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EFFECTS OF METHYLPREDNISOLONE PULSE THERAPY ON INSULIN INJECTIONS IN PATIENTS WITH INSULIN – DEPENDENT DIABETES MELLITUS*

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SUMMARY: Satman İ, Fıçıcıoğlu C, Karşıdağ K, Yılmaz T, Dinçdağ N, Koca F, Odabaşı F, Aydın A, Devrim S, Haktan M. (Metabolism-Nutrition Unit, Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine, and Diabetes Unit, Department of Internal Medicine, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey). Effects of methylprednisolone pulse therapy on insulin injections in patients with insulin-dependent diabetes mellitus. Turk J Pediatr 1996; 38: 419-429.

In this study we evaluated 31 insulin-dependent diabetes mellitus (IDDM) patients (ages 12.1 ± 3.4 years, 18 males/13 females) who started on multiple subcutaneous insulin injections (MSII) within six weeks of diagnosis and achieved either complete (CR: no insulin requirement and near-normoglycemia for at least two weeks) or incomplete (ICR: minimum 50% decline in insulin requirement while maintaining near-normoglycemia for two weeks or more) remissions within the first 12 weeks of the MSII trial. Methylprednisolone pulse therapy (MP) was administered four times per day by IV bolus at a dose of 30 mg/kg (max. 1000 mg) on alternate days.

Eleven patients did not accept "MP-pulse" therapy; therefore, we followed these cases (7 males/4 females) as the control group. During the first year of follow-up, 13 patients from the "MP pulse" group achieved CR (3 males/1 female) or ICR (5 males/4 females) in 3.5 to 14 months. Remission occurred in only two of the control group cases (1 male CR for 17 days and 1 female CR for 7 months). Of those with CR in the "MP-pulse" and control groups, all were greater than 12 years of age, and all but one in the "MP-pulse" group were males. The stimulation capacity of beta cells (as defined by percentage increase in serum C-peptide levels after glucagon injection) among CR

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cases was found to be higher than that of non-remitted (NR) cases ($p < 0.05$ at onset, $p < 0.001$ during MSII-induced remission and $p < 0.05$ at the end of the first year of follow-up). Although patients with CR or ICR had higher beta cell reserves than NR cases at onset, only CR cases could sustain this capacity during the MSII-induced remission phase and one year after "MP-pulse" therapy. From this preliminary study, we conclude that "MP-pulse" therapy, may lead to prolonged near-normal beta cell function or partly preserved residual beta cell reserve during the MSII-induced remission phase of IDDM. The beneficial effects of MP could be seen clearly in patients diagnosed during the late childhood years. *Key words: insulin-dependent diabetes, methylprednisolone immuno therapy.*

Insulin-dependent diabetes mellitus (IDDM) is thought to result from the auto-immune destruction of beta cells in genetically susceptible individuals[1]. Beta-cell destruction is mediated by cells from the immune system and their products due to loss of self-tolerance[2,3].

Life prolongation achieved by the introduction of insulin has not changed the mostly unavoidable face of long-term complications[4]. The ability to secrete even a small amount of insulin seems to have a favorable influence on metabolic control and on the delay of onset of microvascular complications. As a logical approach, several immuno-interventions, ranging from aggressive immunosuppression to mild immunomodulation, have been tried in the past 15 years[5]. Immunointervention trials during the early clinical phase have failed because the pathological process has started months or even years before clinical onset, and many of the patients have only a limited beta-cell reserve at diagnosis[6]. On the other hand, 10 to 30 percent of patients may experience a naturally occurring complete remission as defined by clinical and metabolic characterizations[7]. Interestingly, in approximately half of new-onset patients, remission can be induced by intensive insulin therapy within the first few weeks or months of the disease[8]. Over the last 10 years our group has been involved in studying the potential value of the remission period for immunotherapeutic interventions.

The aim of this study was to prolong the remission period with the use of an immunosuppressive agent, methylprednisolone. Our study is the first to consider the effects of methylprednisolone pulse (MP-pulse) on multiple subcutaneous insulin injection (MSII)-induced remission in patients with IDDM.

