

Blood biochemical changes in mice after administration of a mixture of three anesthetic agents

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ABSTRACT. Currently, from the viewpoint of animal welfare, anesthesia or analgesia is required during experimental procedures in animals that are likely to cause pain. A part of these anesthetics have been reported to influence a blood biochemical level. It is important for us to understand the effect of the anesthetic on blood biochemistry when we choose the anesthetic agent to be used in experiments. In this study, we examined the blood biochemical changes in mice after administration of a new mixture of three anesthetic agents –medetomidine / midazolam / butorphanol (MMB). We subcutaneously administered two dose combinations of MMB (0.45 / 6 / 7.5 and 0.9 / 12 / 15 mg/kg) in mice, followed by administration of atipamezole, for reversal of anesthetic effects, after 1 hr. Thereafter, blood biochemistry was assessed at 1, 4 and 24 hr after MMB administration. We observed that MMB administration caused a transient increase in blood sugar, inorganic phosphorus, potassium and creatine kinase levels. These, however, returned to the reference range 24 hr after MMB administration. In conclusion, MMB changes the levels of some blood biochemical parameters, but not to an extent that would threaten health. However, when using laboratory animals, this effect of MMB may influence the experimental results, depending on the experimental content. Hence, the choice of anesthetic agents used in laboratory animals should be based on detailed knowledge of their pharmacological effects.

KEY WORDS: anesthesia, atipamezole, butorphanol, medetomidine, midazolam

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Prevention of pain and distress is essential when experimental animals are used for biomedical research. This can be achieved by the use of appropriate anesthetics and analgesics [26]. Most general anesthesia methods consist of a combination of injection and inhalational anesthetics. In the case of laboratory mice, the intraperitoneal (i.p.) or subcutaneous (s.c.) administration route is usually chosen [1, 4, 34]. Although injection anesthesia, using either a single agent or a drug combination, can be used, control of the depth of anesthesia by intravenous drug administration is difficult. Because the duration and depth of anesthesia are affected by the characteristics of the mouse (strain, age, gender, individual variation, etc.) and the surrounding conditions (temperature, kind of surgery, etc.). So, it is difficult to decide the dose of matching all mice [8, 14, 21, 22, 39]. Therefore, despite prior dosage testing, maintenance of anesthesia by injection anesthetics often remains difficult. In addition, there are individual differences in anesthetic effect, which can lead to overdosage in some animals [1, 4, 38].

In laboratory animals, pentobarbital sodium or mixed anesthesia with ketamine (K)/ xylazine (X) has been widely

used until recently. However, ketamine was labeled as a narcotic according to a revised law in 2007. Hence, its use became highly regulated, making it necessary to employ qualified persons, such as a narcotics researcher and the facility's drug application specialist when using ketamine. In addition, pentobarbital sodium is likely to cause a decrease in blood pressure or inhibition / cessation of respiration at the depth of anesthesia that is required for surgery, which can be fatal. Furthermore, the analgesic effect of pentobarbital used as a single agent is reportedly weak [12, 25, 32]. Besides this, due to their metabolism in the liver, injection anesthetics are known to cause changes in blood levels of metabolic enzymes released by the liver [35].

The most common volatile anesthetic used in laboratory rodents is isoflurane [26], which is supplied through to the respiratory tract of the animal by a dedicated vaporizer via a carrier gas. These volatile anesthetics allow rapid anesthesia induction and washout, which facilitates regulation of the concentration of the anesthetic in each individual animal. Monitoring of the vital signs of the animal, as indicative of the depth of anesthesia, allows regulation of the concentration of volatile anesthetics and minimizes the risk of death. Volatile anesthetic devices for use in laboratory rodents have already been developed and are available for use [26, 33]. In addition, recent anesthesia devices come with built-in exhaust gas collection devices [10, 11, 15], thus protecting users from inhalation of the exhaust gas [20, 29–31]. However, the use of these devices is limited by the number of animals

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Table 1. List of drugs used in this study

