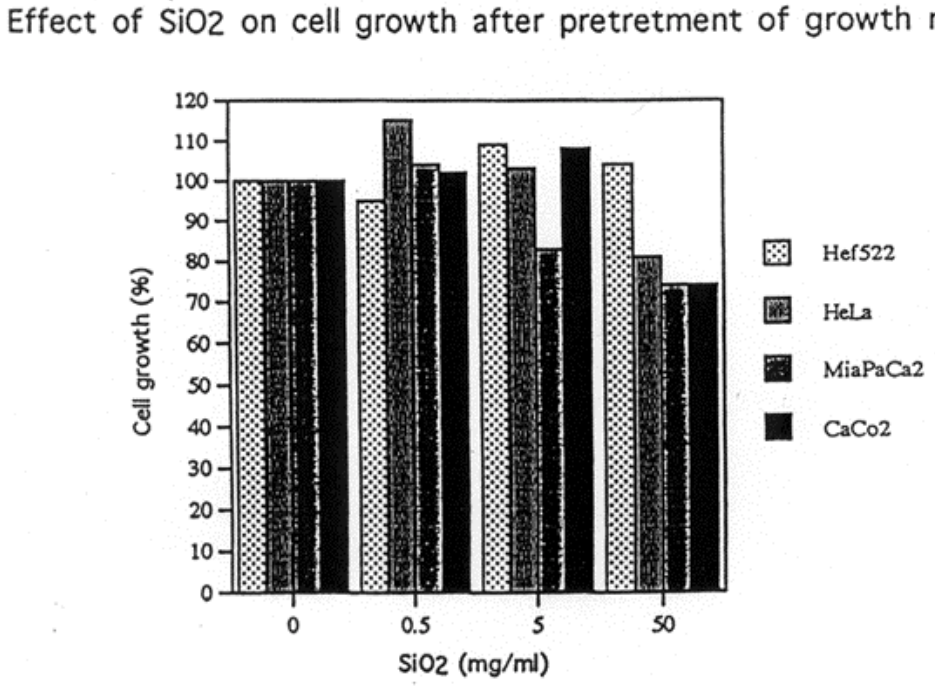
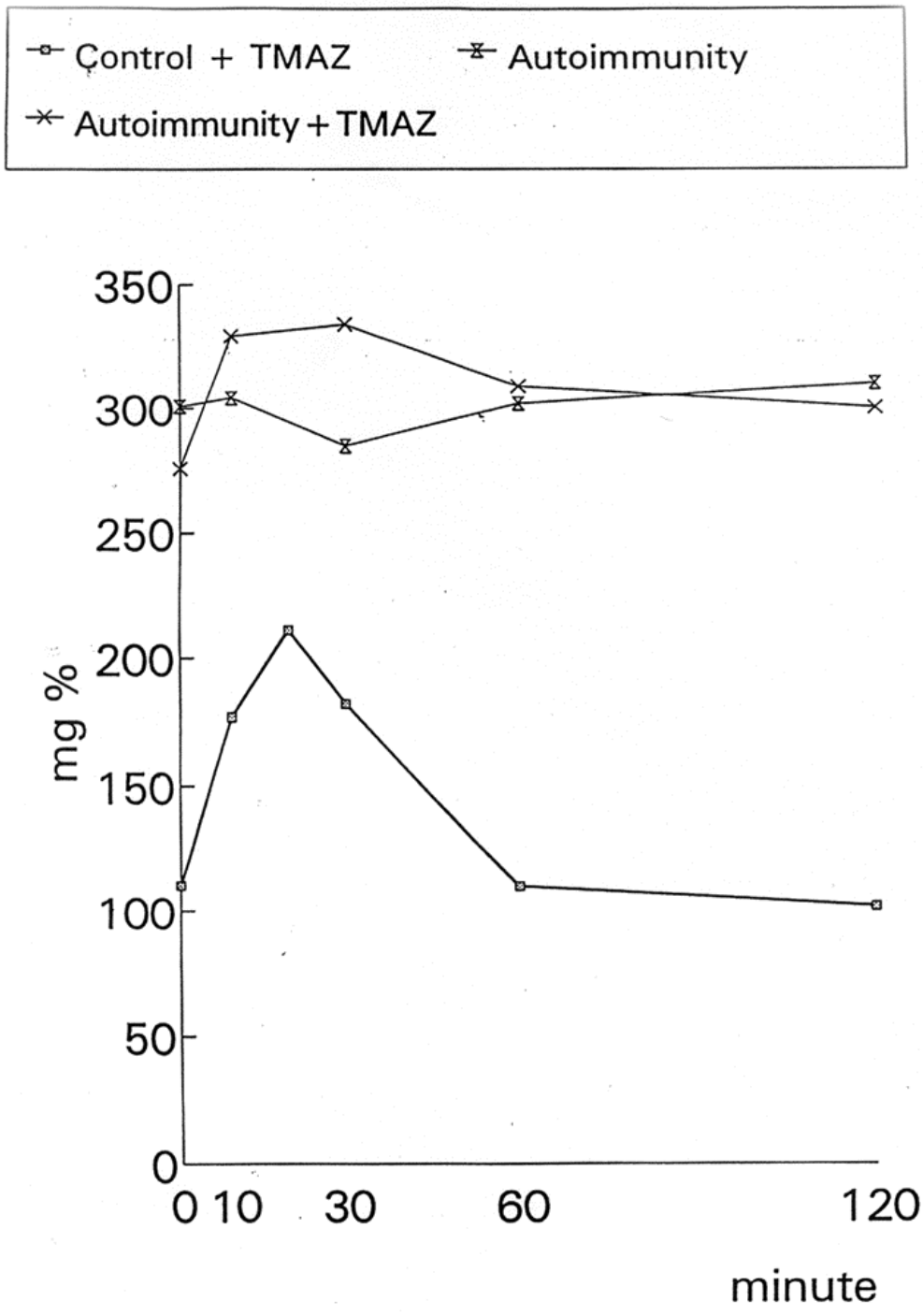

