

# SYNERGISM IN COMBINED USE OF ORAL ANTIHYPERGLYCEMIC DRUGS

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**The aim of the investigation** was to study the capabilities of combined use of metformin and vildagliptin, and its effect on glycemia and oxidative stress parameters in patients with newly diagnosed type 2 diabetes mellitus (DM 2).

**Materials and Methods.** The study involved 37 patients with DM 2, they were divided (after two-week metformin dose titration) into two groups by simple random sampling. Group 1 patients (n=18) were administered metformin at a dose of 1700 mg a day, group 2 patients (n=19) — the combination of metformin (1000 mg a day) with vildagliptin (50 mg a day). Group 3 — a control group (n=20) consisted of subjects without carbohydrate metabolism imbalance. We studied glycemia indices (fasting blood plasma glucose, glycosylated hemoglobin), the level of molecular products of lipid peroxidation (diene and triene conjugates, malon dialdehyde), intensity of oxidative protein modification by the level of carbonyl derivatives, superoxide dismutase and catalase activity before and 3 months after therapy.

**Results.** Combined use of metformin (1000 mg a day) and vildagliptin (50 mg a day) in patients with newly diagnosed DM 2 showed the synergism of their antihyperglycemic and antioxidant effect. The mentioned combination of drugs compared to metformin monotherapy (1700 mg a day) enables to reduce side effect rate and significantly increase catalase activity.

**Conclusion.** The results of combined use of metformin and vildagliptin demonstrated the capability of DM 2 therapy based on rational choice of the combination of antihyperglycemic drugs relying on their synergistic properties.

**Key words:** oral antihyperglycemic drugs; pharmaceutical synergism; newly diagnosed diabetes mellitus.

Type 2 diabetes mellitus (DM 2) needs early, timely and adequate therapy from the disease onset. Metformin is deservedly considered to be an agent of choice to initiate the therapy. Its basic mechanism is in the increased glucose intake by muscular and adipose tissue cells due to insulin binding to receptors and increased activity of glucose transporters GLUT-1 and GLUT-4 [1]. Currently, both antihyperglycemic and antioxidant characteristics of the agent have been proved to have a significant pleiotropic effect since oxidative stress plays an important role in DM 2 pathogenesis and progression. Metformin molecule was found to be able to inhibit the formation of advanced glycation end products, known as AGEs, bind alpha-oxoaldehydes (methyl glyoxal and glyoxal) regardless of their antihyperglycemic effect. Metformin molecule instead of the molecules of such amino acids as lysine, arginine, cysteine also binds to malone dialdehyde (MDA). Therefore, protein structures are prevented from damage by lipid peroxidation (LP) products, and there is no oxidative protein modification (OPM) [2].

The role of gastrointestinal tract in DM 2 pathogenesis was considered as early as in XIX c., but only at the turn

of XX c. there was discovered the so called disturbed incretin response in DM 2 patients related to inadequate insulin secretion on oral glucose load [3]. Glucagon-like peptide-1 (GLP-1) is incretin stimulating insulin release in response to food glucose loading. It is reduced incretin response to glucose loading that has been proved [4] to underlie DM 2 pathogenesis rather than the excess of dipeptidyl peptidase-4 (DPP-4) destroying it. And only in the process of a long-term hyperglycemia and insulin-resistance increase, DPP-4 starts to accumulate and destroy a small amount of GLP-1 that, in its turn, makes a certain contribution to DM 2 progression.

Vildagliptin is one of the first preparations from dipeptidyl peptidase-4 (iDPP-4) inhibitors preventing from incretin breakdown in a patient's body, the mechanism of antihyperglycemic effect of which is well studied [5].

Despite metformin and vildagliptin have different antihyperglycemic mechanisms, there is one general target — GLP-1. Experimental and clinical studies have showed that metformin is supposed to raise GLP-1 level by its synthesis growth having no effect on DPP-4 [6–8]. The preparation causes direct enhancement of secretory function of intestinal L-cells by increasing transcription of

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gene-receptors to GLP-1 [9], as well as reduces renal excretion of GLP-1 [10].

Thus, all mentioned mechanisms indicate unidirectional antihyperglycemic effect of metformin and vildagliptin.