Material and Methods

Selection of Patients

The study group consisted of 31 patients ranging in age from six to 19 years. Subjects were considered for recruitment in the study if they were six to 20 years of age, were of normal body weight, had IDDM diagnosed according to the WHO criteria[9] within the preceding six weeks, and developed a remission phase within 12 weeks of MSII therapy. Patients were started on 55 percent carbohydrate, 30 percent fat and 15 percent protein diets. Twenty patients were enrolled in

the "MP-pulse" group. Eleven patients who did not accept "MP-pulse" therapy were followed as the control group. Patients were admitted to the hospital during MSII therapy and were not discharged until their metabolic control was assessed after MP-pulse therapy. After discharge they self-monitored their blood glucose were contacted frequently by telephone for adjustments in insulin doses.

The study was approved by the local Ethical Committee, and an informed consent was obtained from patients or their parents.

MSII Therapy

Therapy consisted of regular human insulin injections before each main meal (3 doses) plus an intermediate-acting (NPH) insulin injection at bedtime for up to 12 weeks. Insulin doses were adjusted, aiming for preprandial glucose level of less than 140 mg/dl. Glucose profiles were monitored at least four times daily using a portable glucometer and glucose strips.

Remission

Remissions were defined according to the following criteria:

1. Compelete Remission (CR):
   - Preprandial glucose levels less than 140 mg/dl.
   - HbA1c less than 7.5 percent.
   - No insulin requirement for at least two weeks.
2. Incomplete Remission (ICR):
   Less than 50 percent of initial insulin requirement in spite of near-normal metabolic control for two weeks or more.

MP-Pulse Therapy

Methylprednisolone (MP) was administered four times per day on alternate days at a dose of 30 mg/kg (max. 1000 mg/day) by intravenous bolus.

Methods

Plasma glucose was measured by glucose oxidase and HbA1c was determined by a column chromatographic method. A commercially available RIA kit (DPC) was used for C-peptide determinations. Stimulated C-peptide levels were defined as a peak increase within 10 minutes of glucagon (30 µg/kg, max. 1000 µg I.V. bolus) injection. ICA (islet-cell antibody) was assayed by Botazzo's indirect IF technique, while CD4 (helper-inducer T cells) and CD8 (suppressor-cytotoxic T cell) evaluations were performed by EPICS CS apparatus (Coulter Electronics Inc. U.K.).

Statistical Evaluation

Student's t-test (paired or unpaired) and chi-square test were used for statistical analyses whenever required. A p value of less than 0.05 was accepted as significant.

Results

The clinical characteristics of the "MP-pulse" and control groups at entry (at clinical onset) listed in Table I.

Table I: General and Laboratory Characteristics of Patients at Entry*

General Characteristics	"MP-pulse" Group	Control Group
	n: 20	n: 11
Age (years)	11.7 ± 3.6	11.8 ± 3.1
Sex (m/f)	11/9	7/4
BMI (kg/m^2)	16.9 ± 2.9	16.3 ± 2.2
Occurrence with DK/DKA (%)	50	45
Family history of diabetes (%)	35	36
Laboratory Characteristics		
Blood glucose (mg/dl)**	338.4 ± 124.3	343.0 ± 119.3
HbA1c (%)	121.1 ± 4	12.0 ± 3.5
FCR (ng/ml)	0.51 ± 0.36	0.47 ± 0.25
SCR (ng/ml)	1.13 ± 0.78	1.11 ± 0.38
ICA positivity (%)	45	64
CD4/CD8 (n: 16)	1.9 ± 0.8	–
Insulin requirement (IU/kg/day)	0.73 ± 0.29	0.69 ± 0.13

* Results given as mean ± SD. (No significant difference other than ICA was found between the groups).
** Mean preprandial blood glucose level.

DK : Diabetic ketosis.
DKA: Diabetic ketoacidosis.
BMI : Body mass index.
FCP : Fasting C-peptide level.
SCP : Peak stimulated C-peptide level within 10 minutes of 1 mg glucagon injection.

Participants in the "MP-pulse" therapy included 11 males and nine females, while the control group consisted of 11 patients (seven males and four females). All differences between groups were insignificant at entry except for ICA. The ratio of CR/ICR after MSII therapy in the "MP-pulse" group was 10/10 (7 males and 3 females/4 males and 6 females) and 5/6 (2 men and 3 women/5 men and 1 woman) in the control group (Table II). The mean duration from disease onset to remission was 8.8 and 8.9 weeks in the "MP-pulse" and control groups, respectively. Laboratory characteristics during MSII-induced remission were not significantly different between the two groups, except for ICA positivity.