Effect of SiO2 on cell growth after pretretment of growth medium
Cell growth (%)
120
110
100
90
80
70
60
50
40
30
20
10
0
0
0.5
5
50
SiO2 (mg/ml)
Hef522
HeLa
MiaPaCa2
CaCo2

Fig. 3.



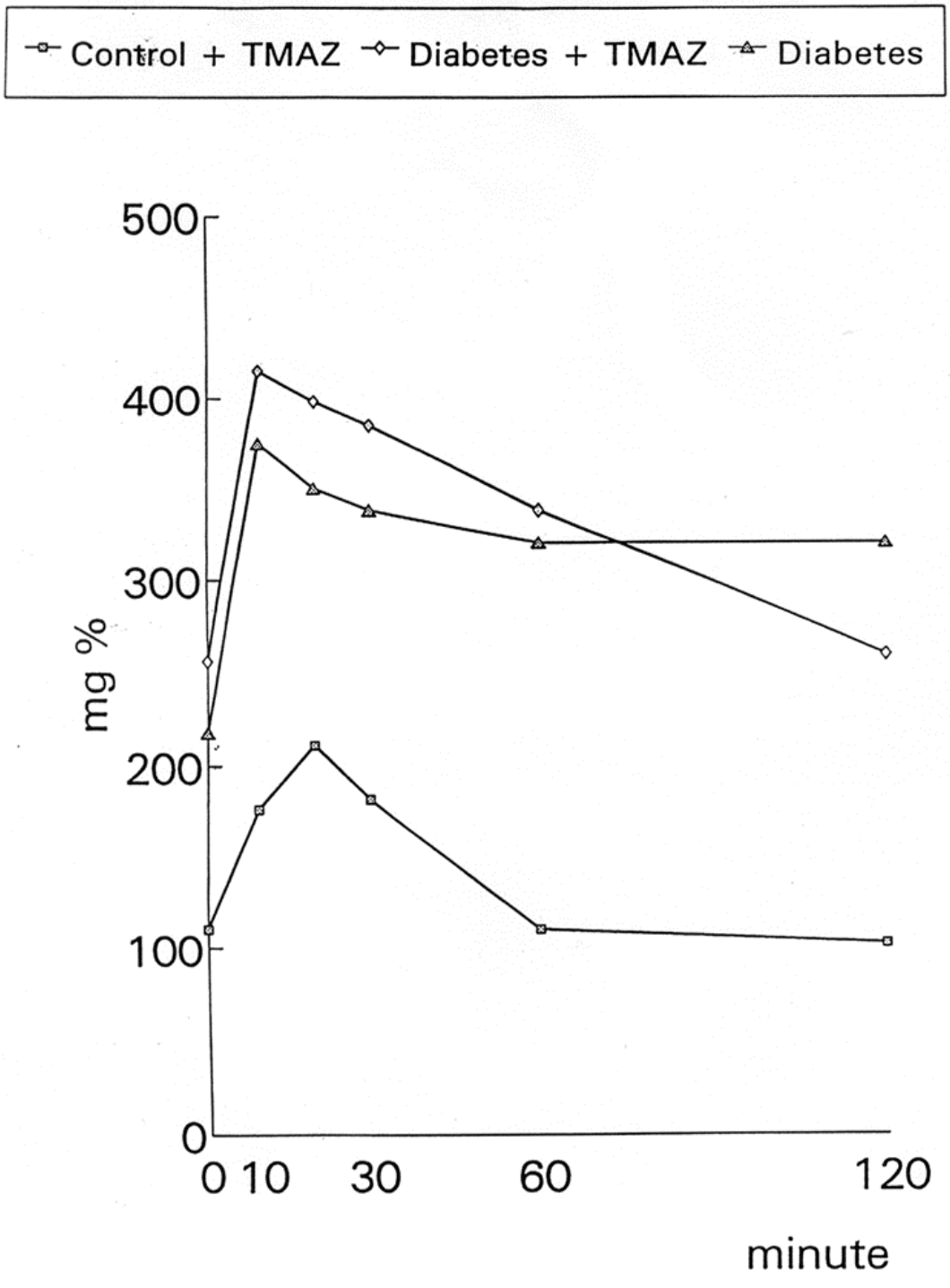
Blood glucose concentration in NOD mice

Fig. 4.

Body weight of CBA mice before and after treatment

Group of mice	Body weight (g)
Control	33.75
Diab. + O	24.6
Diab. + TMAZ	28.2

Fig. 5.



Blood glucose concentration in CBA mice

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