Group name	Drug	Product concentration	Agent dose	Administration route
Control	Saline	–	–	subcutaneous
	Saline	–	–	subcutaneous
Iso sa (+)	Isoflurane	2%	4 l/min	inhalation
	Atipamezole*	5.0 mg/ml	1.8 mg/kg	subcutaneous
Iso sa (–)	Isoflurane	2%	4 l/min	inhalation
	Medetomidine	1.0 mg/ml	0.45 mg/kg	
MMB Low	Midazolam	5.0 mg/ml	6.0 mg/kg	
	Butorphanol	5.0 mg/ml	7.5 mg/kg	subcutaneous
	Atipamezole*	5.0 mg/ml	0.9 mg/kg	
	Medetomidine	1.0 mg/ml	0.9 mg/kg	
MMB High	Midazolam	5.0 mg/ml	12.0 mg/kg	
	Butorphanol	5.0 mg/ml	15.0 mg/kg	subcutaneous
	Atipamezole*	5.0 mg/ml	1.8 mg/kg	
	Medetomidine	1.0 mg/ml	0.9 mg/kg	
Pent	Pentobarbital sodium	64.8 mg/ml	48.6 mg/kg	intraperitoneal
K / X	Ketamine	50 mg/ml	75 mg/kg	
	Xylazine	20 mg/ml	10 mg/kg	intraperitoneal
Med	Medetomidine	1.0 mg/ml	0.9 mg/kg	
Mid	Midazolam	5.0 mg/ml	12.0 mg/kg	subcutaneous
But	Butorphanol	5.0 mg/ml	15.0 mg/kg	

Atipamezole* is only administrated in Experiment 3.

used in the experiment, because of the number of possession device. Therefore, large-scale animal experiments using isoflurane anesthesia may be difficult, due to number of devices that would be required and the laboratory space required to store them. Further, a transient increase in blood sugar levels with isoflurane use has been reported [7, 11, 40, 41].

Hence, a new injection anesthesia method, which is not limited by the number of possession devices, not strictly regulated and can adequately control the depth of anesthesia, is strongly needed. It is also important for us to understand the effect of the anesthetic on blood biochemistry when we choose the anesthetic agent to be used in experiments. In this study, we examined the blood biochemical changes in mice after administration of a combination of three anesthetics, medetomidine / midazolam / butorphanol (MMB), which recently attracted attention for injection anesthesia of laboratory animals [13, 17, 19]. Changes in blood biochemistry following MMB administration were compared with those following isoflurane and other injection anesthetic administration (Pentobarbital sodium and K / X).

MATERIALS AND METHODS

Animals and housing conditions: We used male C57BL/6J mice at 8 weeks of age from CLEA Japan, Inc. (Tokyo, Japan). Animals were acclimated for at least 7 days before experimental use. The animals were housed in stable groups of 3 or 4 each, in polycarbonate cages with autoclaved bedding (Aspen Chips, TAPVEI Ltd., Kortteinen, Finland). Each cage had tap water in a polycarbonate bottle, and the mice were supplied with sterilized food (CLEA Japan, Inc.). The ambient temperature was controlled by a central

temperature management system and was maintained from 20 to 26°C. Humidity was maintained between 30 and 70%. Mice were maintained at a 12:12-hr light: dark cycle (light on from 7 a.m. to 7 p.m.). All of the animal procedures were conducted in accordance with the approved guidelines of VET-300 (2014), VET-700 (2014) and CAR-100 (2014) of the Institutional Animal Care and Use Committee at Chugai Pharmaceutical Co., Ltd.

Drugs: We used the following drugs for the experiments. The MMB combination in this study contained medetomidine (Domitol, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan) and butorphanol (Vetorphale, Meiji Seika Pharma Co., Ltd., Tokyo, Japan). Atipamezole (Antisedan, Nippon Zenyaku Kogyo Co., Ltd.) was used as a medetomidine antagonist. The other anesthetics assessed included isoflurane (Escain, Mylan Co., Ltd., Tokyo, Japan), pentobarbital sodium (Somnopentyl, Kyouritu Co., Ltd., Tokyo, Japan), and a mixture of ketamine (Ketalar, Daiichi Sankyo Co., Ltd., Tokyo, Japan) and xylazine (Celactal, Bayer Yakuhin Co., Ltd., Osaka, Japan). All drugs were diluted by saline (Otsuka normal saline, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan). And, all drugs were stored at room temperature.