As discussed above, metformin is a first line drug in a long-term multi-stage transition from one combination to another in DM 2 therapy. In practice, DPP-4 inhibitors are unreasonably administered a few years later, when the destruction process of the pancreas involves a considerable part of beta-cells, and protective effect of iDPP-4 has no opportunity to approve oneself [11].

In addition, there still remain unstudied the problems of synergism of metformin and iDPP-4 (vildagliptin, in particular) effect in their combined use in patients with a short period of the disease, as well as the effect of the mentioned combination on oxidative stress regardless of their antihyperglycemic effect.

**The aim of the investigation** was to study the capabilities of combined use of metformin and vildagliptin, and its effect on glycemia and oxidative stress parameters in patients with newly diagnosed type 2 diabetes mellitus.

**Materials and Methods.** The study was carried out on the clinical site of N.A. Semashko Regional Clinical Hospital and laboratory facilities of Nizhny Novgorod State Medical Academy. 57 patients including 37 patients with DM 2 and 20 patients without metabolic imbalance were examined.

The entry criteria for DM 2 patients were the following: a proven DM 2 case — not to exceed 2 years, age — from 40 to 70 years, glycosylated hemoglobin — from 6.5 to 7.5%, body mass index (BMI) — up to 40. Exclusion criteria were the following: severe DM 2 sequelae, hepatic, renal and cardiovascular abnormalities, the beginning of taking antihyperglycemic drugs later than 3 months before the study initiation.

The study complies with the declaration of Helsinki (adopted in June, 1964 (Helsinki, Finland) and revised in October, 2000 (Edinburg, Scotland)) and approved by the Ethics Committee of Nizhny Novgorod State Medical Academy. Written informed consent was obtained from all patients.

Table 1  
Characteristics of the subjects involved in the study (Me [25; 75])

Parameters	Comparison group	DM 2 patients
Sex (male/female)	10/10	19/18
Mass, kg	88.0 [82.0; 93.0]	88.15 [82.50; 97.75]
BMI	30.05 [29.37; 33.85]	32.65 [28.50; 34.50]
Age, years	57.0 [54.0; 69.0]	60.5 [56.5; 63.5]
FBG, mmol/L	4.45 [4.25; 4.80]	6.50 [6.10; 8.00]*
HbA1c, %	4.90 [4.10; 5.00]	7.20 [6.60; 7.50]*

Note: \* — statistical difference in relation to a control group (p<0.05)

The patients involved in the study (after two-week metformin dose titration) were divided into two groups by simple random sampling. Group 1 patients (n=18) were administered metformin (Glucophage) at a dose of 1700 mg a day, group 2 patients (n=19) — the combination of metformin, 1000 mg, and vildagliptin (Galvus), 50 mg a day. Group 3 — a control group (n=20) — consisted of subjects without carbohydrate metabolism imbalance (Table 1).

We studied glycemia indices: fasting blood glucose (FBG) level in capillary blood by glucose oxidase test on an analyzer Biosen 5030 (Russia), and glycosylated hemoglobin (HbA1c) by liquid chromatography on a chromatograph Bio-Rad 680 (Bio-Rad Laboratories, USA) with standard kits (France).

The concentration of LP molecular products — diene (DC) and triene (TC) conjugates, MDA was studied spectrophotometrically on an analyzer Helios (Thermo Spectronic, USA).

Moreover, we estimated OPM intensity by the level of carbonyl derivatives revealed in the reaction with 2,4-dinitrophenylhydrazine — aldehydedinitrophenylhydrazones (ADPH) and ketonedinitrophenylhydrazones (KDPH), which are respectively early and late markers of oxidative protein destruction according to E.E. Dubinina (1995).

The activity of superoxide dismutase (SOD) was determined according to M. Nischikimi method (1972) modified by E.E. Dubinina et al. (1988), catalase (CAT) — according to Y. Aebi technique (1970) modified by M.A. Korolyuk et al. (1988), S. Chevari et al. (1991). Optical density was measured on a spectrophotometer CФ-2000 (Russia).