No side effects were noted during MP-pulse therapy except for a temporary increase in blood sugar which was normalized by insulin adjustments.

Table III shows the remission results and laboratory findings 12 months after "MP-pulse" therapy and among controls. Sixty-five percent of patients underwent

clinical remission during the first year of followup (4 CR and 9 ICR) in the "MP-pulse" group and 18.2 percent (1 CR and 1 ICR) in the control group ($p < 0.01$).

Table II: Patient Characteristics During MSII-Induced Remission Phase

	"MP-pulse" Group	Control Group
Mean time from onset to remission (weeks)	8.8 ± 26	8.9 ± 1.4
Remission status		
– CR (m/f)	7/3	2/3
– ICR (m/f)	4/6	5/1
total (CR/ICR)	10/10	5/6
Blood glucose (mg/dl)	121.8 ± 50.3	107.5 ± 17.6
HbA1C (%)	7.1 ± 2.3	7.6 ± 1.1
FCP (ng/ml)	0.68 ± 0.45	0.53 ± 0.31
SCP (ng/ml)	1.20 ± 0.68	1.14 ± 0.52
ICA positivity (%)	33	62
CD4/CD8 (n: 15)	1.5 ± 0.5	
Insulin requirement (IU/kg/day)	0.31 ± 0.12	0.29 ± 0.17

* No significant difference was defined between groups except ICA positivity.

CR : Complete remission.
ICR: Incomplete remission.

Table III: Patient Characteristics After "MP-Pulse" Therapy

	"MP-pulse" Group	Control Group
Duration of remission (months) (Mean total CR and/or ICR) Range	6.6 ± 4.6 (3.5-14 months) $p < 0.01$	3.1 ± 2.3 (17 days-7 months)
Remission status		
– CR (m/f)	3/1	1/10
– ICR (m/f)	5/4	0/1
Total (CR/ICR)	4/9 $p < 0.01$	1/1 1/1
Laboratory findings (at 12th month)		
Blood glucose (mg/dl)	121.8 ± 50.3	178.0 ± 40.6 $p < 0.01$
HbA1c (%)	9.2 ± 3.6	10.5 ± 1.9
FCP (ng/ml)	0.64 ± 0.29	0.36 ± 0.23
SCP (ng/ml)	1.17 ± 0.60	0.68 ± 0.43 $p < 0.02$
ICA positivity (%)	3.5%	5.5% $p < 0.01$
CD4/CD8 (n: 10)	1.7 ± 0.3	–
Insulin requirement (IU/kg/day)	0.31 ± 0.14 (n: 16)	0.66 ± 0.28 $p < 0.01$ (n: 11)

* NS: Not significant.

The ratio of helper-inducer to suppressor-cytotoxic T-cells (CD4/CD8) in the "MP-pulse" group increased at onset, markedly declined during the MSII-induced remission phase, and although slightly increased at the first year, remained lower than the initial value ($p < 0.01$ onset vs. MSII remission, $p < 0.05$ MSII remission vs. "MP-pulse", and $p < 0.05$ onset vs. "MP-pulse"). Unfortunately, we were not able to follow-up CD4/CD8 profiles of control patients because of limited resources.

At the end of the first year, the mean insulin requirement of patients in the "MP-pulse" group was 0.31 IU/kg/day, and 0.66 IU/kg/day in the control group ($p < 0.01$).

Another reflection of this difference appeared in the serum C-peptide levels. Patients in the "MP-pulse" group had higher beta-cell reserves than control cases, as indicated by both fasting and stimulated C-peptide levels ($p < 0.01$ and $p < 0.02$, respectively).

Discussion

The ultimate aim of the ongoing research in the field of immunotherapy for IDDM is to provide non-insulin dependence and prevention of long-term complications without upsetting patients' quality of life[7]. Early immunosuppressive therapy with azothioprine may prevent the rapid decline of endogenous insulin secretion and increase the remission rate during the first year[11]. No long-term benefits of either azothioprine or cyclosporin therapy have been reported, and after discontinuation of the drug the insulin dose is similar in treated and non-treated groups[12-15]. This fact is more or less true for other immunotherapeutic modalities, such as plasmapheresis[16], prednisone[17], antithymocyte globulin[18], anti-CD5[19] and nicotinamide alone or in combination with steroids or cyclosporin[20, 21]. Therefore, researchers have addressed the problem at the preclinical phase, aiming at prevention of IDDM before marked beta-cell loss[22-28].