Preparation of the drugs: All drugs were prepared according to Table 1 and were diluted in saline. Preparation of MMB was conducted in accordance with previous reports [3, 12, 18, 37]. Each MMB dose was determined by pre-examination that evaluated MMB singly administrated in mice; MMB Low dose was the minimum dose that enabled surgery, and the MMB High dose was the maximum single dose that did not result in death. All mice were warmed in

Table 2. Blood biochemical levels 1 hr after commencement of the experiment in male mice

Item	Control (N=3)	Iso sa (+) (N=6)	Iso sa (-) (N=6)	Pent (N=6)	K/X (N=6)	MMB Low (N=7)	MMB High (N=19)
GLUC (mg/dl)	276.3 ± 14.8	289.8 ± 22.5	356.0 ± 55.5	251.8 ± 31.2	653.5 ± 47.1 ^{b)}	721.4 ± 55.2 ^{b)}	714.8 ± 65.4 ^{b)}
IP (mg/dl)	6.9 ± 0.3	5.1 ± 0.4 ^{a)}	7.8 ± 1.1	8.1 ± 0.6	10.4 ± 0.7 ^{b)}	10.5 ± 0.4 ^{b)}	10.5 ± 0.5 ^{b)}
Ca (mg/dl)	9.1 ± 0.3	9.6 ± 0.2 ^{b)}	9.6 ± 0.1 ^{b)}	9.4 ± 0.3	9.0 ± 0.1	9.1 ± 0.2	9.1 ± 0.2
LD (U/l)	228.7 ± 18.9	252.8 ± 83.4	187.3 ± 58.8	214.8 ± 62.9	203.2 ± 50.3	268.3 ± 82.5	278.6 ± 108.4
CK (U/l)	271.0 ± 81.7	197.5 ± 34.5	68.5 ± 15.7	224.0 ± 63.2	188.3 ± 44.1	348.6 ± 63.8	604.4 ± 150.7 ^{b)}
Na (mmol/l)	147.7 ± 0.6	145.0 ± 0.9	145.3 ± 1.0	150.3 ± 1.2	144.8 ± 1.3	144.1 ± 1.3 ^{a)}	145.7 ± 2.2
K (mmol/l)	4.4 ± 0.2	4.6 ± 0.2	5.5 ± 0.8 ^{b)}	5.4 ± 0.2 ^{a)}	6.1 ± 0.4 ^{b)}	6.6 ± 0.4 ^{b)}	6.5 ± 0.6 ^{b)}
Cl (mmol/l)	116.3 ± 1.5	113.0 ± 1.5 ^{a)}	110.2 ± 0.8 ^{b)}	115.5 ± 1.4	110.3 ± 3.1 ^{b)}	109.3 ± 1.8 ^{b)}	109.8 ± 1.9 ^{b)}

The value is mean ± standard deviation. The statistics processing uses the parametric Dunnett's multiple comparison tests. a) $P < 0.05$ vs. Control, b) $P < 0.01$ vs. Control.

the all experiments. The dosage of other drugs followed the guidelines of the Institutional Animal Care and Use Committee at Chugai Pharmaceutical Co., Ltd.

Evaluation of the depth of anesthesia: Three parameters were used to assess the depth and duration of anesthesia, namely, the righting reflex, abdominal region pinch reflex and pedal withdrawal reflex in the hind limbs. Recovery of the righting reflex was defined as the time when the animals began to roll over. For the pedal withdrawal reflex, the interdigital web of limbs on both sides was lightly pinched and pulled using atraumatic forceps [17, 19]. For the abdominal region pinch reflex, a 1 cm region of the abdominal skin was lightly pinched using hooked forceps. Absence of all reflexes was defined as an adequate depth of surgical anesthesia. The depth of anesthesia was evaluated at 10, 30 and 60 min after commencement of the experiment. All reflex tests were carried out and assessed by the same operator.

Experimental design: Animals were divided into the following experimental groups: Iso group, MMB Low group, MMB High group, Pent group, K / X group, medetomidine (Med) group, midazolam (Mid) group and butorphanol (But) group (Table 1). In addition, the Iso group was divided into the saline administered group (Iso sa (+)) and not administered group (Iso sa (-)), to confirm the effect of saline administration. A control group was also included, in which a necropsy was performed under isoflurane anesthesia 1 hr after saline administration.

Experiment 1: Blood sample for biochemical measurements was collected from abdominal portion of vena cava under isoflurane anesthesia in all groups, other than the MMB High group that maintained an adequate depth of surgical anesthesia. Necropsies were performed on mice to confirm the influence on organ of MMB in the Control group (N=3), Iso sa (+) group (N=6), Iso sa (-) group (N=6), Pent group (N=6), K / X group, (N=6), MMB Low group (N=7) and MMB High group (N=19) 1 hr after the experiment was commenced. Blood biochemical levels (blood sugar (GLUC), inorganic phosphorus (IP), calcium (Ca), lactate dehydrogenase (LD), Creatine Kinase (CK), sodium (Na), potassium (K) and chlorine (Cl)) at the time of necropsy were compared between groups.