The object of the research was blood and plasma of patients and control subjects. The above mentioned indices were studied when the patients were involved in the study and 3 months after continued taking of the drugs.

The results were processed by conventional statistical techniques using Statistica 10.0 (v10.0.228.8 STA999K347150-W, StatSoft Inc., USA). Distribution of samplings was assessed by Shapiro–Wilk and Kolmogorov–Smirnov tests. Since all the samplings under study had distributions different from normal, we used nonparametric Wicoxon and Mann–Whitney criteria to determine statistical significance of the differences between dependent and non-dependent samplings respectively. The data were presented as median, lower quartile (25th percentile) and upper quartile (75th percentile).

**Results and Discussion.** Dynamics of glycemia indices studied after 3-month treatment was unidirectional in both groups: a metformin monotherapy group and a combined therapy group. The patients of both groups had stable significant decrease of fasting plasma glucose

Table 2

## Dynamics of glycemic indices, side effect rate and patients' compliance (Me [25; 75])

Index	Metformin (n=18)			Metformin with vildagliptin (n=19)		
	Before treatment	3 months after therapy	p	Before treatment	3 months after therapy	p
FBG, mmol/L	6.50 [6.11; 8.00]	5.55 [4.95; 5.95]	0.023	6.55 [6.15; 7.55]	5.60 [5.33; 6.70]	0.018
HbA1c, %	7.00 [6.30; 7.50]	6.00 [4.90; 7.00]	0.009	7.1 [6.10; 7.40]	5.80 [4.50; 6.90]	0.005
Side effects	In 6 subjects			In 1 subject		
Readiness to continue therapy	15 subjects			19 subjects		

Note: p — the level of statistical significance of differences between the samplings "before treatment" and "3 months after therapy".

and glycosylated hemoglobin levels in relation to the initial value (Table 2) that is consistent with literature data [1, 5].

It is worth mentioning that the degree of blood plasma glucose decrease in a combined therapy group did not significantly differ from that in a metformin monotherapy group. Antihyperglycemic effect similar to monotherapy is likely to be reached against a combined therapy at a lower dose of metformin that indicates not just unidirectional as shown by other researchers [5, 10] but a synergistic antihyperglycemic

action of metformin and vildagliptin. Moreover, combined use of metformin and vildagliptin contributes to reduced rate of side effects (diarrhea, tympanitis) and increased patients' treatment compliance (See Table 2).

The results of a examination indicated that even a short duration of the disease in DM 2 patients triggers a "cascade" of oxidative stress reactions (Fig. 1) — other researchers have also found it [2]. The signs of oxidative stress are the following: the increment in LP activity, OPM intensification, a decreased activity of antioxidant system.

After 3 months of metformin administration, as well as the combination of metformin and vildagliptin we recorded a reduced level of LP molecular products (DC and TC) in relation to the initial level (DC:  $p=0.017$  — in a group with metformin;  $p=0.006$  — in a group with metformin and vildagliptin; TC:  $p=0.033$  and  $p=0.02$ , respectively). However, there were no significant differences in the dynamics of the mentioned indices in the groups of mono- and combined therapy, despite different doses of metformin (Fig. 2).

MDA level also decreased in relation to the first examination data in patients of both groups ( $p=0.0004$  — in a group with metformin;  $p=0.0007$  — in a group with metformin and vildagliptin), there being no significant difference between the groups (See Fig. 2).

There was found the tendency towards a decreased

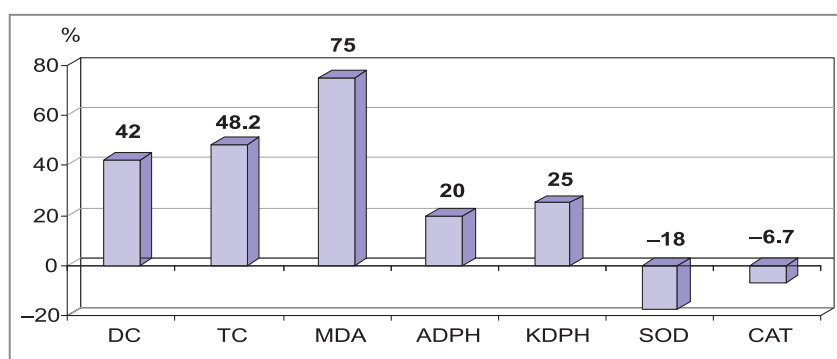


Fig. 1. The excess of LP, OPM molecular products content, and decreased activity of antioxidant enzymes in patients with newly diagnosed DM 2 in relation to a control group in a primary study