On the other hand, intensive insulin therapy (IIT) either with continuous subcutaneous insulin infusion (CSII) or MSII started very early after the onset of IDDM (within the first few weeks) may induce remission in approximately 50 percent of patients[6, 7, 29]. The beta-cell recovery leading to a full remission with IIT depends not only on the magnitude of improved endogenous insulin secretion due to removal of the suppressive effect of glucotoxicity on the intact beta cells and allowing them to rest but also on the relieved body's sensitivity to insulin[30, 31].

The diagnosis of IDDM is very easy; careful follow-up and awareness of the possibility may be sufficient to notice the remission phase. Finally, patients in remission are destined to be "definitive" diabetics if left without intervention. For this reason, the ethical argument for intervention, mostly at the preclinical stage, is not fair[32].

Because autoimmune diseases in general are favorably influenced by a course of corticosteroids, it seemed reasonable to investigate whether steroids may

improve residual beta-cell function and prolong the remission phase in IDDM. One can expect increased insulin resistance during steroid therapy, but slowed down or halted beta-cell destruction at remission may suggest that this is not as severe a problem as suspected to warrant intervention with corticosteroids soon after clinical onset[32].

The increased CD4/CD8 ratio in our "MP-pulse" group, which indicates active autoimmunity, declined at remission ($p < 0.01$). Similarly, metabolic parameters (glycemia, HbA1c) and endogenous insulin secretion (fasting and stimulated C-peptide levels) improved during remission in both groups. The remission findings of our patients are proof of decelerated autoimmunnity at this phase.

A factor that, though unproven, may contribute to the maintenance of endogenous insulin secretion during remission is regeneration of beta cells[33]. The selected immunosuppressive agent in our study, MP, has a well-known regenerative effect on beta cells, as showed experimentally[17]. We previously revealed that long-term oral MP treatment during the remission period of IDDM prolonged this phase for at least 18 months in 60 percent of treated patients. Although we did not see severe side effects during the course of oral MP, these patients had to undergo long-term hospitalization. "MP-pulse therapy" is one of the therapeutic modalities applied for many autoimmune diseases. e.g. rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

At one-year of follow-up, about two-thirds of patients in the "MP-pulse" group reached remission, while the percentage of remitted patients among the control group was only 18 percent ($p < 0.01$). No patient under the age of 12 years was found among CR patients. Approximately 80 percent of ICR patients were over 10 years of age (Fig. 1).