Experiment 2: Necropsies were performed on Control group (N=3), Iso sa (-) group (N=6), MMB High group

(N=12), Pent group (N=6), K / X group (N=6), Med group (N=6), Mid group (N=6) and But group (N=6) mice 1 hr after commencement of the experiment. Thereafter, CK isoenzyme levels were compared between groups.

Experiment 3: Changes in biochemical parameters in the Iso sa (+) (N=18), MMB Low (N=19) and MMB High groups (N=27) over time were compared in each group. In addition, 1 hr after commencement of anesthesia, mice in all the groups were given atipamezole (Iso sa (+) and MMB High; 1.8 mg/kg, MMB Low; 0.9 mg/kg) for recovery from anesthesia (Table 1). Then, necropsies were performed in 6–7 animals in each group at 1, 4 and 24 hr after commencement of the experiment.

Blood biochemical level measurements: After blood collection, the blood was poured into a tube containing heparin lithium and centrifuged at 1,900 G and 15°C for 10 min, and the resultant plasma was collected. Plasma was analyzed for blood biochemical levels by Chemical automatic analyzer TBA-120FR (Toshiba Medical Systems, Co., Ltd., Otawara, Japan). The CK isoenzyme was measured using a CK isoenzyme kit (Titan gel CK (QG)) with automatic electrophoretic device, Epalyzer 2 (Helena Laboratories, Co., Ltd., Beaumont, TX, U.S.A.).

Data analysis: All results are expressed as mean ± standard deviation. All data were analyzed by parametric Dunnett's multiple comparison tests with SAS software ver.5.0 (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS

In the MMB Low group, surgical level anesthesia was achieved in 50% of all mice at 10 min and 60 min. In Pent and K / X groups, all mice recovered from anesthesia within 1 hr after administration of the anesthetics. Hence, necropsies were performed in all the groups immediately after isoflurane anesthesia, other than the MMB High group that maintained an adequate depth of surgical anesthesia. In the Med, Mid and But groups, an adequate surgical depth of anesthesia was not achieved. In addition, death due to anesthetic overdosage did not occur in any of the mice in this study.

Changes in blood biochemical levels 1 hr after start of the experiment: In experiment 1, GLUC, IP and K levels

increased 1 hr after commencement of the experiment in the K / X ($P < 0.0001$), MMB Low ($P < 0.0001$) and MMB High groups ($P < 0.0001$) and beyond the reference range (Table 2) [5]. In addition, CK increased in the MMB High group ($P = 0.0058$). On the other hand, LD levels did not change in any of the groups and within the reference range (Table 2) [5]. In the Iso sa (+), Iso sa (-) and Pent groups, blood biochemical levels did not change significantly or were within the reference range (Table 2) [5].

Change in CK isoenzyme levels 1 hr after commencement of the experiment: In experiment 2, CK in the MMB High ($P = 0.0036$) and Mid groups ($P = 0.027$) increased and in the Med group ($P = 0.0503$) had a tendency to increase at 1 hr after start of the experiment. Skeletal muscle type isoenzyme of CK (CK-MM) also had a tendency to increase in the MMB High group ($P = 0.0993$). In addition, the brain-type isoenzyme of CK (CK-BB) increased in the Med group ($P = 0.0003$), while CK-MM increased in the Mid group ($P = 0.0184$) (Table 3).

Changes in blood biochemical levels 1, 4 and 24 hr after MMB administration: In experiment 3, the changes in blood biochemical parameters seen in experiment 1 returned to the levels seen in the Iso sa (+) group at 24 hr after anesthetic administration (Table 4) (Supplementary Table 1). The levels of GLUC ($P < 0.0001$), IP ($P < 0.0001$) and K ($P < 0.0001$) increased at 1 hr after MMB administration in the MMB Low and High groups. CK increased only in the MMB High group ($P < 0.0001$). All the blood biochemical parameters, except GLUC, returned to the level in the Iso sa (+) group 4 hr after MMB administration. There were some statistically significant changes in other blood biochemical items (ALP, TCHO, TG, TP, UN, Ca, Na and Cl) as well. However, these changes were not considered to be important (Supplementary Table 2), because they were unrelated to the dose of anesthetic administered or the passage of time, and they were within the reference range [5]. An additional experiment proved that there were no gender related differences in the results (Supplementary Tables 3 and 4).