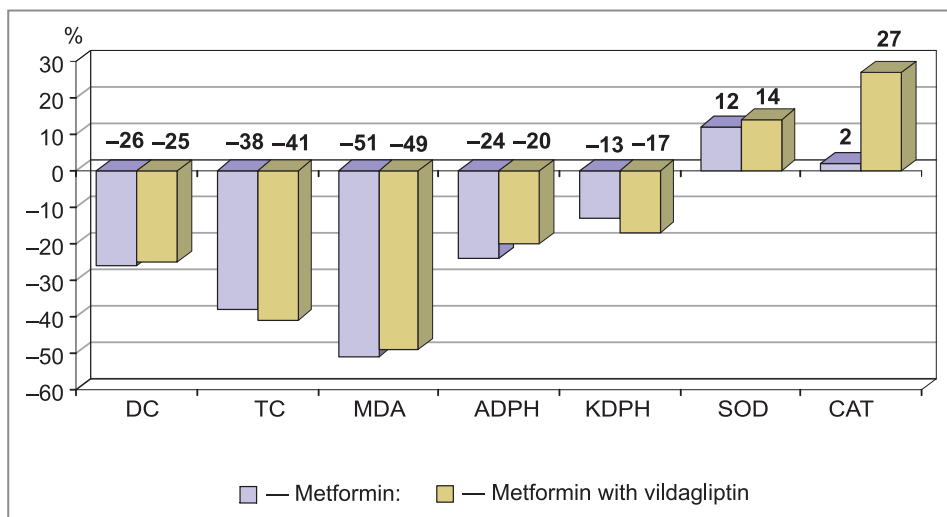
formation of OPM products — aldehyde- and ketone-dinitrophenylhydrazones — in both groups, with no statistical difference between the groups.

Moreover, both metformin monotherapy and its combination with vildagliptin promoted the activity of antioxidant enzyme system (SOD and CAT) concerning the initial level.

In particular, both groups demonstrated increased SOD activity ( $p=0.035$  — in a group with metformin;  $p=0.01$  — in a group with metformin and vildagliptin) (See Fig. 2). In addition, intergroup differences in the dynamics of the above-noted index were not statistically significant.

However, in a group of combined therapy (metformin and vildagliptin) there was a sharp increase of catalase activity (twofold) in relation to the initial level ( $p=0.03$ ), while in a monotherapy (metformin) group such changes appeared to be statistically insignificant. This fact has been proved by the findings of other researchers [12].

Oxidative stress control is known to consist not only in LP and OPM activity decrease but also in antioxidant enzyme system stimulation. While an antioxidant effect of metformin is able to get out LP products directly of the body [2], the mechanism of vildagliptin effect on oxidative stress is not related to glycemia normalization, as was shown above [3], nor yet the increased activity of antioxidant enzymes, in particular, catalase, which in its



**Fig. 2.** Dynamics of LP, OPM indices and activity of antioxidant enzymes 3 months after therapy in relation to the results of a primary study

turn, prevents from MDA appearance — one of integral markers of oxidative stress [13].

The findings indicate that both metformin and its combination with vildagliptin equally effectively reduce the intensity of LP and OPM processes increasing the activity of antioxidant enzymes. Therefore, a lower dose of metformin as a part of a combined therapy has the similar to monotherapy effect indicating the synergism of an antioxidant effect of the drugs under study. In addition, the combination of metformin and vildagliptin more significantly than metformin monotherapy increases the activity of the second stage of an antioxidant protection — catalase.

**Conclusion.** Combined use of metformin, 1000 mg, and vildagliptin, 50 mg a day, in patients with newly diagnosed type 2 diabetes mellitus shows the synergism of their antihyperglycemic and antioxidant effect. The mentioned combination of drugs compared to metformin monotherapy (1700 mg a day) enables to reduce side effect rate and significantly increase the activity of the second stage of antioxidant protection — catalase.