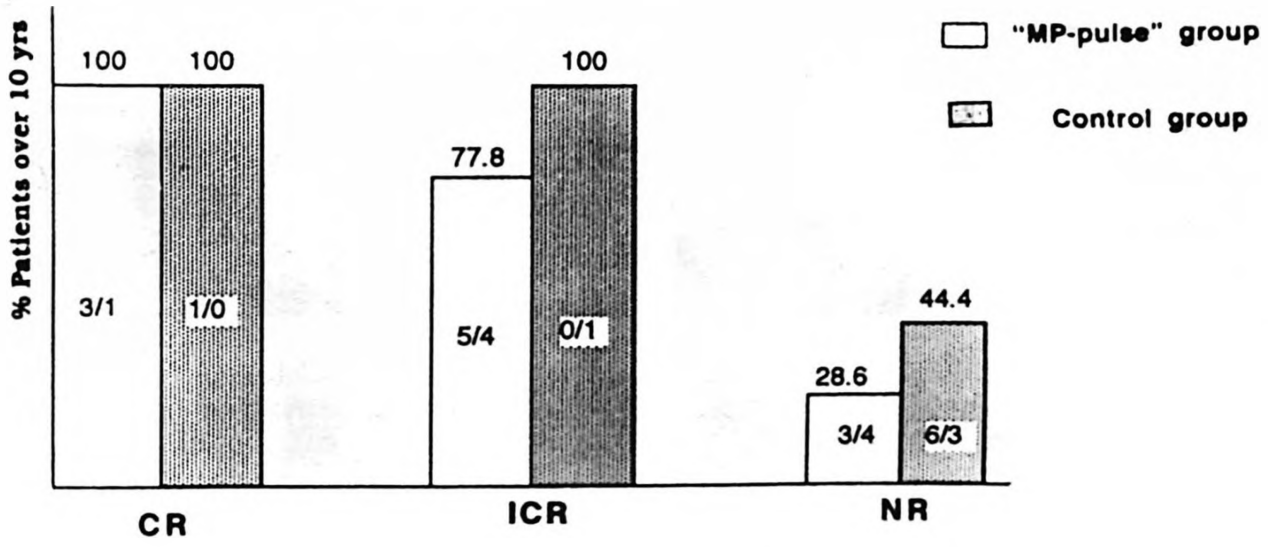

Fig. 1: Percentage of patients greater than 10 years of age among CR, ICR and NR patients (*figures inside each column indicate male/female ratio). CR: chronic remission. ICR: incomplete remission. NR: non-remitted cases.

In the first study with azothioprine, Harrison et al.[11] reported that seven out of 13 patients (mean age 25 years) were remitted completely. Later studies with the same drug in younger-onset patients (mean age 11 years) did not show such a high CR rate[6, 12]; thus the age of the patient at entry was considered as a possible predictor of outcome. The disease may progress more rapidly in younger than older children. In our study three out of four CR cases in the "MP-pulse" group and one patient who developed CR in the control group were males. Previous studies by our groups have revealed that male patients, especially in older-age-onset IDDM, may have better metabolic outcomes and a higher possibility of remission with IIT and immunotherapy[7, 32, 34]. This finding requires further confirmation.

CR patients had higher beta cell reserves than did ICR and NR patients at onset, as defined by percentage increase in serum C peptide after glucagon stimulation ($p < 0.05$ vs. NR, although CR vs. ICR was not significant). This reserve did not change significantly at the first year of follow-up; ICR patients had a lower capacity for beta-cell stimulation than did CR patients during MSII-induced remission ($p < 0.001$). However, this reserve was difficult to preserve later. NR patients had a limited beta-cell reserve at onset which could not be maintained during follow-up (Fig. 2).

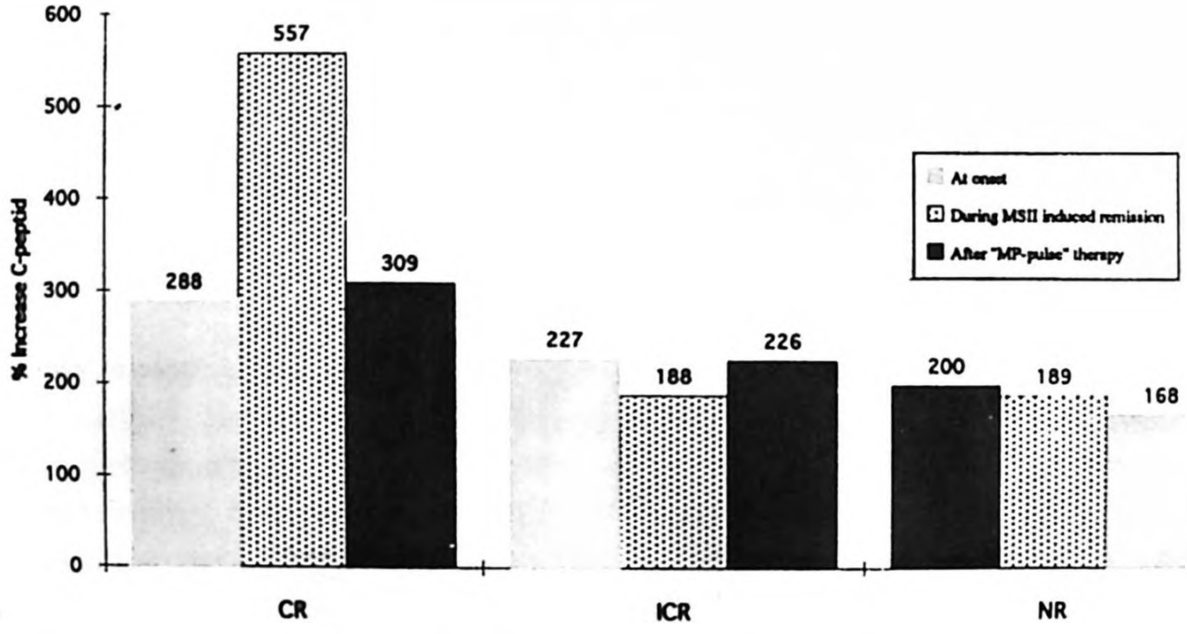

Fig. 2: Mean percentage increase in C-peptide level after glucagon stimulation in "MP-pulse" group. (*$p < 0.01$, **$p < 0.02$, ***$p < 0.05$, ****$p < 0.01$ vs. each onset).