DISCUSSION

In this study, Iso sa (+) group and Iso sa (-) group of the comparison group of the anesthesia were anesthetized with isoflurane; the isoflurane is frequently used during surgery, because of its easy control of anesthesia depth and its relative safety compared to injection anesthesia [36]. A previous report indicated that isoflurane increases GLUC levels by decreasing insulin secretion [7, 11, 40, 41] and the surgical stress itself conduces to the hyperglycemia [9]. So, the GLUC level in the Control, Iso sa (+) and Iso sa (-) groups might have been influenced by the surgery stress. The reason for the elevated GLUC and K levels in the MMB Low, MMB High and K / X groups is inhibition of insulin secretion of the pancreatic β -cells and closing of the ATP-dependent potassium ion channels of the cell membrane by $\alpha 2$ -adrenoreceptor agonists; Medetomidine and Xylazine [2-4, 6, 11, 13, 18, 23, 36, 41] (Table 2). Since pentobarbital does not affect insulin secretion, GLUC levels in the Pent

group become the level in the control group (Table 2) [5, 16, 27, 40]. Further, atipamezole has been reported to have $\alpha 2$ -adrenoreceptor blocking effects [24]. In the MMB High group, the blood level of GLUC and CK remained high at 3 hr after atipamezole administration (Table 4). This suggests the possibility that the dose of atipamezole used in this study was not enough to reverse the effects of MMB. According to a previous report, it requires a long time for the GLUC level to decrease when the dose of atipamezole on medetomidine is not enough [27]. All blood biochemical levels finally became the level in the Iso sa (+) group or returned to reference levels at 24 hr after administration of the anesthetic agents (Table 4).

The blood levels of GLUC, IP and K in the K / X group were increased, as in the MMB administration group. The K / X combination contains xylazine, which stimulates on the $\alpha 2$ -adrenoreceptors, similar to that of medetomidine [3, 4, 13, 18] (Table 2). However, CK levels in the K / X group did not increase. And, it is thought that the effect of MMB administration was beyond the influence of the s.c. administration. Because, the CK level of the MMB High group was higher than the Control group by s.c. administration. Muscle twitching is reported to be caused by $\alpha 2$ -adrenoreceptor stimulants, the degree of muscle twitching varying according to the route of drug administration [28]. Hence, the difference in CK levels between the K / X and MMB groups is probably related to the difference in the method of administration of the drugs. CK levels increased in both the Med and Mid groups. Therefore, because of the cumulative effect of both medetomidine and midazolam, CK levels in the MMB group probably increased to a greater extent than in the K / X group (Table 3).

To clarify which drug in the MMB combination caused the increase in CK, CK isoenzymes in each group were examined. We observed increases in CK levels in the MMB High, Med and Mid groups (Table 3). In the Mid group, CK-MM levels were elevated, similar to what was seen in the MMB High group. CK-BB levels in the Med group were also clearly high in comparison with the Control group. This indicates that the rise in CK level in the MMB High group was due to the combined influence of both medetomidine and midazolam. In addition, it appears that most of the changes in blood biochemistry, except for the change in CK levels, were mainly due to medetomidine (Supplementary Table 1).

In this study, administration of MMB in mice caused a temporary rise in the levels of blood biochemical parameters. This, however, did not seem to adversely affect the health of the mouse. Our results highlight the fact that when MMB is used to anesthetize animals for experiments, the effects of the anesthetic agents on blood biochemistry should be considered during experimental data analysis. In addition, use of MMB in pathological animal models, such as diabetes models, requires caution, because GLUC levels increase greatly with MMB.

Our study suggests that the anesthetic and analgesic agents that are usually used for animal experiments may cause changes in the blood biochemical parameters that are commonly measured as experimental data. Hence, it is important to be aware of the effects of the drugs before their use.

Table 3. CK and CK isoenzyme levels in plasma 1 hr after anesthetic drug administration in male mice