The findings enable to optimize the therapy of patients with newly diagnosed type 2 DM based on a rational choice of the combination of antihyperglycemic drugs taking into account their synergistic properties able to reduce a therapeutic dose and side effect rate, increase patients' treatment compliance and prevent the disease progression in the examined cohort of patients.

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## References

1. Nikonova T.V. Slow-release Metformin (Glucophage Long) in management of type 2 diabetes mellitus. *Farmateka* 2012; 3: 49.
2. Esteghamati A., Eskandari D., Mirmiranpour H., et al. Effects of metformin on markers of oxidative stress and

antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin Nutr* 2013; Apr; 32(2): 179–185, <http://dx.doi.org/10.1016/j.clnu.2012.08.006>.

3. Rizzo M.R., Barbieri M., Marfella R., Paolisso G. Reduction of oxidative stress and inflammation by blunting daily acute glucose fluctuations in patients with type 2 diabetes: role of dipeptidyl peptidase-IV inhibition. *Diabetes Care* 2012 Oct; 35(10): 2076–2082, <http://dx.doi.org/10.2337/dc12-0199>.

4. Pala L., Ciani S., Dicembrini I., et al. Relationship between GLP-1 levels and dipeptidyl peptidase-4 activity in different glucose tolerance conditions. *Diabetic Med* 2010; 27(6): 691–695, <http://dx.doi.org/10.1111/j.1464-5491.2010.03010.x>.

5. Hamamoto S., Kanda Y., Shimoda M., et al. Vildagliptin preserves the mass and function of pancreatic  $\beta$  cells via the developmental regulation and suppression of oxidative and endoplasmic reticulum stress in a mouse model of diabetes. *Diabetes Obes Metab* 2013; 15: 153–163, <http://dx.doi.org/10.1111/dom.12005>.

6. Hinke S.A., Kuhn-Wache K., Hoffmann T., et al. Metformin effects on dipeptidylpeptidase IV degradation of glucagon-like peptide-1. *Biochemical Biophys Res Commun* 2002; 291: 1302–1308, <http://dx.doi.org/10.1006/bbrc.2002.6607>.

7. Yasuda N., Inoue T., Nagakura T., et al. Enhanced secretion of glucagon-like peptide-1 by biguanide compounds. *Biochemical Biophys Res Commun* 2002; 298: 779–784, [http://dx.doi.org/10.1016/S0006-291X\(02\)02565-2](http://dx.doi.org/10.1016/S0006-291X(02)02565-2).

8. Mannucci E., Ognibene A., Cremasco F., et al. Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care* 2001; 24: 489–494.

9. Maida A., Lamont B.J., Cao X. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor- $\alpha$  in mice. *Diabetologia* 2011 Feb; 54(2): 339–349, <http://dx.doi.org/10.1007/s00125-010-1937-z>.

10. Halimi S., Schweizer A., Minic B., et al. Combination treatment in the management of type 2 diabetes: focus on vildagliptin and metformin as a single tablet. *Vasc Health Risk Manag* 2008; 4(3): 481–492.

11. *Algoritmy spetsializirovannoy meditsinskoj pomoshchi bol'nym sakharnym diabetom* [Algorithms of specialized



medical aid for diabetic subjects]. Pod red. Dedova I.I., Shestakovoy M.V. [Dedov I.I., Shestakova M.V. (editors)]. Moscow; 2013; 120 p.

12. Pavlović D., Kocić R., Kocić G., et al. Effect of four-week metformin treatment on plasma and erythrocyte antioxidative defense enzymes in newly diagnosed obese patients with type

2 diabetes. *Diabetes Obes Metab* 2000 Aug; 2(4): 251–256, <http://dx.doi.org/10.1046/j.1463-1326.2000.00089.x>.

13. Men'shchikova E.B., Zenkov N.K., Lankin V.Z., et al. *Okislitel'nyy stress: patologicheskie sostoyaniya i zbolevaniya* [Oxidative stress: pathological conditions and diseases]. Novosibirsk; 2008; 284 p.