The mean total duration of remission for consecutive CR and/or ICR in the "MP-pulse" group was found to be remarkably longer than in the control group ($p < 0.01$). Figure 3 depicts life-table analyses of "MP-pulse"-treated and control groups. The "MP-pulse" group had better metabolic control and significantly higher

beta-cell reserve than the control group at follow-up. Our results revealed that male gender, disease onset during late childhood and a higher beta cell reserve at clinical onset may point to a possible complete and/or long-term remission. This confirms the previous findings reported by our group, Schiffrin et al.[35, 36] and Karjalainen et al.[37] It seems that ICA positivity is not related to the likelihood of remission. ICA differences of our MP and control groups were thought to be due to limited numbers of patients enrolled in the study.

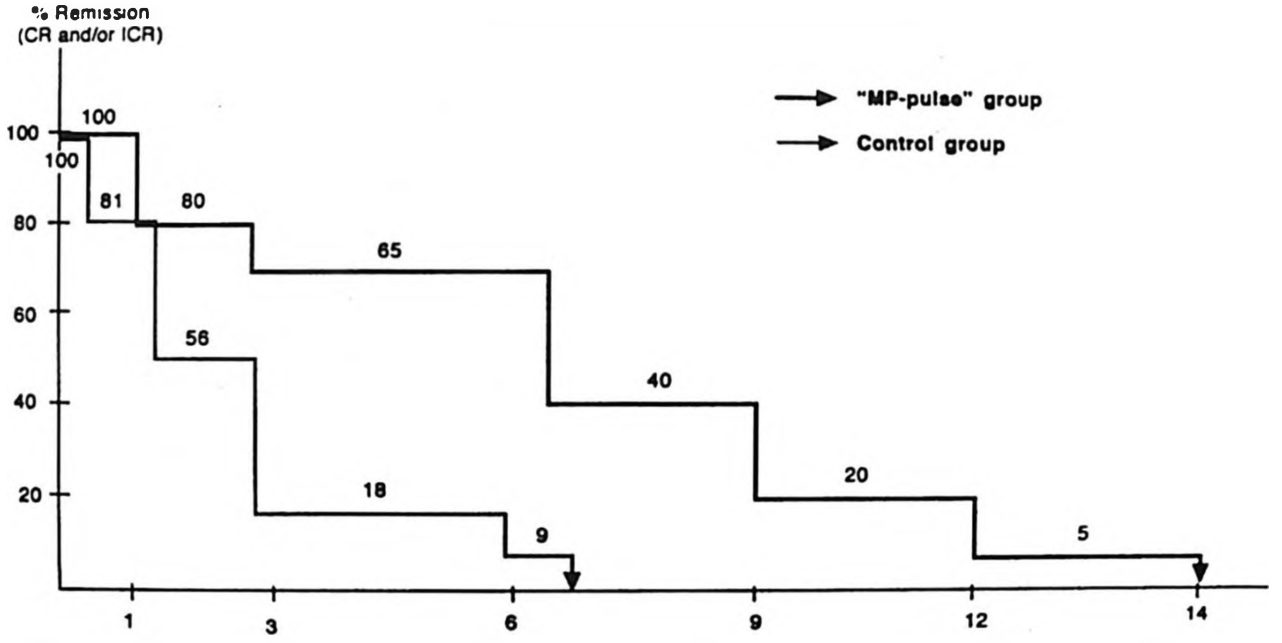

Fig. 3: Life table analyses for total remission in "MP-pulse" and control groups. Figures in parentheses indicate number of cases in remission in each time-period.

This work was conducted as part of a development plan relevant to immune-interventional trials in the remission period of IDDM. To our knowledge this study is the first to investigate the influence of "MP-pulse" therapy specifically on MSII-induced remission. The preliminary results suggest that "MP-pulse" therapy may have utility in prolonging the remission phase by helping to preserve beta-cell function from further deterioration during the MSII-induced remission phase of IDDM, particularly in patients diagnosed after early childhood ages.

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