Item	Control (N=3)	Iso sa (-) (N=6)	MMB High (N=12)	Pent (N=6)	K / X (N=6)	Med (N=6)	Mid (N=6)	But (N=6)
CK (U/l)	271.0 ± 81.7	68.5 ± 15.7	572.9 ± 138.3 ^{a)}	224.0 ± 63.2	188.3 ± 44.1	584.7 ± 296.9	616.0 ± 297.3 ^{a)}	240.2 ± 134.0
CK-MM (U/l)	190.0 ± 71.0	28.0 ± 14.5	394.2 ± 111.2	114.6 ± 39.0	84.8 ± 25.2	306.0 ± 243.8	484.1 ± 232.9 ^{a)}	149.6 ± 124.8
CK-BB (U/l)	77.1 ± 9.0	37.3 ± 10.5	157.9 ± 45.2	106.1 ± 27.9	92.4 ± 19.9	259.0 ± 130.3 ^{b)}	125.2 ± 68.0	86.0 ± 22.3
CK-MB (U/l)	3.9 ± 2.0	3.2 ± 1.1	20.8 ± 16.0	3.3 ± 0.9	11.2 ± 5.6	19.8 ± 19.2	6.8 ± 3.1	4.6 ± 2.0

The value is mean ± standard deviation. The statistics processing uses the parametric Dunnett's multiple comparison tests. a) $P < 0.05$ vs. Control, b) $P < 0.01$ vs. Control. CK-MM: Skeletal muscle type isoenzyme of CK, CK-BB: Brain type isoenzyme of CK, CK-MB: Myocardial type isoenzyme of CK.

Table 4. Changes in blood biochemical parameters 1, 4 and 24 hr after anesthetic administration in male mice

Item	Iso sa (+)			MMB Low		
	1 hr (N=6)	4 hr (N=6)	24 hr (N=6)	1 hr (N=7)	4 hr (N=6)	24 hr (N=6)
GLUC (mg/dl)	289.8 ± 22.5	258.3 ± 30.0	290.2 ± 27.9	721.4 ± 55.2 ^{b)}	473.8 ± 66.2 ^{b)}	284.3 ± 28.7
IP (mg/dl)	5.1 ± 0.4	7.8 ± 1.6	8.1 ± 0.4	10.5 ± 0.4 ^{b)}	8.4 ± 0.9	7.3 ± 0.5
Ca (mg/dl)	9.6 ± 0.2	9.4 ± 0.1	9.8 ± 0.3	9.1 ± 0.2 ^{b)}	9.4 ± 0.2	9.5 ± 0.2
LD (U/l)	252.8 ± 83.4	327.8 ± 54.8	194.5 ± 92.2	268.3 ± 82.5	340.7 ± 94.7	140.5 ± 20.4
CK (U/l)	197.5 ± 34.5	364.2 ± 209.3	145.0 ± 136.0	348.6 ± 63.8	437.7 ± 59.7	78.0 ± 27.3
Na (mmol/l)	145.0 ± 0.9	149.3 ± 1.0	147.3 ± 0.8	144.1 ± 1.3	145.5 ± 2.4 ^{a)}	148.5 ± 0.5 ^{a)}
K (mmol/l)	4.6 ± 0.2	3.8 ± 0.2	3.6 ± 0.4	6.6 ± 0.4 ^{b)}	4.4 ± 0.2 ^{b)}	3.4 ± 0.2
Cl (mmol/l)	113.0 ± 1.5	115.7 ± 1.6	112.8 ± 1.5	109.3 ± 1.8 ^{b)}	110.8 ± 3.8 ^{a)}	115.2 ± 1.5 ^{a)}

Item	MMB High		
	1 hr (N=13)	4 hr (N=7)	24 hr (N=7)
GLUC (mg/dl)	724.9 ± 74.1 ^{b)}	453.4 ± 91.6 ^{b)}	214.3 ± 33.3 ^{b)}
IP (mg/dl)	10.5 ± 0.5 ^{b)}	8.6 ± 1.0	7.9 ± 0.8
Ca (mg/dl)	9.1 ± 0.2 ^{b)}	9.4 ± 0.2	9.5 ± 0.1
LD (U/l)	269.7 ± 128.4	346.7 ± 74.2	136.9 ± 43.0
CK (U/l)	577.7 ± 170.9 ^{b)}	826.7 ± 206.0 ^{b)}	130.4 ± 128.5
Na (mmol/l)	145.7 ± 2.5	148.9 ± 2.6	150.3 ± 1.0 ^{b)}
K (mmol/l)	6.6 ± 0.6 ^{b)}	4.2 ± 0.3 ^{a)}	3.9 ± 0.3 ^{b)}
Cl (mmol/l)	110.2 ± 2.1 ^{a)}	114.6 ± 2.4	116.7 ± 1.7 ^{b)}

The value is mean ± standard deviation. The statistics processing uses the parametric Dunnett's multiple comparison tests.

a) $P < 0.05$ vs. Iso sa (+), b) $P < 0.01$ vs. Iso sa (+).

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